Keynote Address - Terry Orr-Weaver  Research taking flight from foundational biology.  T.L. Orr-Weaver  Whitehead Institute, Dept. of Biology, MIT.

Many exciting questions in biology remain to be solved. In Drosophila there is a wealth of fascinating biological processes whose regulation and mechanisms can be deciphered, building from a ground work of powerful tools developed by the Drosophila community for multidisciplinary approaches. This biology and community provide an excellent training environment for PhD students and career opportunities for postdocs. Examples will be provided from this lab of choices of problems and approaches to gain fundamental insights into the control of meiosis, the oocyte-to-embryo transition, and DNA replication.

Plenary Session 1  Mechanisms and roles of tumor-suppressive cell competition.  T. Igaki  Graduate School of Biostudies, Kyoto University, Kyoto, Japan.

Normal epithelial cells often exert anti-tumor effects against nearby oncogenic cells. In Drosophila imaginal epithelia, clones of oncogenic mutant cells for apico-basal polarity genes scribble (scrib) or discs large (dlg) are actively eliminated by cell competition when surrounded by wild-type cells. It has been shown that JNK signaling plays a crucial role in this cell elimination; however, the initial event occurring at the interface between normal cells and polarity-deficient cells remained unknown. Through a genetic screen in Drosophila, we identified the ligand Sas and the receptor-type tyrosine phosphatase PTP10D as the cell-surface ligand-receptor system that drives tumor-suppressive cell competition. At the interface between wild-type “winner” and polarity-deficient “loser” clones, winner cells relocalize Sas to the lateral cell surface while loser cells relocalize PTP10D to the lateral cell surface. This leads to trans-activation of Sas-PTP10D signaling in loser cells that restraints EGFR signaling, thereby enabling elevated JNK signaling in loser cells to trigger cell elimination. These findings uncover the mechanism by which normal epithelial cells recognize and eliminate oncogenic neighbors by cell competition. I will also discuss our recent data on the roles of tumor-suppressive cell competition in normal development and homeostasis, as well as on the mechanisms of other types of cell competition.

Plenary Session 1  Lost in translation – RNA processing defects impact synaptic metabolism in neurodegeneration.  D.C. Zarnescu  Dept Molec & Cell Biol, Univ Arizona, Tucson, AZ.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron death and muscle atrophy. Due to the presence of disease causing mutations and its association with pathological aggregates in 97% of ALS cases, the RNA binding protein TDP-43 has emerged as a major molecular denominator in disease. Using a systems approach we have uncovered TDP-43 dependent synaptic deficits caused by mRNA sequestration and translation inhibition in fly and patient derived motor neurons. Overexpression of specific mRNAs sequestered by TDP-43 in insoluble aggregates restores microtubule stability and the synaptic vesicle cycle at the neuromuscular junction, which in turn mitigates locomotor deficits and increases lifespan. Transcriptome and translatome profiling further support a model whereby synaptic and metabolic genes are dysregulated in degenerating motor neurons. Indeed, mitochondrial and ATP production deficits appear to be compensated by increased glycolytic input, which mitigates ALS phenotypes across multiple models. Collectively, these findings indicate that RNA based mechanisms can explain key functional deficits in motor neuron disease and inform therapeutic strategies.

Plenary Session 1  Mechanisms of Odor-coding and its Manipulation to alter Behavior.  A. Ray  Molecular Cell & Systems Biology, Univ California, Riverside, Riverside, CA.

There are several mysteries about how the sense of smell guides behaviors like finding food, avoiding predators and mating. The olfactory system uses one of the most sophisticated arrays of chemical sensors in nature: numerous transmembrane receptors that detect tiny amounts of odorants with specificity and generate complex patterns of neural activity in precisely wired circuits. We use Drosophila as a model to understand molecular and cellular coding principles underlying olfactory behavior. In order to do so we compliment genetics and neurophysiology with computational approaches like Machine Learning that allows for a system-wide understanding of odor-coding. We find that different receptors and neurons contribute to different types of olfactory behaviors, which in turn gets modified with experience. This knowledge serves as a model to understand odor-coding in other insects like disease-transmitting mosquitoes and agricultural pests, enabling discovery of odorants that can act as next-generation repellents to protect against malaria and agricultural loss. Using Drosophila we have also uncovered a parallel non-receptor pathway, which detects odorants that can be absorbed through
the membrane into a cell. This pathway is slow-acting, more ancient than transmembrane receptors, and is highly conserved across eukaryotes.

**Plenary Session 1 Reproductive Capacity Evolves in Response to Ecology through Common Developmental Mechanisms.** Didem P. Sarikaya5, Sam Church1, Laura P. Lagomarsino4, Karl M. Magnacca3, Steve L. Montgomery6, Don P. Price7, Kenneth Y. Kaneshiro8, Cassandra G. Extavour1,2 1) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge MA; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge MA; 3) Department of Evolution and Ecology, University of California, Davis CA; 4) Shirley C. Tucker Herbarium, Louisiana State University, Baton Rouge CA; 5) O'ahu Army Natural Resources Program, Schofield Barracks HI; 6) Waipahu, HI; 7) School of Life Sciences, University of Nevada Las Vegas, Las Vegas NV; 8) Department of Biology, University of Hawai‘i at Manoa, Honolulu HI.

The process of evolution by natural selection relies on heritable variation in traits that confer differential fitness. One such trait is lifetime reproductive capacity, or the total number of offspring that an individual can give rise to in its lifetime. In *Drosophila* reproductive capacity in females is determined in large part by the number of ovarioles, the egg-producing subunits of the ovary. Ovariole number is a quantitative trait that is highly variable across *Drosophila*, and is largely heritable, but also displays some phenotypic plasticity under different environmental conditions, including nutritional input. The greatest variation in *drosophilid* ovariole number occurs in the Hawaiian *Drosophila*, where ovariole number can range from one to over 100 ovarioles per ovary. We report, for the first time, insights into the developmental mechanisms regulating ovariole number and its evolution among Hawaiian Drosophila. We find evidence that the developmental mechanism principally responsible for controlling ovariole number in *D. melanogaster* also regulates ovariole number in natural populations of Hawaiian Drosophilids. Using comparative phylogenetic methods, we show that there is a trade-off between ovariole number and egg size, that reductions in ovariole number evolve convergently concurrent with habitat shifts to specific food sources, and that ovariole number variation among species with different food sources is best explained by adaptation to specific ecological niches.

**Plenary Session 1 The genomic basis of adaptation in *Drosophila*: sex, poison and other dramas.** A. Yassin Institute of Systematics, Evolution and Biodiversity, CNRS - MNHN, Paris, FR.

Adaptation shapes natural genetic variation and determines the evolutionary fate of organisms. However, the underlying mechanisms that promote or constrain adaptation are still unclear. The great diversity of drosophilid flies offers a unique opportunity to genetically dissect ecologically relevant traits using advanced tools. Here, I will focus on two traits that have recurrently evolved in multiple *Drosophila* species. In *D. erecta* and more than 20 species of the *montium* group, females have a contrasting color dimorphism with one morph potentially mimicking males. In *D. sechellia* and a subspecies of *D. yakuba* inhabiting different oceanic islands, flies are strictly associated with the toxic fruits of the same host plant (*Morinda citrifolia*). Such an association is a complex adaptation since it requires the simultaneous evolution on the same genome of preference and tolerance traits to *Morinda* chemicals. In both cases, a combination of population genomics, genome mapping and functional analyses identified recurrent and idiosyncratic genetic changes underlying the evolution of convergent phenotypes. These findings provide new insights on the genetic mechanisms underlying the evolution of adaptive traits.

**Plenary Session 1 The guts of Wnt signal transduction.** Yashi Ahmed Dept Molecular and Systems Biolo, Dartmouth Med Sch, HANOVER, NH.

The signal transduction pathway activated by the secreted ligands Wnt/Wingless is essential for growth and patterning in metazoans, and aberrantly activated in the vast majority of colorectal cancers. We use a Drosophila model to study core principles that underlie Wnt pathway activation and to identify novel therapeutic targets to combat Wnt-driven disease. We have focused on a central regulatory complex comprised of the key tumor suppressor Adenomatous polyposis coli (APC), the essential scaffold protein Axin, and a regulator of Axin that is among the most promising therapeutic targets, the ADP-ribose polymerase Tankyrase. In addition, we are testing the long-held tenet that Wingless function requires spread from its site of synthesis to form a gradient that specifies distinct cell fates as a function of ligand concentration. Surprisingly, this view was recently upended by the completely unexpected finding that tethering of Wingless to the membrane produces adults with nearly normal external morphology, indicating that the spreading of Wingless is largely dispensable for tissue patterning. To confirm that Wingless spreading is also unnecessary for the patterning of internal organs, we are studying the adult gut, in which Wingless gradients are established during development and persist through adulthood, despite the weekly turnover of the intestinal epithelium.

**Plenary Session II Non-conventional autophagy in the prothoracic gland mediates a larval nutritional checkpoint through alteration of cholesterol trafficking.** M.B. O’Connor, Xueyang Pan Gen, Cell Biol, Dev, HHMI, Univ Minnesota, Minneapolis, MN.

In *Drosophila*, the timing of post-embryonic developmental transitions is modulated by various environmental conditions.
such as nutrient availability. During the L3 stage, starvation causes developmental arrest and/or death if larvae do not first pass through two nutrient-dependent checkpoint known as critical weight (CW) and minimal viable weight (MVW). The molecular mechanism responsible for inducing developmental arrest prior to, but not after, satisfying these checkpoints is not understood. In this study, we demonstrate that starvation strongly induces an autophagy-like process within the PG prior to achieving CW/MVW, but this response is strongly muted as larvae pass through the checkpoint. We show that this autophagy-like process requires many Atg genes and can be suppressed by activation of the insulin/TOR pathway or knockdown of Atg gene expression in the PG leading to precocious pupariation and death. In contrast, if autophagy is hyper-activated in the PG of well-fed larvae after CW/MVW it causes developmental delay. Furthermore, we provide evidence that this autophagy-like process blocks production of the steroid hormone ecdysone by altering the trafficking of cholesterol, its primary precursor. The non-conventional aspect of this process is revealed by an apparent alteration in the fusion of autophagic vesicles with lysosomes. Instead, a dynamic Atg8 positive tubular network is induced in the PG before, but not after, passage through CW/MVW. In addition to confirming non-conventional autophagy as a gatekeeper of metamorphosis, we further find that Anaplastic Lymphoma Kinase (Alk), a receptor tyrosine kinase, works as an autophagy suppressor in late L3 nutrition-restricted (NR) animals. Alk expression in the PG is low during the early L3 stage and then increases as larvae surpass CW/MVW. Expression of constitutively active Alk in the PG prevents NR-induced autophagy in the early L3 stage, while knocking down Alk enables autophagy to be markedly stimulated during late L3 NR animals. In summary, our findings suggest that a nutritionally-regulated non-conventional autophagic-like process, specifically in the major endocrine organ of larva, is one mechanism by which the CW/MVW checkpoints delay development if sufficient energy reserves have not been sequestered to ensure successful maturation of the juvenile larva into the adult fly.

Plenary Session II Sexual interactions and the evolution of species isolating barriers. L.C. Moyle1, D.M. Castillo1,2, J.S. Davis1 1) Biology, Indiana University, Bloomington, IN; 2) Molecular Biology and Genetics, Cornell University, NY.

Sexual selection is frequently proposed as a powerful driver of speciation. Nonetheless, this role for sexual selection remains controversial; both empirical and theoretical work indicate that sexual selection can enhance or inhibit the evolution of reproductive isolation, depending upon genetic and ecological conditions.

Drosophila offers a powerful model to examine the mechanistic associations between sexually selected traits and species isolating barriers, and the reproductive and ecological conditions that could shape both. Flies remain the exemplar model for speciation studies, including of reproductive isolation, and genetic and physiological mechanisms of pre- and postcopulatory sexual interactions are well understood in several species.

Focusing largely on postcopulatory reproductive traits, we have been evaluating the role of sexually selected traits in the expression of isolating barriers, using male competition assays, and genetic and genomic analyses. We find positive phenotypic and genetic associations between male sexual performance within and between species, two necessary conditions for sexual selection to drive speciation. In addition, we find evidence that stronger conspecific sperm precedence has evolved in response to reproductive contact with heterospecifics (reinforcing selection), with both phenotypic and genetic consequences for sexual selection on male postcopulatory performance within species.

Our data indicate that sexually selected postcopulatory traits, such as sperm precedence, can both enhance reproductive isolation between species and respond to selection on reproductive isolation, indicating these traits might play a critical role in connecting sexual selection to the evolution of new species.

Plenary Session II Regulation of stem cell number in the intestine. Elena Lucchetta, Benjamin Ohlstein Dept Genetics & Development, Columbia Univ, New York, NY.

Intestinal stem cells (ISCs) serve as the powerhouse of the intestinal epithelium, the most actively self-renewing tissue in mammals. High turnover organs such as the intestine rely on the function of resident stem cells for survival in face of constant digestive, chemical and bacterial insults. As such, tissue homeostasis is intimately linked to stem cell number. Stem cells, by definition, self-renew. Therefore, stem cell replacement is thought to be driven by symmetric divisions of existing stem cells. However, alternate secondary mechanisms to symmetric divisions have remained largely unexplored. To probe alternative modes of ISC replacement in the intestinal epithelium, we developed a physiologically relevant starvation assay that elicits a rapid and severe loss of ISCs in the Drosophila posterior midgut. Using our assay, we demonstrate that, in areas nearly void of progenitor cells, ISCs are rapidly replaced upon re-feeding by depolyploidization of enterocytes through the process of amitosis. Current work is directed at identifying underlying mechanisms that are responsible for initiation and progression of amitosis.

Plenary Session II Highways for repair: nuclear actin filaments and myosins relocalize heterochromatic DNA breaks to the nuclear periphery. Christopher P. Caridi, Carla D’Agostino, Taehyun Ryu, Grzegorz Zapotoczny, Laetitia Delabaere, Xiao Li, Varandt Y. Khodaverdian, Nuno Amaral, Emily Lin, Alesandra Rau, Irene Chiolo Molecular and Computational Biology
Heterochromatin is largely composed of repeated DNA sequences prone to ectopic recombination. In Drosophila cells, ‘safe’ homologous recombination repair of heterochromatic double-strand breaks (DSBs) relies on a specialized pathway that relocates repair sites to the nuclear periphery before recruiting the strand invasion protein Rad51. The mechanism responsible for this movement was unknown. We discovered that relocation is dependent on Arp2/3-dependent dynamic actin filaments that start polymerizing at heterochromatic damage sites and reach the nuclear periphery. Relocalization also requires nuclear myosins and the myosin activator Unc45, which is recruited to repair sites in a Smc5/6-dependent manner. These components are responsible for the directed motion of heterochromatic breaks from the heterochromatin domain to the nuclear periphery. Arp2/3, actin nucleation and myosins are also required to relocalize heterochromatin DSBs in mouse cells, revealing conserved pathways. Defects in nuclear actin polymerization and myosin motor function result in heterochromatin repair defects and widespread chromosome rearrangements, revealing the importance of the relocalization pathway in genome integrity. These findings identify the formation of de novo nuclear actin filaments and associated myosins as effectors of chromatin dynamics required for heterochromatin repair in multi-cellular eukaryotes.

Plenary Session II Effects of the gut microbiota on host behavior and homeostasis. Julien Royet IBDM, Aix Marseille University, Marseille, _, FR.

Since eukaryotes live in an environment heavily contaminated by microorganisms, it is not surprising that they have forged, over the times, extremely complex and intimate relationships. It is also expected that eukaryotes have developed mechanisms to perceive the presence of bacteria and to adapt their immune response, their physiological status or even their comportment accordingly. Many reports have shown that bacteria can interact with eukaryotic nervous system, either for the benefit of the microbe that alters the host's behavior or to the benefit of the host that adapts its behavior to the infection. However, in most cases, the molecules and mechanisms underlying the dialog between bacteria and their host nervous system were not identified and their mode of action poorly understood. We will present our latest data dissecting the cellular and molecular mechanisms by which one single microbiota-derived compound, called peptidoglycan, influences the behavior, the physiology and the homeostasis of its infected host.

Plenary Session II Looking at chromosomes. J. Erceg1, J. AlHaj Abed1, A. Goloborodko2, R. B. McCole1, G. Nir1, I. Farabellia, C. Perez Estradaa, B. R. Lajoie5, G. Fudenberg2,4, N. Abdennur2, M. Imakaev2, S. C. Nguyen1, B. J. Beliveau1,7, H. M. Sasaki1,7, P. Yina,1,2 E. LiebermanAiden6,8,9, M. A. Marti-Renom5,10,11,12, J. Dekker5,13, L. A. Mirny2, C. Wu1 1) Harvard Med Sch, Boston, MA; 2) MIT, Boston, MA; 3) Barcelona Inst of Science and Technology, Barcelona, Spain; 4) Baylor College of Medicine, Houston, TX; 5) Univ Massachussetts Med Sch, Worcester, MA; 6) Univ of California, San Francisco, CA; 7) Wyss Inst, Boston, MA; 8) Broad Inst, Cambridge, MA; 9) Rice Univ, Houston, TX; 10) Centre for Genomic Regulation, Barcelona, Spain; 11) Univ Pompeu Fabra, Barcelona, Spain; 12) ICREA, Barcelona, Spain; 13) HHMI, Worcester, MA.

Our studies of genome organization have led us in a number of directions. One involves the application of haplo-type resolved Hi-C to explore the structure of homolog pairing in Drosophila. Another has led to the development of two single-molecule super-resolution imaging technologies (OligoSTORM and OligoDNA-PAINT). I will describe our progress in both these areas and, time permitting, touch on our exploration of sequence ultraconservation and its potential relevance to homolog pairing. This work was supported by NIH (C-TW, LM, JD, ELA, PY), HHMI (JD), ERC (MAM-R), the EMBO (JE), William Randolph Hearst (RBM), Damon Runyon (BB), and Uehara Memorial (HS) Foundations/Organizations, and the Ministerio de Economia, Industria y Competividad (IF), with the imaging work carried out in collaboration with Bruker Nano Inc. Finally, we apologize to our colleagues whose names could not be included as authors due the guidelines for abstracts. Those colleagues are: Steven Callahan, Carl Ebeling, Mohammed A. Hannan, Eric F. Joyce, Soun Lee, Sheikh Russell, Wren Saylor, John Schreiner, T. Niroshi Senaratne, Jeff Stuckey, and Michael E. Talkowski. *SCN is currently at University of Pennsylvania.

1 Determination of the interaction of STAT92E, Zfh2 and Wingless to regulate regeneration after radiation damage in Drosophila melanogaster. Shilpi Verghese, Tin Tin Su Molecular Cellular and Developmental Biology, University of Colorado, Boulder, CO.

The wing imaginal disc in the developing Drosophila larvae which forms the presumptive adult wing consists of pouch, hinge and notum. The pouch gives rise to the distal structure wing blade, notum to the body wall and hinge to the structure that connects the two. We have been studying regeneration in Drosophila larval wing imaginal discs after damage by ionizing radiation. Our published work found that the hinge is the source of cells that help regenerate the pouch to restore the normal structure. We identified STAT92E (Drosophila STAT3/5 or ‘STAT’) and Wingless (Drosophila Wnt1 or ‘Wg’) as activities required cell autonomously for the regenerative properties of the hinge cells (Verghese and Su, 2016). Our recently published work shows that while most larvae regenerate normally after radiation damage, around 20% regenerate with gross abnormalities forming ectopic wings that arises from the notum (Verghese and Su, 2017). We found that STAT activity increased after IR damage and that both STAT and Wg are also required for the formation of ectopic discs. The picture that emerges is one in which activities like STAT increase after radiation damage and fulfill essential roles in rebuilding the tissue. But such efforts must be continued in such a way that one and only one wing disc is regenerated. Our current efforts center on
understanding how STAT activity is controlled for faithful regeneration and the identification of downstream target(s) of STAT in regeneration. In this regard, we identified Nurf-38, which encodes a member of the Nucleosome Remodeling Factor complex, as opposing STAT to modulate the frequency of ectopic disc growth. We also detect the ectopic induction of Zfh2, a transcription factor that is normally expressed in the hinge, in the cluster of cells that resolve to form the ectopic discs. Our preliminary data show that down-regulating Zfh2 reduces the ability to regenerate the pouch and to form ectopic wings, suggesting that Zfh2 plays an important and previously un-detected role during normal and abnormal regeneration. During wing disc development, Wg initially induces Zfh2 (Whithworth and Russell, 2003) but later in development, Zfh2 is required to maintain Wingless expression in a subset of cells called the inner ring (Perea et al., 2013). Zfh2 was identified as an effector of STAT signaling in the Drosophila wing disc (Ayala-Camargo et al., 2013) and is induced in a STAT dependent manner during regeneration (Fortezza et al., 2016). We hypothesize that STAT92E, Zfh2 and Wg also interact to provide regenerative plasticity to the hinge cells during normal and abnormal regeneration. The results of ongoing experiments that address this hypothesis will be presented.

2 High-dimensional microbiome interactions shape host fitness. A. Gould¹, V. Zhang¹, L. Lamberti², E. Jones³, B. Obadia¹, A. Gavryushkin², J. Carlson³, N. Beererwenkel², W. Ludwig³ 1) Molecular & Cell Biology Dept, UC Berkeley, Berkeley, CA; 2) Computational Biology Dept, ETH Zurich; 3) Physics Dept, UC Santa Barbara.

With hundreds of species interacting with each other as well as with specific proteins and cells in our body, the microbiome is a complex ecosystem within a complex organism, neither of which we fully understand on their own, let alone in combination. Central questions of this relationship are how high microbial diversity is maintained in the gut and how this diversity impacts host fitness. Here we show that interactions between bacteria are major determinants of host physiology and the maintenance of diversity. We performed a complete combinatorial dissection of the naturally low-diversity Drosophila gut microbiome using germ free flies colonized with each possible combination of the 5 core species of bacteria, forming a 5-dimensional cube. We then measured the resulting microbial community abundances and fly fitness traits, including (i) development, (ii) reproduction, and (iii) aging. Notably, the fly gut environment promotes microbial diversity, which accelerates development, reproduction, and aging. From these measurements we calculated the impact of microbial interactions on fly fitness as the triangulations of the 5-cube, following the combinatorial geometry approach of Beererwenkel-Pachter-Sturmefels. Single species are not predictive of host phenotypes such as aging when in diverse communities, contradicting Koch's postulates where a single pathogen causes a disease. Furthermore, we find evidence that higher-order interactions are widely prevalent and contribute to the maintenance of microbial diversity, which ecologists have recently predicted. Important for evolution, a positive feedback exists between microbial community stability and host fitness, which may poise a population for divergence of hosts and the emergence of host-specific microbiomes.

3 Plasmamembrane-localization of apoptotic caspases for non-apoptotic functions. A. Amcheslavsky¹, S. Wang², C. Fogarty¹, J. Lindblad¹, Y. Fan¹, A. Bergmann¹ 1) Molecular, Cell and Cancer Biology, UMass Med Sch, Worcester, MA; 2) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 3) University of Birmingham, School of Biosciences, Edgbaston, Birmingham, UK.

Caspases are best characterized for their function in apoptosis. However, they also have non-apoptotic functions such as apoptosis-induced proliferation (AiP) where caspases release mitogens for compensatory proliferation independently of their apoptotic role. We report that the unconventional myosin, Myo1D, which is known for its involvement in left/right development, is an important mediator of AiP in Drosophila. Mechanistically, Myo1D translocates the initiator caspase Dronc to the basal side of the plasmamembrane where Dronc promotes the activation of the NADPH-oxidase Duox for ROS generation and AiP in a non-apoptotic manner. We propose that the basal side of the plasmamembrane constitutes a non-apoptotic compartment for caspases. In summary, we identified a novel function of Myo1D for AiP and tumorigenesis, and reveal a mechanism by which cells sequester apoptotic caspases in a non-apoptotic compartment at the plasmamembrane.

4 Tango7 and dark regulate mutually-exclusive subcellular domains of caspase activation during development. S. Neuman¹, Y. Kang¹,², A. Bashirullah¹ 1) Pharmaceutical Sciences Division, University of Wisconsin-Madison, Madison, WI; 2) Vollum Institute, Oregon Health & Sciences University, Portland, OR.

Caspases perform critical functions in both living and dying cells; however, how caspases perform physiological functions without killing the cell remains unclear. Here we identify a novel physiological function of caspases at the cortex of Drosophila salivary glands. In living glands, activation of the initiator caspase dronc triggers cortical F-actin dismantling, enabling the glands to stretch as they accumulate secreted products in the lumen. We demonstrate that tango7, not the canonical Apaf-1-adaptor dark, regulates dronc activity at the cortex; in contrast, dark is required for cytoplasmic activity of dronc during salivary gland death. Therefore, tango7 and dark define distinct subcellular domains of caspase activity. Furthermore, tango7-dependent cortical dronc activity is initiated by a sublethal pulse of the inhibitor of apoptosis protein (IAP) antagonist reaper. Our results support a model in which biological outcomes of caspase activation are regulated by differential amplification of IAP antagonists, unique caspase adaptor proteins, and mutually exclusive subcellular domains of caspase activity.
5 Regulation of *Wolbachia* by host autophagy across multiple cell-types in *Drosophila melanogaster*. M. Deehan1, P. Fineis1, H. Frydman1,2 1) Biology, Boston University, Boston, MA; 2) National Emerging Infectious Disease Lab NEIDL, Boston University, Boston, MA.

Autophagy is a conserved intracellular degradation pathway involved in recycling cytoplasmic constituents, including protein aggregates or damaged organelles such as mitochondria. Beyond its role in cytoplasmic maintenance, autophagy has been shown to act as an innate immune response targeting intracellular pathogens to the lysosome for degradation. While much work currently investigates autophagic-pathogen interactions, little is known about how autophagy interacts with endosymbionts. *Wolbachia*, a vertically transmitted obligate endosymbiont, is estimated to infect upwards of 40% of insect species including *Drosophila*. Previous studies showed that flies treated with rapamycin, a known autophagy inducer, led to decreased *Wolbachia* density in larva but increased density in the germline. This indicated that *Wolbachia* densities may be modulated by host autophagy in a cell-type dependent manner. Expressing RNAi against multiple autophagy proteins in a cell-type specific manner we showed increased *Wolbachia* density in the hub and polar cells, a somatic cell population in the testis and female germline respectively. Knockdown of autophagy in the germline reduced *Wolbachia* density in the germline. Further investigation into how autophagy negatively regulates *Wolbachia* in the hub reveals multiple autophagic pathways which regulate *Wolbachia* density differently. Selective autophagy utilizing a specific subset of core autophagy proteins targets *Wolbachia* for degradation. Conversely, a subset of autophagy proteins described in canonical autophagy positively effects *Wolbachia* densities. We describe how *Wolbachia*, an obligate endosymbiont interacts with host autophagy differently across multiple cell types and further describe how different types of autophagy within the same cell-type regulate *Wolbachia* density differently. This research provides novel insights into host-endosymbiont interactions including host degradation of endosymbionts by autophagy and how *Wolbachia* uses alternative forms of this process for their benefit.

6 Stretch follicle cells utilize lysosomal machinery to eliminate nurse cells by phagoptosis. A.A. Mondragon1,2, A. Yalonetskaya1, A.J. Ortega1, Y. Zhang1, O. Naranjo1, J. Elguero1, K. McCall1 1) Biology, Boston University, Boston, MA; 2) Molecular Biology, Cell Biology, and Biochemistry, Boston University, Boston, MA.

Apoptosis, autophagic cell death, and necroptosis are the most heavily studied types of regulated cell death; however, there are many other forms of cell death. One such form is phagoptosis. In phagoptosis, one cell utilizes phagocytosis machinery to kill a nearby cell that would otherwise continue living. In the Drosophila ovary there are 15 nurse cells that support the oocyte throughout development. The nurse cells and oocyte are surrounded by a layer of follicle cells. In late stages of oogenesis the nurse cells are encompassed by a subset of follicle cells called stretch follicle cells. Interestingly, we have recently shown that the stretch follicle cells utilize phagocytosis machinery (Draper, Ced-12, and the JNK pathway) to promote nurse cell death through phagoptosis; however, the exact mechanism of how the stretch follicle cells induce nurse cell death remained elusive. Through live imaging, an in vivo engulfment detector, and an RNAi screen, we have determined that the stretch follicle cells utilize lysosomal machinery to acidify and breakdown the nurse cells. Interestingly, live imaging of egg chambers with probes and GFP fusion proteins as well as an in vivo engulfment detector has demonstrated that nurse cells are not engulfed piece-wise despite the requirement of phagocytosis machinery, but are instead surrounded and acidified extracellularly. We have found that MITF, a transcription factor responsible for lysosomal biogenesis, is required for the enrichment of lysosomal machinery used for the acidification and breakdown process and V-ATPases are required in the stretch follicle cells for nurse cell acidification. GFP fusion proteins and antibody staining show that V-ATPases become enriched in stretch follicle cells in late oogenesis and localize to the apical side of the plasma membranes of the stretch follicle cells to acidify the nurse cells that they surround. Following acidification, the stretch follicle cells release cathepsins, lysosomal proteases, to breakdown and process the nurse cells. We are exploring phagoptosis in other tissues by ectopically expressing these genes to determine if this machinery is sufficient to induce phagoptosis. Altogether this work further characterizes a novel form of cell death and illustrates the importance of lysosomal components acting through a non-autonomous mechanism to control the death of neighboring cells.

7 Peroxisomes join the fight against infection. F. Di Cara1, A. Sheshachalam1, N. Braverman2, R. Rachubinski1, A. Simmonds1 1) Cell Biology, University of Alberta, Edmonton, Alberta, CA; 2) Research Institute of the McGill University Children's Hospital, Montreal, Quebec, Canada.

Peroxisomes are conserved ubiquitous organelles deputy to complex lipid metabolism and reactive species turnover. Since lipids and reactive species are pivotal signaling molecules in innate immunity, we investigated the unexplored role for peroxisomes in innate immune responses against microbial infection in *Drosophila* and in the mouse system. Using genetic, genomic and cell biology approaches, we show the requirement for peroxisomes in microbe engulfment, in fly and mouse. Both cultured macrophages and adult flies with impaired peroxisomes have a reduced capacity to respond to pathogens, defects in immune signaling (as NF-KB and MAPK mediated responses) and reduced viability due to high microbial load and gut delamination. Metabolomic analysis demonstrates that peroxisomes produce lipid species (as docosahexaenoic acid) and reactive oxygen species (as NO) to induce phagocytosis and activate the systemic immune responses. All together our finding demonstrate that functional peroxisomes are essential for the activation of, and defense by, the innate immune system in fly and mouse.
8 Functional analysis of cAMP-producing toxins in *Drosophila* identifies several chemical inhibitors. Annabel Guichard¹, Prashant Jain¹, Ruth Schwartz¹, Mahtab Moayeri², Curtis Sera¹, Jammal Abu-Kazneh¹, Janet Liu¹, Beatriz Cruz-Moreno¹, Bernice Aguilar³, Steven Chin¹, Steven Leplla², Victor Nizet⁴, Ethan Bier⁴ 1) Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA92093-0349, USA; 2) Microbial Pathogenesis Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, Maryland 20892-3202, USA; 3) Department of Pediatrics, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0687, USA.

cAMP-producing toxins such as anthrax Edema Factor (EF, a highly active adenylate cyclase) or Cholera toxin (Ctx, an ADP-ribosyl transferase that stimulates endogenous adenylate cyclases) cause severe symptoms during infection, such as edema and profuse diarrhea, respectively. We have previously established transgenic *Drosophila* lines expressing these toxins, and found that they both inhibit Rab11-dependent trafficking. Because Cadherins rely on Rab11 and its downstream effectors (such as Rip11 and Sec15) to reach apical site at the plasma membrane, inhibition of this small GTPase by EF or Ctx results in weakened cell-cell junctions, thus contributing to disease symptoms. Making use of an array of systems, including *Drosophila* wings and salivary glands, human endothelial cells and mice, we analyzed the mechanism by which cAMP overload results in Rab11 inhibition. We found that EF and Ctx act after the GTP loading step, and block association with downstream factors. The cAMP effector Epac and its partner Rap1, but not PKA, are the predominant mediators of EF in this process. Interestingly, artificial stimulation of Arf6 - a small GTPase that controls junction disassembly through endocytosis- mimics EF-induced phenotypes. Conversely, inhibition of Arf6 expression partially blocks EF or Ctx-induced phenotypes. Further suggesting that Arf6 and Epac act as mediators of EF, chemical inhibitors of these two proteins block the effect of EF in human cells and mice. Thus, our study uncovered promising leads for the treatment of infectious pathologies involving cAMP overload.

Reference: Anthrax edema toxin disrupts distinct steps in Rab11-dependent junctional transport
Research Article | published 25 Sep 2017 PLOS Pathogens
https://doi.org/10.1371/journal.ppat.1006603


Extrinsic control of neuronal activity is essential to determine the neural circuits underlying behavior. Despite the extensive use of optogenetics in targeted activation of neurons, the limits of optogenetic tools necessitates the use of other modalities such as temperature to control neuronal activity. However, few known temperature sensitive proteins (e.g., transient receptor channels TRPA1 and TRPM8) operate in the physiological range of common preparations. The recent discovery of temperature sensitivity of GR28bD indicates the potential for the orthologs of GR28bD in other Drosophila species to be temperature responsive, and thus be used as tools. We are studying the temperature response properties of orthologs of *D. melanogaster* GR28bD in 5 species of *Drosophila* that share 80 to 98% amino acid identity. To test thermosensitivity, we use the heat box, which can detect the temperature preference of flies with a resolution of 2°C. We overexpressed each of the orthologs pan-neuronally using the nSyb-GAL4 driver and subjected them to temperature steps of 2°C from 24-40°C. At the temperature of activation of the mis-expressed gene products, we expect a reversible paralysis of flies. Our results show that GR28bD is responsive from 34-36°C. The orthologs from *D. simulans, D. yakuba* and *D. pseudoobscura* which are 98, 96 and 85% identical to *D. melanogaster* GR28bD, are responsive from 30-34°C. The ortholog from *D. willistoni* with 81% identity with GR28bD is responsive from 34-38°C. Finally, flies expressing the 80% identical *D. mojavensis* ortholog were not paralyzed up to 40°C, and hence are not thermosensitive within the specified temperature range. We are also studying the molecular properties of GR28bD and its orthologs. We heterologously expressed the genes in *Xenopus laevis* oocytes. Results show that GR28bD conducts a cation non-specific temperature sensitive inward current upon expression in oocytes. Additionally, GR28bD alters the activity of *D. melanogaster* motor neurons, and the effect can be observed with co-expression of calcium indicator, GCaMP6f. Currently, we are assaying the temperature response properties of the orthologs in oocytes and in motor neurons to explore their possibilities of being used as independent tools in multiple cell types.

10 The *Drosophila* small conductance potassium channel (SK) negatively regulates nociception. S.E. Mauthner¹, K.C.E Walcott², A. Tsubouchi³, J.L. Robertson², W.D. Tracey¹ 1) Department of Biology, Indiana University Bloomington, Bloomington, IN; 2) Duke University Medical Center, Duke University, Durham, NC; 3) The University of Tokyo, Graduate School of Arts and Sciences, Tokyo, Japan.

Inhibition of nociceptor activity is important for the prevention of spontaneous pain and hyperalgesia. To identify the critical K⁺ channels that regulate nociceptor excitability we performed a forward genetic screen using a *Drosophila* larval nociception paradigm. Knockdown of three K⁺ channel loci, the small conductance calcium-activated potassium channel (SK), seizure and tiwaz, resulted in marked hypersensitive nociception behaviors. In more detailed studies of SK, we found that hypersensitive phenotypes could be recapitulated with a genetically null allele. Importantly, the null mutant phenotype could be rescued with tissue specific expression of an SK cDNA in nociceptors. Optical recordings from nociceptive neurons showed a
A significant increase in mechanically activated Ca\(^{2+}\) signals in SK mutant nociceptors. SK showed expression in peripheral neurons. Interestingly, SK proteins localized to axons of these neurons but were not detected in dendrites. Our findings suggest a major role for SK channels in the regulation of nociceptor excitation and they are inconsistent with the hypothesis that the important site of action is within dendrites.

11 Humidity sensing in *Drosophila*. D. Frank\(^1\), A. Enjin\(^2\), E. Zaharieva\(^1\), G. Jouandet\(^1\), S. Mansourian\(^2\), M. Stensmyr\(^2\), M. Gallo\(^1\) 1) Neurobiology, Northwestern University, Evanston, IL; 2) Department of Biology, Lund University, Lund, Sweden.

Many terrestrial organisms can only prosper within a specific range of air humidity. For example, small poikilotherms like *Drosophila melanogaster* risk desiccation (and death) when exposed to hot, dry conditions even for relatively short periods of time. Hence, a fly's ability to detect and appropriately respond to humidity changes can be critical for survival. The *Drosophila* antenna is a hub for the senses, containing receptor neurons for mechanical, olfactory, thermal and humidity stimuli. Neurons expressing the ionotropic receptor IR40a have been recently implicated in the selection of an appropriate humidity range, but while previous work indicates that insect hygroreceptors may be made up by a 'triad' of neurons (with a dry- a cold- and a humid-air responding cell), IR40a expression included only cold- and dry-air cells. Here, we report the identification of the humid-responding neurons that complete the hygrosensory triad in the *Drosophila* antenna and express the ionotropic receptor IR68a. Next, we follow the projections of hygrosensory neurons to the brain, and show that they form distinct glomeruli in the posterior antennal lobe. Here, a spatial map of neural activity represents related features of the external environment, with adjacent 'hot', 'cold', 'dry', and 'humid' glomeruli. This organization may allow for both unique and combinatorial sampling by central relay neurons, and we indeed find evidence for each. Our results further our understanding of humidity sensing in the *Drosophila* antenna, uncover neuronal substrates for the processing of temperature and humidity stimuli in the brain, and illustrate the logic of how ethologically relevant combinations of sensory cues can be processed together to produce adaptive behavioral responses.

12 Multisensory integration in the *Drosophila* mushroom body. J. Li, I. Christofferson, S.J. Caron Department of Biology, University of Utah, Salt Lake City, UT.

Multisensory integration is a function of all brains. Despite its fundamental importance, the mechanisms of connectivity underlying multisensory integration remain poorly understood. To uncover these mechanisms, we are using the numerically simple *Drosophila melanogaster* mushroom body, an associative brain center that consists of 2,000 neurons called the Kenyon cells. Recent evidence suggests that the mushroom body processes olfactory, visual and gustatory information. From a preliminary screen through the FlyLight collection of GAL4 transgenic lines, we identified six projection neurons that connect the visual, gustatory, olfactory, hygrosensory and thermosensory systems to the mushroom body. We used the GRASP technique to show that the identified projection neurons form synapses with a subpopulation of Kenyon cells called the \(\alpha/\beta_{\text{posterior}}\) Kenyon cells. We are in the process of determining how individual \(\alpha/\beta_{\text{posterior}}\) Kenyon cells integrate inputs from this group of projection neurons. Thus far, our results suggest that the \(\alpha/\beta_{\text{posterior}}\) Kenyon cells are multimodal: they are not dedicated to a single sensory modality, rather they integrate inputs across different modalities. Given that many fundamental design principles are conserved from invertebrates to vertebrates, it is likely that the mechanisms of connectivity underlying multisensory integration in the *Drosophila* mushroom body will also apply to the more complex associative brain centers.

13 On the ORigin of olfactory worlds. M. Korageorgi\(^1\), S. Lebreton\(^2\), M. Paris\(^3\), C. Minervino\(^2\), M. Cavey\(^2\), K.P. Siju\(^4\), I.C. Grunwald Kadow\(^1\), T. Matsunaga\(^1\), N.K. Whiteman\(^1\), N. Gompel\(^2\), Benjamin Prud'homme\(^2\) 1) Integrative Biology, University of California, Berkeley, USA; 2) Developmental Biology Institute of Marseille (IBDM), France; 3) Ludwig-Maximilians University of Munich, Faculty of Biology, Biozentrum, Germany; 4) TUM School of Life Sciences Weihenstephan Technical University of Munich, Germany.

Behavior is among the first traits to evolve when insects adapt to new ecological niches. Olfaction is one sensory modality used to translate environmental signals into species-specific behaviors during this process. Yet, little is known about the genetic basis responsible for the evolution of novel olfactory-driven behaviors. We leveraged an evolutionary transition to herbivory within the Drosophilidae, specifically we studied the reproductive shift from rotting to living plant tissues in *Drosophila suzukii* and *Scaptomyza flavia* and their relatives as model systems to address how olfactory-driven behavior evolves. We first introduced neurogenetics and the CRISPR technology in *D. suzukii* and found that the odorant receptor subsystem is involved in the evolution of its egg-laying behavior. Interestingly, we also observed in *D. suzukii* a specific upregulation of orthologs of odorant receptors that detect fresh fruit odors in *D. melanogaster*. This transcriptomic signature suggests that targeted changes in the expression of chemoreceptor repertoires could drive the evolution of novel adaptive olfactory-driven behaviors. The results of this work are compared with the evolution of olfactory-driven behaviors in another drosophilid that has transitioned to herbivory, the leafminer *Scaptomyza flavia*, in which targeted changes in its olfactory repertoire have also been observed. Together, these results will help us understand the role of olfaction in a fundamental ecological shift in insects, the evolution of herbivory.
14  **Sing me a new song - towards the neural basis of fly courtship song evolution.**  Yun Ding¹, Joshua Lillvis¹, Jessica Cande¹, Gordon Berman¹, Ben Arthur¹, Min Xu¹, Barry Dickson¹, David Stern¹  ¹Janelia Research Campus, HHMI, Ashburn, VA 20147; ²Rollins Research Center, Emory University, Atlanta, GA 30322.

The neural basis for behavior evolution is poorly understood. Since brain anatomy is often conserved, functional comparisons of homologous neural circuitry may illuminate behavior evolution, but these experiments are difficult to accomplish in most animals. Here, we compare the function of homologous neurons driving fruit fly courtship song by exporting neurogenic reagents that label and manipulate identified neurons in *Drosophila melanogaster* to *Drosophila yakuba*. We found that a homologous descending neuron with conserved electrophysiological properties can drive multiple song types in both species, but that its primary function is to drive different song types in each species. These divergent songs are produced in a conserved social context, suggesting that this descending neuron may receive conserved inputs. Song evolution resulted from changes in the sensitivity of circuitry downstream of the descending neuron. This experimental approach can be generalized to other neural circuits and therefore provides a framework for studying how the nervous system has evolved to generate behavioral diversity.

15  **A circuit for the experience of mating in *Drosophila*.**  L. Shao¹, P. Chung¹, A. Wong¹, C. Kent¹,², X. Long¹, I. Siwanowicz¹, U. Heberlein¹  ¹Janelia Research Campus, HHMI, Ashburn, VA; ²Department of Biology, York University, Toronto, ON, Canada.

The behavior of female *Drosophila* changes profoundly after mating due to the effects of sperm and seminal fluid proteins transferred from males during copulation. Here, we describe and characterize the “female mating experience”, which is induced by the act of copulation per se. Mating with non-ejaculatory males transiently decreases receptivity and increases oviposition. We identify a three-layered circuit underlying this mating experience. Abdominal neurons expressing the mechanosensory channel Piezo convey the signal of copulation to a pair of female-specific ascending neurons, LSANs, in the ventral nerve cord. LSANs relay the mechanosensory information of copulation to neurons expressing Myoinhibitory Peptide in the dorsal brain. Females in which neurotransmission in any layer of this circuit is blocked behave as if they had not mated, although they copulated with males that do not ejaculate. We speculate that the mating experience is a mechanism by which females evaluate the quality of matings in order to adjust future behavior.

16  **From perception to reaction: a novel neuronal pathway to encode motion and regulate forward walking speed in *Drosophila*.**  J. Eliason, M. Isaacson, A. Nern, K. Shinomiya, G. Rubin, M. Reiser  Janelia Farm Research Campus, Ashburn, VA.

The major goal of neuroscience is to connect sensory input with behavioral output by ascribing function to neurons and neuronal circuits. The visual system of *Drosophila melanogaster* provides an excellent model to study the conversion of sensation into action. The anatomy of neurons in the visual system has been known for over one hundred years, and flies have a variety of interesting visually-guided behaviors. Vision is vital for exploration, courtship, navigation, and predator elusion. Yet it is only in the past decade or so that advancements in genetic tools, collaborations, and technology have allowed researchers to make substantial progress and associate neuronal anatomy and connectivity with behavioral relevance.

The neuronal infrastructure that makes motion vision possible is of particular interest. Motion vision represents the brain’s ability to make complex calculations from sensory stimuli. No one neuron or photoreceptor can perceive movement on its own or provide any information about speed or direction. But by coordinating within a network, neurons convert their simple photon signals to a perceived motion and refine the qualities and properties of the movement against a visually noisy background.

By creating new genetic tools and using innovative anatomical and behavioral techniques, we have identified and characterized specific neural correlates which encode translational back-to-front motion and influence the behavioral output, “walking speed.” The identified neuronal circuit includes a previously-unknown neuronal type we have named LPC1. LPC1 encodes back-to-front and up translational motion, regulates forward walking speed, and regulates surge behavior during flight. This circuit also demonstrates important principles of motion vision mechanics, e.g. how the brain processes rotational and translational motion separately or how the brain interprets motion signals from varying directions. By mapping the function and connectivity of visual neurons, we demonstrate the greater understanding to be gained regarding how sensory neurons cooperate to encode stimulus information and produce appropriate reactions.

17  **TDRDSP promotes germline differentiation through post-transcriptional gene regulation in cytoplasmic RNA granules.**  Caitlin Pozmanter, Shekerah Primus, Mark Van Doren  Biology, Johns Hopkins University, Baltimore, MD.

The RNA-binding protein Sex lethal (SXL) is essential for sex determination in both the germline and the soma, however little
is known about its targets in the germline. Previous work in our lab identified **tudor domain-containing protein S-prime (tdrd5p)** as a novel germline target of Sxl. **tdrd5p** RNA and protein levels are greatly enriched in males in a manner dependent on Sxl and TDRD5P protein levels are increased in females when putative SXL binding sites in the **tdrd5p** mRNA are mutated. Additionally, we demonstrated that **tdrd5p** is capable of promoting male identity in the germline and loss of **tdrd5p** causes male fertility defects. While this data suggested a role for **tdrd5p** specifically in males, we have recently found that **tdrd5p** functions in the female germline as well, although it appears to act later in female germline differentiation. Thus, **tdrd5p** plays a role in male germ cell sexual identity but may also act to control germline differentiation in both sexes.

TDRD5P contains a TUDOR domain most closely homologous to Drosophila **tejas** and mouse TDRDS. Like some other Tudor-domain proteins, TDRD5P localizes to peri-nuclear cytoplasmic punctae in the germline, similar to RNA “bodies” that regulate RNA metabolism and post-transcriptional gene regulation. Interestingly, TDRD5P forms a hollow shell surrounding these bodies, suggesting it could act as a molecular gatekeeper or to organize distinct RNA bodies relative to one another. The “TDRD5P body” is localized adjacent to, but is distinct from, VASA-containing nuage. TDRD5P shows partial co-localization with the **Processing (P)-Body** protein RNA Decapping Protein 1 and exhibits genetic interaction with the Deadenylase **twin** and the miRNA pathway member **gawky**, suggesting that TDRD5P may be involved in RNA degradation in P-**Bodies**. We also find that smaller TDRD5P-containing punctae co-localize with another Tudor-domain protein, **Survival Motor Neuron (SMN)**, suggesting that TDRD5P might be involved in the biogenesis of dynamics of RNPs. We are generating the reagents necessary for live imaging of TDRD5P punctae to test this hypothesis. We are also identifying other proteins with which TDRD5P associates in these bodies and searching for mRNA targets that are regulated by TDRD5P.

18 **Distinct RNP Classes in the Drosophila Germ Plasm Orchestrate Differential RNA Regulation.** C. Ruesch, E. Gavis

Molecular Biology, Princeton University, Princeton, NJ.

Post-transcriptional gene regulation plays an important role in the establishment of cell fates, particularly during early embryogenesis. Formation of large ribonucleoprotein (RNP) granules serves to organize RNA molecules on the basis of their regulatory requirements. The **Drosophila** germ plasm is a specialized cytoplasm at the posterior of the embryo containing many large RNP granules containing factors that are required for establishing germline fate. At least two types of granules can be distinguished. The first, which we term founder granules, contains oskar (osk) mRNA along with Staufen protein. Founder granules provide for the local production of Osk protein, which recruits additional protein and RNA components to assemble the second type of granule, called germ granules. Germ granules are incorporated into the primordial germ cells (pole cells) as they bud from the posterior of the embryo and are required for germline development. In contrast, founder granules remain largely outside of the pole cells. Aberrant targeting of osk to pole cells by packaging in germ granules impedes germline development, demonstrating the importance of segregating osk to founder granules. We have investigated how partitioning of mRNAs into different RNPs within the germ plasm regulates their fate, by characterizing the distinct behaviors of germ granules and founder granules in the early embryo. We find that unlike germ granules, founder granules are not actively transported during pole cell budding. Instead, osk is degraded in the germ plasm by a mechanism that is distinct from the bulk degradation of maternal RNAs, including the population of osk and germ granule transcripts that remains outside of the germ plasm. The use of an alternative degradation mechanism for osk in the germ plasm may serve to protect germ plasm-localized germ granule RNAs that are destined for the pole cells. In addition, we observe remodeling of founder granules, but not germ granules, that accompanies osk degradation in the germ plasm. We are currently investigating the link between founder granule remodeling and osk degradation to elucidate the cause-effect relationship between these two events and how they influence or are influenced by access of degradation machineries to osk.

19 **Dm Ime4 regulates chic splicing in Drosophila spermatogenesis.** Antonio Rockwell1, Cintia Hongay2 1) Biology, Clarkson University, Potsdam, NY; 2) Biology, Clarkson University, Potsdam, NY.

A developmental strategy used by multicellular organisms is to divide cell populations into distinct functional units separated by physical boundaries. Recent data from our lab show that Drosophila Inducer of Meiosis 4 (**Dm ime4**) expressed in somatic cyst cells plays a role in the integrity of germline cyst boundaries. **Dm ime4** encodes an evolutionarily conserved mRNA methyltransferase that catalyzes the N6mA non-editing modification of RNA. This enzyme has been shown to be essential for Arabidopsis and murine embryonic development and our lab showed that its depletion in Drosophila leads to reduced viability and reduced fertility. Using spermatogenesis as our model system, we show that **Dm Ime4** is abundant in the pair of somatic cyst cells that envelop the developing germline cyst cells throughout spermatogenesis. The somatic cyst cells are known to provide the germline cysts with a physical boundary known as the somatic permeability barrier. Disruption of the barrier results in defects in germline differentiation, cell signaling, cell death, and infertility. Our data show that reduced levels of **Dm ime4** results in misregulation of profilin (**chic**), an essential protein for proper function of the somatic permeability barrier. **Our recent findings provide the first report of direct regulation of a transcript (chic) by Dm ime4 and the biological consequences of incorrect splicing of this transcript in spermatogenesis.**

20 **Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA binding factor Rbm15/Spenito to other components of the m6A machinery.** Tina Lence1, Philip Knuckles2,3, Irmgard Haussmann4, Dominik Jacob5, Nastasja...
previously unexplored mechanism in the study of genital coevolution. This work highlights the need to consider the role of pleiotropy in coevolutionary relationships between these two sexual characters, in spite of the apparent morphological distinctness of male and female genitalia. Genitalia evolve rapidly, and it is common to see interspecific differences in both male and female genital structures. When these features of females and males are morphometrically correlated across species, the relationship is usually assumed to be driven by selective mechanisms. An oft-overlooked alternative explanation for concurrent changes in male and female genitalia is pleiotropic linkage. The degree to which coevolving genital structures are genetically independent has not been elucidated in any species. We investigated the genetic underpinnings of two possibly coevolving genital structures in the Drosophila melanogaster subgroup. In a prior study, the gene network required for posterior lobe formation was found to have been co-opted from a larval structure during the origination of the male structure. Using gene knockdown, in-situ hybridization, antibody staining, and enhancer analysis, we investigated whether this genetic network is shared between the posterior lobe and the oviscapt pouch. Surprisingly, we discovered that patterning genes from the posterior lobe network, and even the enhancers of these genes, are also involved in the patterning of the oviscapt pouch during the development of this female structure. These data suggest that the necessarily-shared genetic history of males and females could in part explain the simultaneous origin and size relationship of these two sexual characters, in spite of the apparent morphological distinctness of male and female genitalia. This work highlights the need to consider the role of pleiotropy in coevolutionary relationships between the sexes, a previously unexplored mechanism in the study of genital coevolution.

Alternative Transcription (AT) expands transcriptome diversity by adding transcript isoforms to a single gene and affects a very large fraction of animal genes. Its misregulation has been attributed to human diseases including cancer. However, whether AT plays an important role in the evolution of fundamentally new developmental gene functions remains largely unknown. We found that a conserved Zic family segmentation gene, **odd-paired (opa)**, evolved a new function in the specification of head-to-tail embryo polarity via AT. The sand fly-related midge *Clogmia albipunctata* lacks the unique genes that specify head-to-tail polarity in other dipteran insects, such as *bicoid* in the fruit fly *Drosophila melanogaster* or *panish* in the midge *Chironomus riparius*. Using expression profiling of anterior and posterior embryo portions, we found that one of three alternative embryonic *Clogmia opa* transcripts, *Cal-opa C*, is expressed maternally and localized at the anterior pole of freshly laid eggs. Knockdown of *Cal-opa C* resulted in embryos with perfect tail-to-tail polarity (double abdomen) and duplicated germ cells. Conversely, ectopic expression of *Cal-opa C* mRNA at the posterior pole induced head-to-head polarity (double head). The other alternative transcripts, *Cal-opa A* and *Cal-opa B*, were expressed in a conserved pattern during the blastoderm stage. Of these, *Cal-opa B* was required for segmentation but also sufficient to induce head development when expressed posterior in preblastoderm embryos. Our findings demonstrate that a single gene can evolve a fundamental new gene function via AT. Given that AT is a widespread phenomenon, its role in gene evolution deserves more attention.

24  **Evolving doublesex expression correlates with the origin and diversification of male sexual ornaments in the *Drosophila immigrans* species group.**  G.R. Rice1,2, O.Y. Barmina2, K Hu2, A.V. Kopp2  1) Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 2) Evolution and Ecology, University of California at Davis, Davis, CA.

Male ornaments and other sex-specific traits present some of the most dramatic examples of evolutionary innovations. Comparative studies of similar but independently evolved traits are particularly important for identifying repeated patterns in the evolution of these traits. Male-specific modifications of the front legs have evolved repeatedly in Drosophilidae and other Diptera. The best understood of these novel structures is the sex comb of *Drosophila melanogaster* and its close relatives. Here, we examine the evolution of another male foreleg modification: the sex brush, found in the distantly related *Drosophila immigrans* species group. Similar to the sex comb, we find that the origin of the sex brush correlates with novel, spatially restricted expression of the **doublesex (dsx)** transcription factor, the primary effector of the *Drosophila* sex determination pathway. The diversity of *dsx* expression patterns in the *immigrants* species group closely reflects the differences in the presence, position, and size of the sex brush. These observations suggest that tissue-specific activation of *dsx* expression may be a common mechanism responsible for the evolution of sexual dimorphism. In particular, for the origin of novel male-specific ornaments.

25  **Centromeres epigenetically mark Drosophila germ line stem cell identity.**  A.A. Dattoli, Ben Carty, Elaine Dunleavy  Biochemistry, National University of Ireland (NUI), Galway, Galway, GALWAY COUNTY, IE.

**Background:** Stem cells divide asymmetrically generating a self-renewing cell and a second daughter cell programmed to differentiate. The stem-to-differentiation switch, occurring during cell division, is disrupted in common human diseases, including cancer and infertility. Epigenetic mechanisms were found to contribute to stem cell maintenance/differentiation. In this regard, Centromeres are epigenetically defined chromosomal domains crucial for genomic integrity and accurate chromosome segregation during cell division. An abundance of evidence in the last few years suggests that centromeres may play a role in stem cell maintenance. Centromeres are specified by the histone H3 variant, CENP-A, assembled in the centromeric nucleosome at the end of mitosis (between telophase and G1 in metazoan), with its loading dependent on the chaperone/assembly factor HJURP (Holliday Junction Recognition Protein).

**Hypothesis:** Centromeric domains are arranged differentially upon division in stem and daughter cells and this differential organization is crucial to establish stem cell identity.

**Results:** Confocal microscopy coupled to quantification analysis of CENP-A in combination with different cell cycle markers revealed that in female *Drosophila* germine stem cells (GSCs) centromeres are assembled in G2 phase. Furthermore, co-localisation mapping showed that a specific mitotic marker, phosphorylation of histone H3 at threonine 3 (H3T3P), is enriched on chromosomes that will be inherited by the daughter cell that will differentiate (cystoblast). Conversely, chromosomes inherited by the GSC retain more CID (*Drosophila CENP-A*) at centromeres. Finally, knock down analysis conducted through the GAL4-UAS system showed that the integrity of the centromere core is crucial in the differentiation of GSCs into cystoblasts in *Drosophila* females. Particularly, this process is strictly regulated by the key centromere assembly factor CAL1 (*Drosophila* homologue of HJURP).

**Conclusion:** Collectively, our results suggest that centromeres play a pivotal role in the epigenetic pathway that specifies stem cell identity/maintenance in *Drosophila melanogaster* germine tissues.

26  **Multi-layered control of gene activities ensures timely exit from stemness during asymmetric neural stem cell division.**  H. Komori1, D. Hamm2, N. Rives-Quinto1, E. Larson2, M. Harrison2, CY. Lee1  1) Life Sciences Institute, University of...
Asymmetric stem cell division allows for the generation of a self-renewing stem cell and an uncommitted progenitor that commits to a progenitor identity and generates differentiated cell types. To transition from a stem cell state into a progenitor state, an uncommitted progenitor must exit from stemness by dismantling the stem cell regulatory network. However, the mechanisms controlling this critical transition remain poorly understood. The type II neural stem cell (neuroblast) lineage in the fly larval brain provides an excellent in vivo paradigm for unraveling the mechanisms that dissolve the self-renewal gene network in the uncommitted progenitor (immature INP) during the exit from stemness. By using regulation of the neuroblast self-renewal gene *deadpan* (*dpn*) as a paradigm, we show that transcriptional control, mRNA decay and competitive antagonism function collaboratively to ensure rapid down-regulation of self-renewal gene activities and timely exit from stemness in the newly born immature INP. We identify two transcription factors Zelda (*Zld*) and Fruitless (*Fru*) that function together with Notch signaling to specify the precise level of *dpn* transcription necessary to sustain self-renewal in neuroblasts but not to impede timely exit from stemness in the newly born immature INP. Furthermore, Brat recognizes the 3'UTR of *dpn* mRNA and functions together with Tis11 and multiple deadenylase complexes to target *dpn* mRNA for decay. Additionally, we demonstrate that Insb decommissions excess Dpn activity by competing with its dimerization partners for binding the Orange motif. While reducing the function of an individual layer of the multi-layered control mechanism has little effect on the exit from stemness in the newly born immature INP, mild reduction in any two layers significantly hinders this transition. Our findings provide a new conceptual framework for efficiently and robustly switching instructive mechanisms from the “ON” state to the “OFF” state, driving critical transitions during development and homeostasis.

Lin28 is a critical factor in the aging of *Drosophila testis* stem cell niche. Sreepih Perinthottathil1,2, Changsoo Kim2, Benoit Biteau1 1) Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 2) School of Biological Sciences and Technology, Chonnam National University, Gwangju, South Korea.

Age-related decline in stem cell function is observed in many tissues from invertebrates to humans. While cell intrinsic alterations impair stem cells, aging of the stem cell niche also significantly contributes to the loss of tissue homeostasis associated with reduced regenerative capacity. Hub cells, which constitute the stem cell niche in the *Drosophila* testis, exhibit age-associated decline in number and activities, yet underlying mechanisms are not fully understood. Here we show that Lin28, a highly conserved RNA binding protein, is expressed in hub cells. We found that its expression dramatically declines in old testis and that *lin28* mutant testes exhibit hub cell loss and defective hub architecture, recapitulating the normal aging process. Importantly *lin28* overexpression prolongs hub integrity in aged testes, supporting the notion that *lin28* decline is a driver of hub cell aging. Mechanistically, we found that the level of *upd*, a stem cell self-renewal factor, is reduced in *lin28* mutant testis and that Lin28 protein directly binds and stabilizes *upd* transcripts, in a let-7 independent manner. Finally, our data demonstrate that Lin28 can physically interact with the IGF-II messenger RNA binding protein (IMP), another factor known to protect *upd* transcript. Altogether, our results suggest that Lin28 acts with IMP to protect *upd* transcripts in hub cells, and reduction of both Lin28 and IMP in old testis leads to decreased *upd* levels, hub cell aging and loss of the stem cell niche.

Stem Cell Cytokinesis is Disrupted with Age Due to Diminished Jak/STAT Activity. K. Lenhart1, B. Capozzoli1, S. Dinardo2 1) Biology Department, Drexel University, Philadelphia, PA; 2) Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

It is well established that many aspects of stem cell function, from rates of proliferation to the ability to self-renew, become disrupted with age. Defects in niche signaling and architecture, as well as changes in cytoskeletal dynamics, have been implicated in promoting age-related decline, yet many of the proximate causes of early-onset aging defects are unknown. We have identified disruption of the modified cytokinesis program within germline stem cells (GSCs) of the testis as the earliest known defect in stem cell behavior within the niche. GSCs progressively fail to abscise, or physically separate from their daughter cells, beginning at 7 days after eclosion, with 30% of all GSC divisions resulting in failed abscission by 15-21 days. This block to cytokinesis has severe consequences, as GSC daughters are no longer consistently released to differentiate and contribute to the tissue. Extensive live imaging and genetic analyses revealed that diminished JAK/Stat responsiveness in aged GSCs controls this age-related defect in the modified cytokinesis program, with this pathway regulating F-actin disassembly at the intercellular bridge between GSC-daughter pairs. We found that increasing JAK/Stat activity, either generally in the niche or specifically within the GSCs themselves, significantly rescued the abscission defect in aged GSCs. Depletion of Stat from aged GSCs led to a near complete block to abscission. Interestingly, we found that loss of even a single functional copy of Stat resulted in substantial defects in the GSC cytokinesis program. Young flies heterozygous for a Stat mutation or a deficiency that included the Stat locus exhibited abscission defects that were indistinguishable from those of 15-21 day aged GSCs, indicating that diminished Stat is sufficient to precociously age stem cells. Taken together, this work has identified the earliest age-related defect in GSCs and has revealed a novel role for an established niche signaling pathway in controlling stem cell cytokinesis and in regulating stem cell behavior with age.
29 Accelerated germline stem cell divisions in Drosophila males upon repeated mating - a novel role for G-Protein signaling. M.M. Moleps, Leon McSwain, Benjamin Parrot, Karl Kudya, Chun Ng, Jennie Nicholson, Alicia Hudson, Vinay Choksi, Cordula Schult1. 1) Cellular Biology, University of Georgia, Athens, Athens, GA; 2) Odum School of Ecology, University of Georgia, Athens, Athens, GA; 3) Duke University School of Medicine, Durham, North Carolina; 4) Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA.

Adult stem cells are extremely significant for regenerative medicine due to their ability to generate specialized cells. Most types of adult stem cells are housed in a specific cellular microenvironment, the niche, which regulates and maintains stem cell fate via a battery of mechanisms, including signaling and asymmetric localization of the mitotic spindle. However, little is known about the regulation of stem cell activity and how stem cells respond to physiological cues. Our lab uses the rate of germline stem cells (GSC) divisions as a measure of their activity. We discovered that males repeatedly mated with virgin females display a significant increase in their division frequency. This phenomenon is age independent but gender specific. We will present evidence that the increase in GSC division frequency is dependent on G-protein signaling, a pathway conserved among all metazoan species. Expressing a dominant negative version of Gα1 or reducing the expression of Gα-subunits in the germline via RNA-Interference (RNAi) eliminated the ability of males to accelerate their GSC divisions upon mating. Consistent with a role for G-protein signaling in this process, expressing RNAi against any of seven G-protein coupled receptors (GPCRs) in the germline also prevented the acceleration of GSC divisions in mated males. These were the Serotonin Receptors 5HT-1A, 5HT-1B and 5HT-7, Metuselah (Mth), Metuselah-like5 (Mth-l5), Octopamineβ 2R (Octβ 2R), and CG12290. We hypothesize that GSCs are receptive to several GPCR ligands and that their activity is regulated via cross-talk among signaling pathways downstream of these GPCRs.

30 Wingless promotes EGFR signaling in follicle stem cells to maintain self renewal. Rebecca Kim, Todd Nystul Anatomy, UCSF, San Francisco, CA.

Adult stem cell niche boundaries must be precisely maintained to facilitate segregation of stem cell and daughter cell fates. However, the mechanisms that govern this process in epithelial tissues are not fully understood. In this study, we investigated the relationship between two signals, Wnt and EGFR, that are necessary for self-renewal of the epithelial follicle stem cells (FSCs) in the Drosophila ovary, but may be downregulated in cells that have exited the niche to allow for differentiation. We found that wingless produced by inner germarial sheath (IGS) cells acts over a short distance to activate Wnt signaling in FSCs, and is restricted from moving beyond the FSC niche boundary. In addition, we show that Wnt signaling functions genetically upstream of EGFR signaling by activating expression of the EGF ligand, spitz, and that constitutive activation of EGFR partially rescues the self-renewal defect caused by loss of Wnt signaling. Collectively, our findings support a model in which the Wnt and EGFR pathways operate in a signaling hierarchy to promote FSC self-renewal. Ongoing studies utilizing long-term ex vivo live imaging of the Drosophila germarium will elucidate the dynamics of these signaling pathways in FSCs over time as well as give insight into how activation of signaling might be coupled with the requirements of the tissue during oogenesis.

31 Vive la resistance: evidence from the Drosophila intestine that multidrug resistance is an ancient stem cell trait. M. Markstein, J. DiRussio, H. Dayton, K. Kolbert, O. Williamson, E. D’Souza, A. Balcianne, S. Kondo. 1) Biology Department, University of Massachusetts, Amherst, MA; 2) Genetic Strains Research Center, National Institute of Genetics, Mishima, Japan.

A remarkable feature of adult stem cells is their ability to regenerate tissue in the wake of injuries that cause daughter cell loss. Most studies on regeneration have focused on “S.O.S.” signals from injured tissue, such as inflammatory cytokines, that instruct stem cells to divide and differentiate to replenish missing daughter cells. However, in order for stem cells to respond to these signals they must survive the injury in the first place. In virtually every mammalian tissue, stem cells have been shown to sustain chemical injuries better than their differentiated daughters by expressing higher levels of transmembrane efflux pumps. Interestingly, this same stem cell-daughter cell difference underlies the ability of cancer stem cells to survive chemotherapeutic drugs that effectively eliminate “bulk” daughter cells, rendering the cancer stem cells multidrug resistant. Although this stem cell–daughter cell difference is widely recognized in both normal and diseased mammalian tissues, the regulatory mechanisms underlying higher stem cell expression of protective chemical efflux genes remain poorly understood.

Here we show that the stem cell–daughter cell efflux difference observed across mammalian tissues is mirrored in the Drosophila intestine. Using a novel quantitative efflux assay, we show that stem cells in the fly intestine efflux small molecules more efficiently than their surrounding enterocyte and enteroendocrine daughter cells. Interestingly, the youngest enterocyte daughter cells, which recent published work shows can undergo amitosis to become stem cells again, retain some of the efflux properties of the stem cells, underscoring the relationship between stemness and efflux ability. We show further that specific transporters are required for both efflux and the ability of stem cells to regenerate tissue in the wake of chemical injury. These results suggest that stem cell multidrug resistance may be an evolutionarily conserved stem cell trait and open the door to using the power of Drosophila genetics to dissect the molecular mechanisms underlying how stem cells mount greater protective responses relative to their differentiated daughter cells.
Adaptive genetic redundancy in Drosophila is driven by a vast reservoir of large-effect alleles. N. Bargh1, M. Dolezal1, A. Jaksic1,2, F. Mallard1, N. Nolte1, K. Otte1, R. Tobler1,2,3, C. Schlötterer1. 1) Institut für Populationsgenetik, Vetmeduni Vienna, Vienna, Austria; 2) Vienna Graduate School of Population Genetics, Vetmeduni Vienna, Vienna, Austria; 3) Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, SA, Australia.

The genetic architecture of adaptive traits is of key importance to understand and predict the evolutionary response. Despite it is widely assumed that most traits have a complex architecture, most adaptive traits characterized so far have a simple genetic basis. We combined experimental evolution with whole genome sequencing to study the architecture of a complex trait in replicated Drosophila simulans populations. After more than 100 generations in a novel hot temperature regime all 10 evolved populations converged to very similar phenotypes (fitness, metabolic rate and lipid content). On the genomic level, however, we noted a highly heterogeneous response with most selected loci increasing only in half of the replicates. The dynamics of the 99 strongly selected loci are not compatible with classic population genetic models. Rather, genetic redundancy among the loci is more compatible with the genomic dynamics and matches the observed phenotypic convergence. With on average every second fly carrying a different adaptive allele, natural D. simulans populations could harbor a vast reservoir of adaptive loci facilitating rapid evolutionary responses. As this adaptive genetic architecture is most likely not limited to temperature adaptation, but may be more common in natural populations, our results have major implications for the experimental design of studies on adaptive variation in natural populations.

The microbiota influences life history variation in Drosophila melanogaster. A. Wise1, M. Koyle1, R. Hughes1, J. Chaston1, P. Schmidt2 1) Brigham Young University, Provo, UT; 2) University of Pennsylvania, Philadelphia, PA. Organismal adaptation to spatially-varying selection is primarily attributed to environmental selection on an animal’s genotype, a model that does not but should account for the role of associated microorganisms (‘microbiota’). In Drosophila melanogaster, a key model for understanding clinal adaptation, the microbiota influence the magnitude of individual life history traits such as energy storage, development rate, and lifespan, but these effects have not previously been linked to spatially varying selection. Here, we present data to support that D. melanogaster at different latitudes naturally bear different amounts of microbes that can substantially influence their adaptive traits. We first show that an isogenic fly line individually reared with different bacterial species on a nutrient-rich diet displays life history strategies that favor either but not both of somatic maintenance or early reproduction. We also show that the abundance of key bacterial taxa in wild Drosophila populations is correlated with the latitude at which the flies were sampled and that host genotype can select a latitude-specific microbiota. Finally, by eliminating or swapping the microbiota between fly lines derived from high- and low-latitude wild populations, we reveal that both the microbiota and host genotype contribute to latitude-specific life history traits. For example, bacteria-free high latitude fly lines invested in somatic maintenance to a greater extent than bacteria-free low latitude fly lines; but the same fly lines bore the same somatic maintenance traits when they were inoculated from birth with a high-latitude microbiota. Taken together, these findings establish the microbiota as an essential consideration in spatially adaptive processes.

A genome-wide association study to identify genetic factors affecting resistance allele formation in CRISPR gene drives. J. Champer1,2, J. Chung1,2, C. Liu1,2, A. Luthra1,2, R. Reeves1,2, Y.L. Lee1,2, J. Liu1,2, Z. Wen1,2, E. Yang1,2, P. Conley1,2, P. Messer1, A. Clark1,2 1) Department of Biological Statistics & Computational Biology, Cornell University, Ithaca, NY; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY. Gene drives could allow for control of vector-borne diseases by directly suppressing vector populations or spreading genetic payloads designed to reduce pathogen transmission. CRISPR homing gene drives work by converting cells heterozygous for the drive allele into homozygotes, increasing the frequency of the drive allele in a population. However, all current CRISPR gene drives in insects form resistance alleles at high rates. Such alleles cannot be converted to drive alleles, and would halt the spread of a drive through a population. Furthermore, it seems likely based on previous results that genetic variation in natural populations would include variation in the tendency to produce resistance alleles. We developed a CRISPR homing gene drive in Drosophila melanogaster and crossed it into the genetically diverse Drosophila Genetics Reference Panel (DGRP) lines, measuring several gene drive parameters. Successful drive conversion in the germline averaged 56±8%, with germline resistance allele formation at 39±10% among the lines. Most strikingly, resistance allele formation post-fertilization in the early embryo averaged 40±19%, with variation in resistance rates among lines ranging from 6% to 81%. To uncover the potential genetic explanation for this variation, we performed a Genome-Wide Association Study (GWAS) using our results in the DGRP lines. We found genetic polymorphisms in 25 genes that were significantly correlated with differences in the embryo resistance allele formation rate. These include Camta, a calmodulin-binding transcription activator, enc, involved in germline mitosis, and Tis11, involved in RNA stabilization. Such genes may increase understanding of how natural variation is involved in resistance allele formation and be good target candidates for manipulation to develop gene drives with reduced rates of resistance allele formation.
35 Comparative analysis of centromeric DNA sequences in Drosophila species.  C-H. Chang1, J. Palladino2, A. Chavan3, C-C. Chen4, S. Cordi5, N. Martins6, C-T. Wu7, B. Mellone2, A.M. Larracuente1 1) Biology, University of Rochester, Rochester, NY; 2) Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT; 3) Department of Genetics, Harvard Medical School, Cambridge, MA.

Single molecule long-read sequencing has significantly improved our ability to assemble repetitive genomic regions, including some complex satellite DNAs and regions of Y chromosomes. However, in Drosophila, the sequences underlying the centromeres have remained enigmatic. Centromeres are defined by nucleosomes containing the centromeric histone variant, CENP-A. Drosophila centromeres are embedded deep in simple satellite repeats and have been recalcitrant to genome assembly. We used long-read sequencing from Pacific Biosciences (PacBio) to create heterochromatin-enriched genome assemblies. We will describe how we used these assemblies to identify the putative centromeres of each chromosome in D. melanogaster. We discovered that each centromere contains simple satellite DNAs interrupted by islands of complex DNA consisting of transposable elements and other AT-rich sequences. We validated our centromere assemblies using ChiPseq to confirm that these islands are enriched for CENP-A and fluorescence in situ hybridization to show that they are indeed centromeric. The centromeres of each chromosome are unique, but show similarities in composition and organization. This is especially true for the sex chromosomes and the dot chromosome—an interesting observation given that previous research suggests that the dot chromosome is derived from an ancestral X chromosome. We used deep PacBio sequencing and our heterochromatin-enriched assembly methods in D. simulans, D. sechellia, and D. mauritiana to identify candidate centromeric contigs in the simulans clade. We show that centromeric DNA changes rapidly between Drosophila species. Long-read sequencing makes it possible to study the detailed structure of the most enigmatic regions of genomes. Our study reveals the genetic composition of Drosophila centromeres and how they evolve on long and short evolutionary timescales, with important implications for understanding centromere drive.

36 The Recombination Landscape of Drosophila virilis under hybrid dysgenesis.  L.W. Hemmer, J.P. Blumenstiel  Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS.

DNA damage in the germline is a double-edged sword. Induced double-strand breaks establish the foundation for meiotic recombination and proper chromosome segregation but can also pose a significant challenge for genome stability. Within the germline, transposable elements are powerful agents of double-strand break formation. How different types of DNA damage are resolved within the germline is poorly understood. For example, little is known about the relationship between the frequency of double-stranded breaks, both endogenous and exogenous, and the decision to repair DNA through one of the many pathways, including crossing over and gene conversion. We aim to use the Drosophila virilis hybrid dysgenesis model to determine how recombination landscapes change under transposable element activation. In this system, a cross between two strains of D. virilis with divergent transposable element loads results in the hybrid dysgenesis phenotype, which includes the germline activation of diverse transposable elements, reduced fertility, and male recombination. However, only one direction of the cross results in hybrid dysgenesis. This allows us to examine recombination in genetically identical F1 females; those with baseline levels of programmed DNA damage and those with an increased level of DNA damage resulting from transposable element proliferation. We are using multiplexed shotgun genotyping to map crossover events to compare the recombination landscapes of hybrid dysgenic and non-hybrid dysgenic individuals. Patterns of recombination appear to be robust during hybrid dysgenesis.

37 Prophage WO genes that alter sperm and kill males in Drosophila.  S. Bordenstein1,2,3,4, J. Perlmutter1, D. Shropshire1, E. Layton1, H. Zhou1, B. Leigh1, A. Brooks1,2, T. Hill3, J. Martinez4, R. Unckless5, F. Jiggins6, S.R. Bordenstein1 1) Department of Biological Sciences, Vanderbilt University, Nashville, TN; 2) Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN; 3) Vanderbilt Institute of Infection, Immunology, and Inflammation, Vanderbilt University, Nashville, TN; 4) Vanderbilt Genetics Institute, Vanderbilt University, Nashville, TN; 5) Department of Molecular Biosciences, University of Kansas, Lawrence, KS; 6) Department of Genetics, University of Cambridge, Cambridge, UK.

The obligate intracellular symbiont, Wolbachia pipientis, selfishly alters fly sperm and egg (cytoplasmic incompatibility) and male embryos (male killing) to increase the fitness of infected females relative to uninfected females. These modifications enhance Wolbachia's maternal spread through host populations and significantly impact speciation and vector control in arthropods. However, the genes underlying the adaptations remain mostly elusive. In this presentation, we report the discovery of three genes in the eukaryotic association module of prophage WO that recapitulate Wolbachia's capacity to cause cytoplasmic incompatibility and male killing in Drosophila. The discovery of these three genes (cifA, cifB, wmk) highlights the significance of phage genes in shaping intracellular symbiont adaptations and informs their potential use in suppressing or modifying pest and vector populations.


Gene expression is not only an intermediate step linking genotypic variation to higher-order phenotypic variation, but it is a complex trait that offers unique opportunities to study the regulation of phenotypic variation. The response to stressful
Phosphatidylserine externalization is associated with developmental and pathological neurite degeneration in Drosophila. Maria Sapar, Hui Ji, Bei Wang, Chun Han Well Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY.

Phagocytic clearance of degenerating neuronal processes is critical for preventing neuroinflammation and for maintaining tissue homeostasis in the nervous system. Aberrant engulfment of neuronal membranes is also associated with early onset neurodegenerative pathology. How neurites are marked for phagocytic engulfment in vivo is poorly understood. Externalized phosphatidylserine (PS) has long been postulated as an eat-me signal that triggers the recognition of degenerating neuronal tissues by phagocytes, but in vivo evidence for the association of PS exposure and engulfment of neuronal processes has been lacking. To determine if degenerating neuronal processes indeed expose PS, we developed an in vivo system in the Drosophila larva to visualize potential PS exposure on peripheral sensory neurons. We found that PS is dynamically exposed on the surface of degenerating dendrites both during developmental pruning and after physical injury. The PS exposure is spatiotemporally correlated with the process of dendrite degeneration and is suppressed when dendrite degeneration is genetically blocked. To understand the functional consequences of PS exposure, we ectopically induced PS exposure in uninjured dendrites by knocking out PS flipases and by overexpressing mammalian phospholipid scramblase TMEM16F. Interestingly, ectopic PS exposure causes different modes of neurite degenerations that differ in larval sensory dendrites and in adult olfactory axons. Lastly, we found that extracellular lactadherin potentiates Drosophila epidermal cells to destruct PS-exposing dendrites and this function of lactadherin is independent of its N-terminal integrin-interaction domain, suggesting the existence of a previously unidentified bridging domain in the rest of the protein. Together, our study provides the first characterization of the in vivo dynamics of PS exposure on degenerating neurites and establishes a functional association between PS exposure and phagocytosis-dependent neurite degeneration in vivo.

Autophagolysosome disruption in a Drosophila model of ALS/FTD caused by C9orf72 expansion mutation. Kathleen Cunningham1, Ke Zhang1,4, Hyun Sung1, Munime Senturk2, Zhongyuan Zuo3, Jeffrey Rothstein4, Tom Lloyd1 1) Neurology, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Developmental Biology Program, Baylor College of Medicine, Houston, TX; 3) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 4) Brain Science Institute, Johns Hopkins University School of Medicine, Baltimore, MD.

A GGGGCC hexanucleotide repeat expansion (G4C2 HRE) in the first intron of the C9orf72 gene has been identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In a RNAseq for modifiers of HRE-mediated degeneration, our lab previously identified a key role of nucleocytoplasmic transport (NCT) disruption in C9-ALS. We have now been investigating which key pathways may mediate neurodegeneration downstream of nucleocytoplasmic transport disruption. In our screen we identified two key regulators of autophagy, Ref(2)p5SQSTM1 and Mitf/TFEB, as modifiers of neurodegeneration. In a Drosophila model of C9-ALS expressing (G4C2)n, we found that Ref(2)p is upregulated and forms large aggregates in motor neurons. Ref(2)p plays a key role in autophagy by binding ubiquitinated proteins and delivering them to the autophagosome for degradation via the lysosome. Surprisingly, we find that knockdown of Ref(2)p rescues degeneration in the fly eye and in motor neurons, while knockdown of Mitf enhances neurodegeneration. Immunofluorescence and western blot analysis of autophagy and lysosome markers demonstrates an expansion of lysosomes and decreased delivery to and digestion of autophagic cargo in the lysosome. Using electron microscopy of the Drosophila eye, we observe a remarkable accumulation of expanded multilamellar bodies and autolysosomes that precedes neurodegeneration. Due to these lysosomal defects, we hypothesized that mislocalization of Mitf from the nucleus downstream of NCT defects may cause dysregulation of lysosomes and autophagy. Indeed, we find by
IF that Mitf is more strongly in the cytoplasm in G4C2 expressing cells. We also find that genetic and pharmacological induction of lysosomal genes downstream of Mitf rescues neurodegeneration. We propose that C9orf72-HRE expression causes Mitf/TFE8 mislocalization from the nucleus and contributes to a feed-forward loop between NCT disruption and protein aggregation. This study suggests that drugs targeting lysosomal proteostasis pathways may have therapeutic potential for C9orf72-mediated ALS and FTD.

41 Activation of BMP signaling in non-motor neurons rescues motor dysfunction in a Drosophila model of Amyotrophic Lateral Sclerosis. Aaron Held1, Asli Sahin1, Robert Reenan1, Diane Lipsonbce2-3, Kristi Wharton1,3 1) MCB Department, Brown Univ, Providence, RI; 2) Neuroscience Department, Brown Univ, Providence, RI; 3) Brown Institute for Brain Science, Brown Univ, Providence, RI.

Several cellular mechanisms likely contribute to Amyotrophic Lateral Sclerosis progression, but determining which changes occur first, and which are secondary consequences has been difficult. At the point of diagnosis in humans, there is typically substantial motor neuron degeneration, limiting studies that might provide insight into the point of disease origin. Animal models of ALS are therefore invaluable for exploring disease progression. We made use of a Drosophila Superoxide Dismutase 1 (dsod1) knock-in model that contains a mutation synonymous to the human SOD1G85R mutation, and exhibits end-stage symptoms that parallel human ALS. End stage dsod1G85R animals display motor dysfunction, substantial neuromuscular junction degeneration, and die shortly after failing to emerge from the pupal case. Interestingly, earlier stage animals have motor dysfunction without defects in neuromuscular junction morphology or a decrease in neurotransmission to muscle. We’ve found that defects in feedback from the peripheral nervous system to the central locomotion pattern generator account for this early locomotor change. This feedback defect could be caused by changes in proprioceptors and/or the integration of their output by interneurons. We were then able to alleviate locomotor phenotypes by activating the BMP signaling pathway in both proprioceptors and excitatory interneurons. This non-motor neuron activation of BMP signaling allows 20% of dsod1G85R animals to emerge from the pupal case and improves end stage motor neuron morphology. Our results suggest that non-motor neurons contribute to motor dysfunction, and that activating cellular processes under the control of BMP signaling in non-motor neurons can alleviate motor phenotypes. Future studies will 1) determine how circuitry changes influence the onset and progression of ALS-like phenotypes and 2) identify which cellular processes regulated by BMP signaling alleviate motor dysfunction.

42 Interaction of LRRK2 with Rab GTPases in vivo. C.J.H Elliott, S. Petridi, A. Cording, C.A. Middleton 1) MCB Department, Brown Univ, Providence, RI; 2) Neuroscience Department, Brown Univ, Providence, RI; 3) Brown Institute for Brain Science, Brown Univ, Providence, RI.

A key question in Parkinson’s is how mutations in LRRK2 lead to neurodegeneration. It has been suggested that LRRK2 may interact with a Rab GTPase, but the exact Rab specified has varied depending on the assay. Previously, we identified an excitotoxic mechanism by which expression of mutant forms of LRRK2 in the dopaminergic neurons led to degeneration of the photoreceptors, and complete loss of visual signalling in old flies. Young flies show an increase in visual response when expressing Rab10 in the dopaminergic neurons reduces the visual response of the lamina (retinal) neurons, while expressing both Rab10 and LRRK2-G2019S increases the neural response ~20 fold. These changes in the neural response are independent of photoreception. Much smaller changes are seen in older flies, when neurodegeneration may already have begun.

Knockdown of Rab10 ameliorates the neurodegeneration seen in old flies expressing LRRK2-G2019S in their dopamine neurons.

Localisation with GFP expression/antibody staining suggests that the dopamine neurons innervating the visual lobes and lamina are Rab10+, but other dopamine neurons show no sign of co-localisation.

LRRK2-G2019S is known to reduce neuronal outgrowth in cell culture and fly sensory neurons, so we hypothesise that expressing it in the dopamine neurons may reduce the branching that we have seen in the lamina. Rab10 has also been linked to neuronal outgrowth and to differentiation of axons and dendrites.

The interaction of these proteins in dopamine neurons in a physiological assay contributes to our understanding of the first steps in the toxic cascade in Parkinson’s.

43 Roles of CaMKII in neurodegeneration caused by depletion of presynaptic mitochondria. K. Shinno1, M. Oka1, S. Hisanaga1, E. Suzuki1, K.M. Iijima1, K. Ando1 1) Department of Biological sciences, Tokyo Metropolitan University, Hachioji, Tokyo, Japan; 2) Gene Network Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan; 3) Department of Genetics, School of Life Science, SOKENDAI, Mishima, Shizuoka, Japan; 4) Department of Alzheimer’s Disease Research, National
Mitochondria contribute to many cellular processes including ATP production and intracellular Ca2+ signaling. In neurons, mitochondria are actively transported to the synapse to meet energetic demands for neurotransmission. Reduction in number or function of mitochondria at the synaptic terminals has been suggested to contribute to the pathogenesis of neurodegenerative diseases. However, how loss of synaptic mitochondria leads to neurodegeneration is not fully understood.

Neuronal knockdown of milton, an adaptor protein for axonal transport of mitochondria, causes depletion of mitochondria from the presynaptic terminal. We previously reported that depletion of mitochondria from the presynaptic terminals in the retina or brain neurons causes age-dependent neurodegeneration. Ultrastructural analyses of these neurons indicate abnormalities in neurotransmitter vesicles. Since Calcium/calmodulin-dependent protein kinase II (CaMKII) plays an important role in synaptic transmission, we investigated the role of CaMKII in neurodegeneration caused by depletion of presynaptic mitochondria.

We found that the levels of the autophosphorylated form of CaMKII were increased by neuronal knockdown of milton. Blocking CaMKII activity significantly suppressed neurodegeneration caused by milton knockdown. Furthermore, expression of a Ca2+-independent form of CaMKII induced age-dependent axon degeneration. These results suggest that elevated CaMKII activity mediates neurodegeneration caused by depletion of presynaptic mitochondria.

We previously reported that milton knockdown enhances neurodegeneration caused by microtubule-associated protein tau, which plays critical roles in several neurodegenerative diseases, including Alzheimer’s disease. We found that knockdown of CaMKII also suppressed enhancement of tau toxicity caused by milton knockdown.

These results suggest that dysregulation of CaMKII may contribute to neurodegeneration triggered by mitochondrial abnormality. Further study will enhance our understanding of the relationship between distribution of mitochondria and synaptic transmission and their roles in brain structural integrity.

44 **Drosophila FMRP modulates energy metabolism and mitochondrial function.** E.D. Weisz1, A. Towheed2, R.E. Monya1, M.S. Toth1, D.C. Wallace1,2, T.A. Jongens1 1) Perelman School of Medicine at the University of Pennsylvania; 2) The Children’s Hospital of Philadelphia.

Fragile X Syndrome (FXS) is the predominant form of inherited intellectual disability and the foremost monogenic cause of autism. At the molecular level, FXS is caused by loss of expression of the *fragile X mental retardation 1* (*FMR1*) gene. Given its high prevalence and known etiology, FXS is an ideal genetic paradigm for the study of the cellular and molecular underpinnings of multifarious forms of intellectual impairment and autism. The *Drosophila* model system is uniquely suited to facilitate the use of rapid genetic and biochemical approaches to probe the processes and pathways involved in FXS pathogenesis. Recently, work from our laboratory implicated brain insulin signaling dysregulation in the development of behavioral and cognitive deficits in the *Drosophila* model of FXS. This finding, along with reports that FXS patients with metabolic disturbances have a higher prevalence of clinically defined autism, prompted us to explore the metabolic implications of loss of *dfmr1* expression.

Here, we demonstrate that *dfmr1* modulates the global metabolome. Despite our previous discovery of increased brain insulin signaling, the results from our metabolomics and biochemical assays indicate that flies with a loss-of-function mutation in the *dfmr1* gene have reduced carbohydrate and lipid stores and are hypersensitive to starvation stress. The observed metabolic deficits cannot be explained by feeding behavior, as we found that the *dfmr1* mutants eat more than their wild-type counterparts in the well-established capillary feeder (CAFÉ) assay. Rather, our data identify *dfmr1* as a regulator of mitochondrial function. Specifically, we show that aberrant mitochondrial function and morphology are involved in FXS pathophysiology. Together, our results illustrate the importance of *dfmr1* for proper maintenance of nutrient homeostasis and mitochondrial function.

45 **Transcriptomic and proteomic profiling of an epilepsy fly model reveals cell non-autonomous downregulation of synaptic proteins.** K. Hope1,2, D. Johnson3, D. Kakhniashvili4, L. Reiter1,5,6 1) Department of Neurology, University of Tennessee Health Science Center, Memphis, TN; 2) Integrated Biomedical Sciences Program, University of Tennessee Health Science Center, Memphis, TN; 3) Molecular Bioinformatics Core, University of Tennessee Health Science Center, Memphis, TN; 4) Proteomics and Metabolomics Core, University of Tennessee Health Science Center, Memphis, TN; 5) Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN; 6) Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN.

Duplication 15q syndrome (Dup15q) is caused primarily by maternally inherited duplications of the 15q11.2-q13.1 region and has a high rate of treatment resistant epilepsy. Previous research focused on the neuronal overexpression of one gene located within 15q11.2-q13.1, *UBE3A*, however none of these mouse models have seizure phenotypes. Recently our lab generated a novel fly model that recapitulates the seizure phenotype of Dup15q where *UBE3A* is overexpressed in glial cells, not neurons, implicating glial cells in Dup15q epilepsy. To investigate the differential effects of *UBE3A* overexpression in glia compared to neurons we employed global protein analysis through liquid chromatography coupled to high-resolution mass spectrometry and global transcriptome analysis through RNA-sequencing of whole fly head extract in repo>*Dube3a* (the fly...
UBE3A homolog) vs elav>Dube3a animals. We reliably measured approximately 2,500 proteins at both the transcript and protein level. By comparing transcriptomic and proteomic datasets we were able to identify genes that were altered only at the transcript level, only the protein level, or both the transcript and protein level in neuronal or glial expressing lines. Gene ontology analysis revealed an enrichment of 26 synaptic proteins downregulated at both the transcript and protein level following overexpression of Dube3a in glia (repo-GAL4), including Synapsin and Sap47. These synaptic proteins were relatively unchanged in neuronal Dube3a overexpression (elav-GAL4), indicating synaptic proteins change in a cell non-autonomous manner upon overexpression of Dube3a in glia. Dysregulation of synaptic proteins may be an underlying cause of epilepsy and we are currently investigating whether altered synaptic protein levels are a common theme among other glia-driven fly seizure models. Glial specific knockdown of the classic Drosophila seizure genes ATPolpha and SesB generated seizure phenotypes, while lethality was observed upon glial-specific knockdown of jitterbug, Letm1, and Zydeco. In summary, cell non-autonomous downregulation of synaptic proteins may play a key role in Dup15q epilepsy and possibly other “gliopathic” epilepsy cases.

46 Exploring C(2)M and its ability to promote assembly of the synaptonemal complex. Kim McKim, Justin Mathew, Mercedes Gyuricza, Nikunj Patel. Waksman Inst, Rutgers Univ, Piscataway, NJ.

A common error in meiosis is aneuploidy, where homologous chromosomes do not properly segregate from each other. This can cause various birth defects in human offspring, including Trisomy 21 (Down Syndrome) and Klinefelter Syndrome. In order to investigate the causes of aneuploidy, we study protein complexes called cohesins, which play an important role in synaptonemal complex (SC) formation and chromosome segregation during meiosis. Cohesins are comprised of four subunits: one Stromalin, one kleisin, and two SMC proteins. In mitosis, there is one cohesin ring responsible for sister chromatid cohesion that uses the Rad21 kleisin protein. In meiosis, there are two types of cohesin rings, one required for sister chromatid cohesion that includes SOLO and SUNN and one required for assembly of the SC that uses the C(2)M kleisin protein and Stromalin. We are testing the hypothesis that formation of a ring with C(2)M, Stromalin, SMC1 and SMC3, is required for SC assembly and thus important for accurate chromosome segregation in meiosis. In order to determine if and how C(2)M functions in a meiotic cohesin complex, mutations of C(2)M were made in sites that were hypothesized to interact with the SMC1 or SMC3 subunits. Surprisingly, two point mutations F90A and F525A, were able to localize properly and rescue the SC formation and nondisjunction phenotypes in a c(2)M null mutant background. In contrast, the L529A point mutation was observed to localize properly in a c(2)M wild-type background, but was not able to localize properly nor rescue the SC formation and nondisjunction phenotypes in a c(2)M mutant background. These results suggest direct interactions between C(2)M and SMC1 are important for SC assembly. Additional point mutations in hypothesized interaction residues between C(2)M and SMC1 or SMC3 are currently being analyzed. In addition, co-IP experiments are underway to determine if C(2)M physically interacts with the other subunits of the cohesin ring and how it regulates SC assembly.

47 Separating the contribution of chromatin versus that of repetitive DNA in centromere specification. J.T. Palladino1, A. Chavan1, A. Sposato1, B. Mellone1,2. 1) Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

Centromeres are essential regions of the genome that mediate the accurate segregation of chromosomes during mitosis and meiosis. Most eukaryotes, centromeres are composed of simple and complex repeats and transposable elements organized into a specialized type of chromatin that contains the histone H3 variant CENP-A. For decades, the centromere has been thought to be specified epigenetically. Yet, the respective contributions of chromatin versus centromeric DNA sequences in centromere specification and function have remained elusive. Here, we test whether ectopic centromeres, which are devoid of centromeric DNA, can be transmitted through multiple cell divisions and whether they can compete with native centromeres in Drosophila. We induced the formation of ectopic centromeres on integrated lacO repeat-arrays to which the CENP-A assembly factor CAL1 is tethered via the Lac Repressor (LacI) tag. Ectopic centromeres formed at both euchromatin and heterochromatin. During tethering, ectopic centromeres sometimes prevailed, causing the inactivation of the endogenous centromere by centromeric DNA double-strand breaks that were sometimes followed by HP1-dependent epigenetic silencing. Upon release of CAL1-LacI, ectopic centromeres were retained throughout development, suggesting centromeric DNA is not required for centromere maintenance or function. These results emphasize the expendability of centromeric DNA in centromere specification and suggest that the presence of the CENP-A epigenetic mark is sufficient for sustained centromere function in the animal.

48 A centrosome asymmetry switch in fly neural stem cells. A. Ramdas Nair1,2, E. Gallaud1,2, A. Monnard1,2, T. Pham1,2, P. Singh1,4, D. Salvador Garcia1,2, A. Ferrand1, C. Cobernard1,2. 1) Biozentrum, University of Basel, Basel, Switzerland; 2) University of Washington, Department of Biology, Seattle, WA; 3) NYU Abu Dhabi, Saadiyat Campus, Abu Dhabi, United Arab Emirates; 4) Department of Bioscience & Bioengineering, Indian Institute of Technology Jodhpur, Rajasthan, India; 5) Division of Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, UK.

Centrosomes, the main microtubule organizing centers (MTOCs) of metazoan cells, contain an older ‘mother’ and a younger
‘daughter’ centriole. Stem cells either inherit the mother or daughter centriole, providing a potential mechanism for biased delivery of cell fate determinants. However, the molecular mechanisms regulating centrosome asymmetry and biased centrosome segregation are unclear. Using 3D-Structured Illumination Microscopy (3D-SIM), we investigated the onset, mechanisms and function of centrosome asymmetry and identified a previously undiscovered centrosome asymmetry switch in fly neural stem cells (neuroblasts). We found that the centriolar protein Centrosin (Cbn) and its upstream regulator the mitotic kinase Polo relocalize from the existing mother to the newly formed daughter centriole in early mitosis. Pericentrin (PCNT)-like protein, however, remains predominantly localized on the mother centriole. Loss of Centrosin, or of the microcephaly-associated protein Wdr62, affects Polo's transitioning to the daughter centriole. We further used nanobody technology to prevent the transitioning of Polo from the mother to the daughter centriole and assayed the functional consequences with live cell imaging. Disrupting the centrosome asymmetry switch in neuroblasts perturbs asymmetric microtubule organizing activity, centrosome positioning and spindle orientation. We conclude that this centrosome asymmetry switch provides a mechanism to ensure that fly neural stem cells always retain the daughter centriole-containing centrosome. Furthermore, the switch might explain the differences in biased centromere inheritance across stem cell systems.

49 Diverse Roles of Actin-Microtubule Crosslinker Shortstop in Cell Division. E. Dewey, C. Johnston  Department of Biology, University of New Mexico, Albuquerque, NM.

Properly executed cell division is crucial to development, maintenance, and longevity of multicellular organisms. Defects in both symmetric and asymmetric divisions can lead to improper developmental patterning, as well as genomic instability, disruption of tissue homeostasis, and cancer. Our research focuses on understanding how regulators of the actin and microtubule (MT) cellular cytoskeleton communicate to orchestrate the orientation and stability of the mitotic spindle, a critical component to proper cell division and key to maintaining tissue homeostasis. Shortstop (Shot) is a member of the spectraplakin protein family found previously to crosslink actin and microtubule filaments, playing a vital role in stabilizing interphase microtubules in both Drosophila and human cell models. We describe a role for Shot in oriented cell divisions, with both tissue culture and in vivo Drosophila epithelial models showing spindle misalignment in Shot knockdowns (KD). Further, we show a role for Shot in spindle assembly in these contexts, demonstrating that spindles do not contain tightly focused poles. Shot KDs also produce defects in chromosomal migration to spindle equator (congression) and chromosomal segregation. We show these activities are mediated not only through traditional Shot roles in stabilization of spindle MTs through crosslinks to actin, but also through a direct interaction of Shot actin binding domain to dynein activator subunit actin-related protein 1 (Arp1) filaments. In line with this hypothesis, only the Shot isoform possessing both MT and actin binding activities is capable of rescue of Shot KD phenotypes. We hypothesize Shot interaction with Arp1 functions to crosslink it to spindle MTs, facilitating activation and stabilization of the MT motor protein Dynein, and promoting its activity in spindle assembly, alignment, chromosomal congression, and chromosomal segregation. In support of this model, live cell imaging experiments show defects in cell division timing under Shot conditions. These timing faults implicate involvement of the spindle assembly checkpoint (SAC), with inhibition of SAC components under Shot KD conditions leading to timing rescue. Further, Shot loss in epithelial tissue in vivo leads to an increase in apoptosis, in line with previous findings linking spindle regulators to cell death. Interestingly however, while previous studies have implicated induction of the Jun kinase (Jnk) apoptotic pathway under spindle regulator KD, Shot apoptosis does not implicate Jnk in this activity. Finally, when Shot KD-induced apoptosis is inhibited, tumorigenic-like conditions result, underscoring the importance of Shot as a key component in development and maintenance of multicellular organisms.

50 Examining chromatin in different states of G0. Y. Ma1, S. Nystrom2, D.J. McKay2, L. Buttitta 1) University of Michigan, Ann Arbor; 2) University of North Carolina, Chapel Hill.

States of cellular withdrawal from the cell cycle or G0 can range from readily reversible to permanently postmitotic. We are interested in how different states of G0 are controlled during development and why some are more reversible than others. Emerging evidence suggests a close relationship between a repressive chromatin structure and the silencing of cell cycle genes during the postmitotic state, but whether there are differences in the chromatin state between reversible and permanent cell cycle exit remains unclear. We have focused our studies on the Drosophila pupal wing at a stage where the cells transition from active proliferation to a postmitotic state. We find there are two stages of G0 in this tissue, a flexible G0 period where cells can be induced to re-enter the cell cycle under specific genetic manipulations and a state we call “robust”, where cells become strongly refractory to cell cycle re-entry. We are using this tissue to compare changes in chromatin organization during proliferation, flexible G0 and robust G0 using Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE)-seq. We find that upon robust G0, key enhancers of specific cell cycle genes become occupied by nucleosomes, likely blocking the access of transcription factors. This emphasizes a role for nucleosome remodeling complexes in establishing and maintaining a robust G0 state. We therefore carried out a pilot screen for nucleosome remodeling factors essential for proper cell cycle exit in the wing and identified Mi-2, the ATPase component of Nucleosome Remodeling and Deacetylase (NuRD) complex, as a potential contributor to robust G0.
51  An alternatively spliced form affecting the Marked Box domain of Drosophila E2F1 is required for proper cell cycle regulation during development. M. Kim, J. P. Tang, N.-S. Moon  Department of Biology, Developmental Biology Research Initiative, McGill University, Montreal, Quebec, CA.

Across metazoans, cell cycle progression is regulated by E2F family transcription factors that can function as either transcriptional activators or repressors. For decades, the Drosophila E2F family has been viewed as a streamlined RB/E2F network, consisting of one activator (dE2F1) and one repressor (dE2F2). Although dE2F1 is largely viewed as a transcriptional activator, previous studies have pointed to a role of dE2F1 in the repression of target genes, which remains poorly understood. Here, we report that an uncharacterized isoform of dE2F1, hereon called dE2F1b, plays an important function during development and is functionally distinct from the widely-studied dE2F1 isoform, dE2F1a. dE2F1b contains an additional exon that inserts 16 amino acids to the evolutionarily conserved Marked Box domain. Analysis of de2f1b-specific mutants generated via CRISPR/Cas9 indicates that dE2F1b plays an important role in proper regulation of G1/S-target gene expression, and may play a repressive role in certain developmental contexts. Key E2F target genes such as cyclin E are deregulated across de2f1b mutant tissues such as salivary glands and imaginal discs where the absence of dE2F1b results in de-repression of target gene expression. In addition, chromatin immunoprecipitation assays revealed that dE2F1b may mediate repression by altering RBF1/dE2F1 recruitment to promoters. Collectively, our data suggest that dE2F1b is a novel and essential member among Drosophila E2Fs, revealing a previously unappreciated complexity in the Drosophila RB/E2F network.

52  Master regulators of the Minute phenotype: translation, growth, and cell competition depend on a regulatory pathway induced by ribosomal protein mutations. M. Kiparaki1,2, C.H. Lee1,2, J. Blanco1, A. Kumar1, V. Folgado1, Z. Jil1, G. Rimesso1, N.E. Baker1  1) Departments of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) equal contribution.

Heterozygous inactivating mutations in ribosomal protein genes have been identified in several diseases, known as ribosomopathies, which have some shared defects including bone marrow failure and growth abnormalities. In Drosophila, heterozygous mutations in many of the ribosomal protein genes lead to the Minute syndrome, which includes smaller adult bristles, developmental delay, slow translation and growth and reduced cell competitiveness in genetic mosaics. During cell competition, ribosomal protein heterozygous mutant cells (Rp/+), called Minute cells) are actively eliminated from mosaic tissues containing wild type cells. In our laboratory, previous genetics screens identified two genes which affect the cell competition between wt and Minute cells. One of them is a ribosomal protein gene (RpS12) and the other one is a bZIP putative transcription factor, called Xrp1. By employing genetic experiments, Click-chemistry and Northern analysis, we showed that both proteins belong to the same regulatory pathway, which when intact is activated in Rp/+ cells and reduces their translational rates, with subsequent effects on their growth and competitiveness. Interestingly, we have shown that Xrp1 is responsible for much of the developmental delay of Minute flies. In parallel, RNA-sequencing analysis revealed that the majority (>80%) of the transcriptome changes that occur in ribosomal protein mutant flies depend on this pathway. A conclusion of our study is that global translational rate defect does not have a primary role as a sensor of Rp mutation. Instead translation is regulated as a downstream consequence of this novel characterized RpS12-Xrp1 pathway. This pathway may be advantageous, to eliminate the Rp mutant cells (pathway ON) by cell competition before translation is affected non-specifically or even allow Rp mutant cells to survive in different contexts (pathway OFF).

53  Erk-dependent control of epithelial morphogenesis. Heath Johnson1, Stanislav Shvartsman2,3, Jared Toettcher1  1) Molecular Biology, Princeton University, Princeton, NJ; 2) Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 3) Chemical and Biological Engineering, Princeton University, Princeton, NJ.

The role of Ras/Erk signaling in cell growth, and differentiation has long been appreciated. In contrast, the roles of Erk signaling in cell motility, collective cell migration, and tissue-level morphogenesis are complex and remain poorly defined. Here, we set out to define the Ras/Erk pathway’s role in orchestrating morphogenetic movements during gastrulation in the early Drosophila embryo. Using an optogenetic input to Ras, we found that Erk activity is sufficient to induce cells to adopt a contractile cell fate at nearly any illuminated location within the embryo. This tissue mimics the gene expression and physical organization of the posterior midgut (PMG), a tissue normally patterned by the Torso receptor tyrosine kinase. We define the transcriptional network by which Erk programs PMG cell fate, leading to the accumulation of apical myosin and tissue contraction at gastrulation. By systematically varying the timing and duration of Erk activity, we define the spatiotemporal features of the Erk signal that is required to program these fates. We find that the early embryo responds to the cumulative load of Erk activity delivered over a two hour window in early embryogenesis, revealing a previously unknown long-term memory of signaling that spans multiple nuclear division cycles. Our work mechanistically defines an Erk-dependent cell fate choice and establishes a model system for interrogating how signaling pathway activity can program large-scale changes in tissue organization in vivo.

Epithelial tissues undergo dramatic changes in shape during development. These changes are driven in large part by contractile forces generated by the actomyosin cytoskeleton of cells. In addition to physically shaping cells and tissues, mechanical forces can also act as cues to influence cell behavior and potentially help coordinate cell behaviors across multicellular tissues. A major obstacle to dissecting the mechanisms of how mechanical forces shape tissues has been the lack of tools for precise manipulation of forces in vivo. To address this, we developed a collection of optogenetic tools to locally and systematically modulate cellular actomyosin contractility in the *Drosophila* embryo. With these tools, we have demonstrated local, light-gated myosin recruitment and cell shape changes in targeted regions of epithelia in the developing embryo. We are optimizing these tools for precise, quantitative manipulation of force generation during epithelial morphogenesis in vivo. These studies will shed light on the roles of mechanical cues in coordinating cell behaviors both during development and in a wide range of physiological processes.

Redundancy in supracellular actomyosin networks yields robust tissue folding. A.C. Martin1, Hannah Yevick1, Norbert Stoop2, Jörn Dunkel2 1) Biology, Massachusetts Institute of Technology, Cambridge, MA; 2) Mathematics, Massachusetts Institute of Technology, Cambridge, MA.

Correct tissue shape is essential for proper tissue function. In many developing systems myosin-driven contractions are harnessed to fold cell monolayers and sculpt shape. For example folding forms both the vertebrate eye and the neural tube. In *Drosophila*, a cell monolayer on the ventral side of the embryo undergoes myosin-dependent constriction to fold the tissue internalizing presumptive mesoderm cells. The ventral furrow establishes a supracellular network of contractile actin-myosin fibers just prior to folding. While the cytoskeletal organization and mechanism of contraction in a single cell is understood, less is known about how the cytoskeleton is patterned across the tissue to achieve robust folding. We have integrated concepts from topological feature analysis to map the connectivity of the previously unquantified network spanning hundreds of cells. Our framework allows us to explore stereotypic properties of the supracellular network and investigate the need for reproducibility of its mechanical connections. We apply both mechanical and genetic perturbations to degrade the network and have identified that there exists multiple network architectures that induce folding. Additionally, we demonstrate the importance of redundant connections in ensuring the folding robustness.

The scaffold protein Canoe and ZO1/Polychaetoid help link cell adhesion and the actomyosin cytoskeleton during tissue formation. L.A. Manning, H. Ronk, M. Peifer Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

During embryonic development, tissue establishment is made possible by cell shape changes and migration. Epithelial cells act as the building blocks for most tissues. They are organized into layered sheets with apical-basal polarity and are connected by adherens junctions near the apical end. During development, organ and tissues are shaped by epithelial cells participating in coordinated cell shape changes and migration. These changes require cells to communicate with each other and to remodel the cytoskeleton. The field has identified many proteins in cell junctions, but their mechanisms of action remain an active area of study. I aim to understand the mechanisms regulating the interaction between cytoskeletal and adhesion molecules in shaping epithelial tissue architecture. The actin binding protein Canoe and its mammalian homolog Afadin mediate adherens junctions and actomyosin linkage. Previous studies suggested Canoe mediates actomyosin linkage during gastrulation, with roles in shaping tissue architecture and maintaining integrity during apical constriction and convergent elongation. We are investigating the role of Canoe and its interaction with the ZO-1 homolog Polychaetoid to explore how the actomyosin cytoskeleton associates with adherens junctions during apical constriction, convergent elongation and collective cell migration during gastrulation. Using a cross disciplinary approach combining genetic manipulations and super-resolution microscopy we determined that reducing canoe gene expression to different levels results in defects in distinct morphological events. Our super-resolution microscopy is beginning to provide us with a greater understanding of the architecture of adhesion molecules, actin and actin regulators in the lateral epidermis in wildtype and how they are altered after Canoe loss. Severe reduction of canoe markedly alters cell shape and therefore disrupts later developmental events such as dorsal closure, head and segment formation. Strikingly, reducing both polychaetoid and canoe gene expression results in even more severe effects, consistent with a cooperative function. These results are consistent with the notion that Canoe acts as an important cytoskeletal-junction linker protein during morphogenesis. This study will provide a deeper understanding how cells maintain tissue integrity while moving as an epithelial sheet, which could contribute insight into wound healing or human embryonic defect prevention.

Epithelial rotation is preceded by planar symmetry breaking of actomyosin and protects epithelial tissue from cell deformations. I. Viktorinova, I. Henry, P. Tomancak Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Saxony, DE.

Symmetry breaking is involved in many developmental processes that form bodies and organs. One of them is the epithelial rotation of developing tubular and acinar organs. However, how epithelial cells move, how they break symmetry to define
their common direction, and what function rotational epithelial motions have remains elusive. Here, we identify a dynamic actomyosin network that breaks symmetry at the basal surface of the *Drosophila* follicle epithelium of acinar-like primitive organs, called egg chambers, and may represent a candidate force-generation mechanism that underlines the unidirectional motion of this epithelial tissue. We provide evidence that the atypical cadherin Fat2, a key planar cell polarity regulator in *Drosophila* oogenesis, directs and orchestrates transmission of the intracellular actomyosin asymmetry cue onto a tissue plane in order to break planar actomyosin symmetry, facilitate epithelial rotation in the opposite direction, and direct the elongation of follicle cells. In contrast, loss of this rotational motion results in anisotropic non-muscle Myosin II pulses that are disorganized in plane and cause cell deformations in the epithelial tissue of *Drosophila* eggs. Our work demonstrates that atypical cadherins play an important role in the control of symmetry breaking of cellular mechanics in order to facilitate tissue motion and model epithelial tissue. We propose that their functions may be evolutionarily conserved in tubular/acinar vertebrate organs.

58 The dynamics of the EGFR signaling activation in the follicular epithelium. N. Revaitis1, N. Pouradier Duteil1, R. Marmion1, M. Niepielko1, B. Piccoli1-2, N. Yakoby1-3 1) Center for Computational and Integrative Biology, Rutgers University, Camden, NJ; 2) Mathematics Department, Rutgers University, Camden, NJ; 3) Biology Department, Rutgers University, Camden, NJ.

Organogenesis is a complex process involving the dynamic interaction among different cellular compartments. The *Drosophila* eggshell, the casing of the developing embryo, is an intricate structure derived from a monolayer of follicular epithelium engulfing the growing oocyte. The secretion of Gurken, a TGF-α-like ligand, from near the oocyte nucleus, activates a uniformly expressed epidermal growth factor receptor (EGFR) in the overlying follicular epithelium. This is a dynamic process that depends on the position of the oocyte nucleus; initially at the posterior end and later at the dorsal anterior of the oocyte. As the nucleus is positioned anteriorly across the follicle cells, the oocyte is continuously growing. Due to the relative location of the Gurken source to the overlying cells, the activation of EGFR is transient. We develop a mathematical model that integrates the shapes of the Gurken source and signaling activation (dPEKR) during oogenesis. Parameters used are based on literature and experimental findings. However, the rate of ligand-receptor complex (K\text{on}) internalization, the diffusion of GRK in the perivitelline space (D), and the quantity of EGFR (R\text{o}) remain elusive. Using CRISPR-Cas9, we developed an EGFR-EGFP line that is phenotypically wild type. Using a combination of ELISA and qRT-PCR, we quantified the levels of the receptor during different stages of oogenesis and early embryogenesis. These flies were used to detect localized EGFR trafficking within cells. We detected significant changes in the distribution of the receptor in the presence of high levels of EGFR signaling. These analyses aid to fine-tune the values for K\text{on}, D, and R\text{o}. The model can be used to predict evolutionary changes that account for the diversity of EGFR activation among species.

59 Differential lateral and basal tension drives epithelial folding through two distinct mechanisms. L Sui1, S Alt2, N Dye3, S Eaton1, F Juelicher4, G Salbreux2, C Dahmann1 1) Department of Biology, University of Technology Dresden, Dresden, Germany; 2) The Francis Crick Institute, London, UK; 3) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 4) Max Planck Institute for the Physics of Complex Systems, Dresden, Germany.

Epithelial folding transforms simple sheets of cells into complex three-dimensional tissues and organs during animal development. Epithelial folding has mainly been attributed to mechanical forces generated by an apically localized actomyosin network that drives pulsatile contractions leading to the apical constriction of cells. The contribution of forces generated at basal and lateral cell surfaces, however, remains largely unknown. Here we show that a local decrease of basal tension and an increased lateral tension, but not apical constriction, drive the formation of two neighboring folds in the developing *Drosophila* wing imaginal disc. Spatially-defined reduction of extracellular matrix density results in local decrease of basal tension, basal cell widening, and formation of the first fold. In the second fold, basal tension is unaltered, but fluctuations in F-actin lead to increased lateral tension and pulsatile contractions of the lateral cell edges that shorten cells and drive folding. Using a 3D vertex model of epithelial mechanics, we show that basal decrease and lateral increase in tension provide two distinct mechanisms that can both generate similar morphological changes during epithelial folding. Our combination of lateral and basal tension measurements with a mechanical tissue model reveals how simple modulations of surface and edge tension drive complex three-dimensional morphological changes.

60 Distinct properties of wasp germ plasm correlate with its divergent complement of localized RNA. Honghu Quan, Jeremy A. Lynch 1) Biological Sciences, University of Illinois at Chicago, Chicago, IL.

Many animals set aside primordial germ cells in the earliest stages of embryogenesis, using maternally provisioned and localized germ plasm. In holometabolous insects, germ plasm is assembled at the posterior of the oocyte. Typically, syncytial nuclei of the embryo that enter the germ plasm cellularize precociously (becoming pole cells), take on germ cell traits, and later migrate to the gonad. Surprisingly, germ plasm and pole cell features are quite diverse among the Holometabola. For example, the germ plasm (aka oosome) in the wasp Nasonia is spheroid, moves freely in the posterior half of the egg, and then produces a single large bud at the posterior pole which then subdivides to give multiple pole cells. This contrasts with the small, stationary polar granules and individual germ cell buds in Drosophila. Later, the Nasonia pole cells take a distinct
migratory path to the gonads. To understand these differences, we have sequenced RNAs of anterior and posterior fragments of Nasonia embryos, and have identified more than 30 posteriorly localized mRNAs potentially involved in germ cell determination. Only a handful of the fly orthologs of these transcripts are localized or have a described germ cell role. Functional analysis has confirmed that several of the wasp specific transcripts are important for the unique properties of the Nasonia oosome. These include the release of the germ plasm from the cortex just after the first syncytial division, the coalescence of the germline material into a spherical mass, and the cell biology of generating an extremely large posterior pole cell bud.

61  **Studying the cis- and trans-regulation of Sex lethal in the germline of Drosophila melanogaster.**  R. Goyal, E. Baxter, M. Van Doren  Department of Biology, Johns Hopkins University, Baltimore, MD.

In *Drosophila*, sex determination is under the control of the switch gene *Sex lethal (Sxl)*. Interestingly, XY (male) germ cells expressing *Sxl* are able to produce eggs upon transplantation into an XX (female) somatic gonad [1], demonstrating that even in the germline, *Sxl* is sufficient for female identity. In both the germline and soma, the presence of two X chromosomes leads to *Sxl* expression. However, the mechanism of counting the X chromosomes in the germline differs at both cis- and trans-levels, and we are investigating this mechanism.

We are performing RNA-FISH against nascent transcripts to understand the dynamics of *Sxl*’s transcriptional initiation in the embryonic germline. Interestingly, we have found that unlike in the soma, *Sxl*’s late, non-sex-specific promoter (*SxlP*) is activated in the primordial germline at least as early as its early, sex-specific promoter (*SxlP*) - at stage 5 of embryogenesis.

Further, the DNA elements regulating *SxlP* in the germline have not been identified. We are performing promoter analysis to investigate which regions of the *Sxl* gene locus are important for its germline expression. We have found that no DNA elements upstream of *SxlP* appear to be sufficient to drive sex-specific *SxlP* expression in the embryonic germline, suggesting that cis-regulation of *Sxl* in the germline is different from that in the soma.

We are also identifying trans-acting factors that regulate *Sxl* in the germline. Based on previous studies, the X chromosome “counting genes” that activate *Sxl* in the soma were not thought to act in the germline. However, we found that knocking down one of these genes, *sisterless A (sisA)*, in the female germline results in an ovarian tumor phenotype and germ cell loss, similar to masculinization of the germline due to loss of *Sxl*. Strikingly, *Sxl* expression is lowered in *sisA* RNAI ovaries and the germline loss phenotype can be rescued by *Sxl* cDNA. Using RNA-FISH, we have found *sisA* expression preceding that of *Sxl* in the germline. Put together, our data suggests that *sisA* lies upstream of *Sxl* in the germline sex-determination pathway as well.

Through this work we aim to understand how intrinsic sex determination is regulated in the germline, and how sexual identity of the germline interacts with sex-specific somatic development to control proper gametogenesis.


62  **SETDB1/EGGLESS maintains female sex-identity in Drosophila germ cells.**  A.E. Smolko, L Kulnane, H Salz  Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH.

In germ cells, the female versus male binary fate decision is initially guided by the sex of the developing somatic gonad. After embryogenesis, extrinsic control is lost and sexual identity is maintained by a cell-intrinsic mechanism. We have previously shown that *Sex-lethal (Sxl)* is the key cell-intrinsic regulator of female germ cell fate. Here, we demonstrate that *Sxl* maintains female germ cell identity via an epigenetic regulatory pathway in which SETDB1 is the required chromatin writer.

Using germline-specific knockdown, we show that the H3K9 trimethyltransferase, SETDB1, and its partner protein, Wde, are required to repress spermatogenesis gene expression in female germ cells. We find that SETDB1 is required for deposition of repressive H3K9me3 marks on a number of spermatogenesis genes. In general, the H3K9me3 marks are limited to the gene bodies of the spermatogenesis genes and do not spread to the neighboring loci. Regional deposition is especially striking at the *rho7* locus, a gene with an ovary and a testis-specific transcription start site. In ovaries, H3K9me3 deposition is limited to the silent upstream testis-specific transcription start site. Furthermore, pathway analysis indicates that *Sxl* is required for deposition of this repressive chromatin mark on these spermatogenesis genes. Together our new studies suggest that sex-specific chromatin modifications ensure that sex-identity is preserved.

63  **The nuclear transport protein Tnpo-SR promotes cell proliferation and oocyte specification in the early Drosophila germline.**  E.T. Ables, T.D. Hinnant  Dept. of Biology, East Carolina University, Greenville, NC.

Germ cell development requires a sophisticated interplay between factors that control cell fate and cell division. In the Drosophila ovary, germline stem cells (GSCs) reside at the anterior tips of strings of progressively more developed oocytes, termed ovarioles. GSCs complete asymmetric mitotic divisions giving rise to a differentiated daughter cell (the cystoblast).
while self-renewing to replenish the stem cell pool. The cystoblast divides four times with incomplete cytokinesis; fifteen daughter cells give rise to endocycling nurse cells, while the remaining cell is specified as the oocyte. GSCs thus provide a continuous source of oocyte precursor cells over the life of the female to ensure reproductive success. The molecular mechanisms that maintain mitotic divisions of the GSC and cystoblast and initiate oocyte differentiation, however, remain unclear. In a genetic screen designed to identify novel regulators of GSC self-renewal and germ cell division, we identified Transportin Serine-Arginine Rich (Tnpo-SR) as a key regulator of germline development. Tnpo-SR has structural domains similar to Importin β proteins and has been implicated in protein transport across the nuclear membrane; however, its cell biological role is not well understood. Here, we demonstrate that Tnpo-SR is necessary for proper cell cycle regulation in GSCs and their differentiating daughters. Tnpo-SR mutant germ cells exhibit longer cycle phases, and eventually fail to divide. Tnpo-SR mutant germ cells also grow unusually large, suggesting that cell cycle control is uncoupled from cell growth. Tnpo-SR mutant GSCs fail to self-renew, likely as a result of altered cell cycle control. Intriguingly, Tnpo-SR mutant cysts fail to specify an oocyte, instead forming 16 nurse cells. Further, loss of Tnpo-SR also blocks endoreplication of differentiated nurse cells, suggesting that Tnpo-SR regulates nucleocytoplasmic transport of a set of proteins that underlie multiple modes of cell cycle control. Taken together, our studies underscore the model that the cell cycle and cell differentiation are inextricably linked, and suggest that Tnpo-SR may participate in the molecular mechanisms that regulate this balance in the mitotically dividing germ line. Future studies will address the mechanism by which Tnpo-SR dictates these functions, providing further insight into the control of cell proliferation and fate.

64 Development of a CRISPR-based meiotic double-strand break repair assay provides insight into regulation of meiotic recombination. Nicola Crown, Jeff Sekelsky Integrative Program for Biological and Genome Sciences, University of North Carolina Chapel Hill, Chapel Hill, NC.

An organism's genome must be accurately replicated and packaged into a new cell to faithfully propagate genetic material from one generation to the next. Aneuploidy, the state of having too many or too few chromosomes, is a leading cause of developmental defects (e.g. Down's syndrome), miscarriages, and failed pregnancies using assisted reproductive technology; therefore, reproductive success is completely dependent on accurate chromosome segregation. During meiosis, accurate chromosome segregation is ensured by using recombination to create crossovers (COs) between homologous chromosomes. Recombination is initiated by a DNA double-stranded break (DSB) that can be repaired either as a CO or a noncrossover (NCO) through a series of structural intermediates. The bifurcation in repair pathways is thought to occur early, likely at or before the time DSBs are formed, and the choice in repair outcome is a critical regulatory point in establishing the distribution of crossovers across the genome. We have developed a CRISPR-based DSB repair assay as a new tool for understanding how repair outcome is decided. This tool has allowed us to probe what happens to repair outcome when genes that are essential for CO formation are mutated, providing insight into the regulation of recombination.

65 The TORC1 inhibitor GATOR1 regulates early meiotic events during Drosophila oogenesis. Youheng Wei, Kuikwon Kim, Lucia Bettedi, Mary A. Lilly National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Meiosis must accomplish two seemingly incompatible goals. First, it must faithfully copy and distribute genetic material to the next generation. Second, it must promote genomic diversity through meiotic recombination, a process initiated by the production of DNA double-stranded breaks. In Saccharomyces cerevisiae, an essential step in the transition to the meiotic cycle, as well as early meiotic progression, is the down-regulation of the nutrient-sensitive target of rapamycin complex 1 (TORC1) by the GTPase-activating proteins toward Rags 1 (GATOR1) complex in response to amino acid starvation. We have determined that this conserved nutrient stress pathway has been incorporated into a developmental program that regulates early meiotic events in metazoans. In Drosophila, the GATOR1 complex inhibits TORC1 activity to slow cellular metabolism and drive the mitotic/meiotic transition in developing ovarian cysts. Here we report that the GATOR1 complex promotes genomic stability through the regulation of meiotic double-stranded breaks in Drosophila and mouse. In Drosophila GATOR1 mutant ovaries, increased TORC1 activity results in an increase in the steady-state number of meiotic double-stranded breaks and the hyperactivation of p53 in the female germ line. Notably, epistasis analysis indicates the hyperactivation of p53 in GATOR1 mutants is downstream of meiotic double-stranded break machinery. Additionally, we determined that GATOR1 mutant embryos are sensitive to gamma-irradiation. Taken together our data support the model that the TORC1 inhibitor GATOR1 facilitates the repair of meiotic double-stranded breaks and suggest a link between metabolism and genome stability during meiosis.

66 Mitochondrial Fragmentation Drives the Selective Removal of Deleterious Mitochondrial DNA in the Drosophila Germline. T. Hurd, T. Lieber, R. Lehmann 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Skirball Institute, Department of Cell Biology, NYU School of Medicine, New York, NY, USA.

Mitochondria are unusual among animal organelles in that they contain their own genomes. Unlike nuclear genomes, mitochondrial genomes are inherited only maternally, are subject to a high mutation rate and undergo little recombination. Therefore, if left alone deleterious mutations would accumulate from one generation to the next. However, special selection mechanisms exist in the female germline to prevent this accumulation. Importantly, while strong purifying selection against
mutant mitochondrial DNA (mtDNA) exists in the germline, it is largely absent in the soma. Hence, somatic mtDNA mutations often accumulate throughout life, causing severe disease in humans. Remarkably, despite its fundamental scientific and medical importance, the molecular mechanisms underpinning mtDNA selection remain poorly understood. Here, using an allele-specific fluorescent in situ hybridization approach to distinguish wildtype from mutant mtDNA, we have visualized germline mtDNA selection for the first time. Selection first manifests in the early stages of Drosophila oogenesis, specifically in differentiating germline cysts. We find that just prior, there is a dramatic decrease in mitochondrial fusion, induced by a reduction in the levels of the pro-fusion protein Mitofusin (also known as Marf in Drosophila). We show that the resulting fragmented phase is necessary to isolate mitochondria and prevent them from sharing their contents, which in turn reduces product complementation and allows mitochondria harboring mutant genomes to be selected against. Remarkably, not only is this prolonged fragmented phase necessary for selection in germline tissues, but promoting fragmentation is also sufficient to induce selection in somatic ovarian tissues where selection otherwise does not appreciably occur. Our results demonstrate that the key distinction underlying the female germline’s capacity to select against deleterious mitochondria is a developmentally regulated period of isolation that germline mitochondria undergo during early oogenesis. Mutations in mtDNA are increasingly being recognized as a major cause of human disease. Understanding how the germline isolates mitochondria to select against deleterious mtDNA mutations may allow for the development of somatic therapies to treat those suffering from mtDNA disorders.


Over the past three decades our knowledge of genes has expanded at a tremendous pace. Synthetic biology is an emerging field of biology that aims to apply this knowledge to building new biological systems by rewiring genetic circuitry. While current synthetic biology is largely limited to microorganisms, harnessing multicellular animals will no doubt provide an exciting opportunity to build more complex circuits in a multicellular context. Here I report my first attempt at synthetic biology using Drosophila melanogaster. I developed transgenic flies that carried biosynthetic pathways of four vitamins: β-carotene and vitamins B2, B5 and B6. Each of the pathways comprised two to eight enzymes to synthesize a vitamin from a common metabolite. Flies expressing these pathways indeed produced substantial amounts of each vitamin and were able to develop and survive on vitamin-deficient diets. Notably, the body color of β-carotene-producing flies was as orange as carrots. The present results suggest that it is a feasible strategy to use genetically engineered insects as a factory of organic compounds. It is also interesting to speculate that certain nutrient deficiencies may be cured by gene therapy just as I did for Drosophila.

68 An expanded toolkit for CRISPR/Cas9 gene editing that complements MiMIC drive strategies. D. Li-Kroeger1,3, S. Cowan1, M. Jaiswal1, Y. He3,4, H. Bellen1,2,3,4 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 3) Neurological Research Institute, Houston, Texas; 4) HHMI, Baylor College of Medicine; 5) Rice University, Houston Tx; 6) TIFR Centre for Interdisciplinary Sciences, Hyderabad, India.

The versatile MiMIC system for disrupting, tagging and manipulating endogenous genes relies on recombination mediated cassette exchange to replace a MiMIC cassette with a SA-GFP-SD tag. This allows one to assess protein distribution as well as conditionally remove the endogenously tagged protein using the deGradFP. It also permits insertion of the SA-T2A g4a-polyA sequence to drive any UAS transgene from the endogenous enhancers and promoters of the tagged locus. A limitation of MiMIC and its CRISPR/Cas9 MiMIC (CRiMIC) counterpart is that suitable coding introns for insertion of the SA-GFP-SD need to be present. Unfortunately, 30% of genes cannot be targeted with this technology as the introns are too small and about 20% of those that can be tagged with GFP produce non-functional proteins. We therefore developed an efficient replacement strategy of the entire endogenous DNA sequences to create loss-of-function alleles with a simple dominant yellow wing marker. Once made, the visibly marked loss-of-function lines allow efficient, scarless cassette swapping to incorporate any DNA sequence desired with reasonable frequency using CRISPR/Cas9 based Homology Directed Repair. Additionally, we provide a series of donor vectors to streamline cloning for incorporation of protein tags and Gal4 via this cassette swapping strategy. We demonstrate the usefulness of this system in various applications including structure function analysis and replacement of loci with orthologous human genes and their putative disease causing variants. Hence we provide an expanded toolkit for CRISPR/Cas9 gene editing that can target virtually any location in the genome for precise genetic modification as long as the genes are smaller than 5kb, which is almost always the case for genes with small or no introns.

69 Pooled-format, genome-wide CRISPR/Cas9 screening in Drosophila cells. Raghuvar Viswanatha1, Zhongchi Li1,2, Claire Hu1, Norbert Perrimon1 1) Department of Genetics, Harvard Medical School; 2) School of Pharmaceutical Sciences, Tsinghua University.

In a pooled-format genetic screen in cultured cells, each cell receives one reagent (e.g. sgRNA) at random from a complex library of reagents. Application of pooled screening in mammalian cells has provided a powerful method for the identification of fitness-essential genes and mutations causing drug resistance. Despite the availability of numerous insect cell lines and
An apparatus for automated, high-throughput, and detailed assessment of individual Drosophila free behavior. W.R. Williamson, M. Peek, P. Breads, B. Cooper, G.M. Card, Janelia Research Campus, Ashburn, VA.

Recent advances in Drosophila genetics facilitate silencing or activation of small groups of neurons. Such manipulations can elucidate neuronal circuitry involved in normal behavior. However, resulting phenotypes may evade detection by conventional methods when occurring at small spatial or temporal scales. Detection may also require many trials due to incomplete penetrance or stimulus specificity. To solve these problems, a device should assay fly behavior quickly and automatically using a flexible stimulus and recording individual fly responses with high-speed video at high magnification. Here, we present FlyPEZ, a device for quantifying visually-driven behaviors. In addition to visual stimuli, FlyPEZ can activate neurons optogenetically with multiple light patterns. As proof of principle for identifying detailed behavior with FlyPEZ, we characterize head and body motion during the optomotor response, model the input-output function for directional jumping in response to a looming stimulus, and discover a new loss-of-function phenotype, which occurs on a millisecond timescale, from genetic silencing of a single visual projection neuron type.

Long-term optical brain imaging in live adult fruit flies. C. Huang¹, J. Maxey¹, S. Sinha¹, J. Savall¹, Y. Gong¹, M. Schnitzer¹, ² ¹James H. Clark Center, Stanford University, Stanford, CA; ²CNC Program, Stanford University, Stanford, CA; ³Howard Hughes Medical Institute, Stanford University, Stanford, CA; ⁴Department of Biomedical Engineering, Duke University, Durham, NC.

Time-lapse in vivo microscopy studies of cellular morphology and physiology are crucial for understanding brain function and plasticity, but long-term imaging in the fly brain, a key model species, has been infeasible. To clear this hurdle, we used laser microsurgery to create a chronic fly preparation for imaging neural architecture, calcium and voltage dynamics for up to 50 days. After surgery, ninety percent of male flies survived ten or more days, and female flies had statistically indistinguishable lifespans from those of control flies that never had surgery. Control studies indicated that our surgical protocol has little to no effect on fly locomotor or odor-avoidance behaviors. To illustrate the utility of our methods, we tracked axonal boutons in the MBON-a3 mushroom body neuron for ten days. We also imaged odor-evoked calcium transients in mushroom body neurons and spontaneous spiking in a specific dopamine neuron for up to seven weeks. Further, by using long-term voltage imaging to resolve individual action potentials, we tracked spiking plasticity in dopamine neurons of flies that experienced mechanical stress. In flies subject to 24 hours of stress, PPL1-a3 but not PPL1-a2 dopamine neurons had higher spike rates than in unstressed control flies. Overall, our chronic preparation is compatible with a variety of recent optical imaging and optogenetic methods for use in head-fixed or freely moving flies. By combining our approach with recently developed robotic methods for automated fly handling, it might be possible in the future to incorporate long-term intravital imaging into large-scale genetic screens based on assessments of fly neurophysiological or behavioral traits. Given the powerful genetic toolkits that already exist for use in fruit flies, our preparation for long-term imaging immediately opens new opportunities to investigate the molecular and cellular mechanisms of how individual neurons respond over time to environmental influences, change across the adult fly lifespan, or alter their properties due to brain disease.

PhotoGal4: a new multi-purpose light-dependent switch for spatiotemporal control of gene expression. L. De Mena¹, P. Rizk¹, C. Cruz¹, P. Trejo¹, J. Nedimyer¹, P. Fernandez-Funez², D.E. Rincon-Limas¹ ¹Neurology, University of Florida, Gainesville, FL; ²Biomedical Sciences, University of Minnesota, Twin Cities, MN.

Tools that enable manipulation or perturbation of gene function in a spatiotemporal manner are critical to define its contribution to normal development and disease. Unfortunately, current inducible expression systems in flies preclude accurate spatiotemporal control of gene expression and do not allow for sub-territorial manipulations within a given tissue. What if transgene expression could be manipulated a la carte with a switch triggered by light? To address this question, we developed a new and powerful photoactivatable gene expression system in Drosophila referred to as PhotoGal4. The light “switch” itself is a sensitive and reversible photosensor called phytochrome B (PhyB), a cytoplasmic chromoprotein that...
controls growth and development in plants. In response to red light, PhyB is activated and moves to the nucleus, but it returns to the inactive state under far-red light. Thus, we assembled a single protein device consisting of several unrelated modules, based on the heterodimerization of PhyB with its cofactor Pif6. To test the system, we capitalized on the well-characterized GMR enhancer to drive specific expression to the Drosophila eye territory. Thus, we engineered flies containing all the elements required to induce transcription of genes by light, and crossed them with a UAS-GFP reporter line to test PhotoGal4 functionality. We found that upon red light stimulation, PhotoGal4 efficiently triggers gene expression in long-term ex vivo cultures of eye discs at different developmental stages. We also found that manipulation of light intensity and duration of the stimuli gives control over reporter dose response. Then, we used a 2-photon microscope and a digital micromirror device (DMD) to specifically illuminate a defined group of cells within the GMR expression domain, while keeping the rest of the GMR territory in the dark. Strikingly, we found robust GFP expression only within the restricted area of illumination. To our knowledge, this is the first time that a targeted personalized sub-pattern of gene expression is induced in a light-dependent manner within time and space dimensions. Thus, we anticipate that PhotoGal4 will be a valuable resource for the Drosophila community to investigate complex and multistage biological, developmental and pathological processes with unprecedented resolution. This work was supported by the NIH grant NS088866 to DERL and by an HHMI-LSRF postdoctoral fellowship to LDM.

73 Microgravity research platform for longitudinal and multigenerational studies in Drosophila. Eugene Boland1, Andy Kurk1, Carlos Chang1, Sharmila Bhattacharya2 1) Techshot, Inc., Greenville, IN; 2) NASA Ames Research Center, Mountain View, CA.

A unique automated husbandry system has been developed by Techshot, Inc. for use onboard the International Space Station - National Laboratory. This platform - called the Multi-use Variable-g Platform or MVP, consisting of 12 experiment modules on two independent carousels, allows an investigator to expose Drosophila to fractional gravity (0-1 xG), unit gravity (1 G), or hyper gravity (1-2 xG) within the same system and environment. The experiment modules, or specifically fly modules, consist of 3 independently controlled food cylinders and 2 flight chambers arranged in such a way as to direct two and a half generations to live automatically within the module. Both flight chambers have day and night video as well as fully programmable lighting.

Our initial validation flight in early 2018 should help open the door to the first obvious question of why Drosophila in space. One answer is the study of immune response. Spaceflight, specifically the microgravity environment, alters both cellular and humoral immune responses in humans. There is a suppression of cell-mediated immunity by reducing production and distribution of leukocytes and activity of natural killer cells, neutrophils and macrophages. Humoral effects include interruption of interferon and interleukin production. The fruit fly model is useful to tease out these mechanisms because it has a highly conserved innate immunity but lacks the cell-mediated acquired and adaptability of the human system although they can be primed to make them less susceptible to subsequent exposure. These traits will be tested with wild-type and immunosuppressed mutant strains at 0xg and 1xg for 34 days on orbit. Egg and larva samples from F1 and F2 generations will be preserved on orbit and live F2 flies and a second egg lay from F2 flies will be returned for both wild-type and mutant flies. Previous work [Marcu, et al., 2011] by much of the same team conducted aboard the Space Shuttle had less than half the time, smaller chambers and no on-orbit control produced the foundational work that we hope to build upon with this and future missions.

With this demonstration flight we hope to prove both the validity of the hardware and model for stress induced adaptation as measured in genetic and/or epigenetic markers upon return of live or preserved specimens.


74 High-throughput System for Quantification of Food Consumption in Drosophila. M.D.LA Jaime1, S. Karott1, M. Garmendia-Cedillos2, T.J. Pohida2, B. Oliver1 1) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD; 2) Signal Processing and Instrumentation Section, Center for information Technology, NIH, Bethesda, MD.

One concern when performing screens where flies are fed, are changes in intake due to palatability or individual variability. In order to detect these differences it is important to accurately quantify intake. Traditionally, quantification of food consumption is performed on a small set of animals, ranging from individual to 10s of animals, but as new high-throughput whole animal screening systems are developed there is a need for an equally high-throughput method for quantifying consumption. For example, our previously described Whole Animal Feeding Flat (WAFFL), can house and feeds flies in a 96-well format. The WAFFL uses liquid chemically defined food (CDF) that can be accessed through pores in the bottom of the plate, and allows up to 96 individual diets to be fed simultaneously, and diets can be change simply by moving the WAFFL to a different food plate. In order to quantify intake in this system we developed a novel high-throughput method “WAFFL Insect Excrement Removal Nano-brushes system” (WAFFL IERN). The WAFFL IERN is a handle with 96 brushes to enable the removal
of the excrement left by the flies that underwent screening in the WAFFL. This system enables us to quantify how much food transited through the fly during a certain period. We use sulforhodamine B, a non-toxic fluorescent dye, as a dietary marker added to the CDF. This allows us to quantify food consumption by measuring fluorescence. We can accurately calculate the total amount of food ingested by the animal by adding the fluorescence measured inside the fly (after maceration) and the fluorescence in the excrement. For example, we quantified difference in consumption between male (n=144) and female (n=144) flies. We found that on average female flies ingested 2.09 μl and males 1.84 μl of CDF over 24 hr time period. The WAFFL IERN and WAFFL provide a convenient and accurate high-throughput method to quantify food ingestion in large scale diet based experiments.

75 Tissue Organization in a Small Multicellular Structure. Jasmin Imran Alsous, Matej Krajnc, Tomer Stern, Rocky Diegmiller Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

The three-dimensional organization of growing structures that are comprised of several cell types is a defining feature of embryonic development. However, the number of tractable experimental systems that can be used to explore these collective dynamics is still very limited. The Drosophila egg chamber – a precursor to the oocyte and a multicellular structure with two distinct cell types is such a system. Each egg chamber is composed of 16 stereotypically-connected germline cells that are enveloped by a follicular epithelium. At its youngest, the egg chamber has ~ 50 follicle cells enveloping the germline cells. However, as the 16-cell germline cluster grows by ~4 orders of magnitude through biosynthesis and uptake of extracellular material, the follicle cells undergo several mitotic divisions and expand to maintain the intact envelopment of the germline cells: at its largest, the germline cluster is enveloped by ~ 1,000 follicle cells. How is the growth of these two tissues regulated? Studies of the mechanics of proliferating epithelia have shown that stretching induces cell division, suggesting that epithelial proliferation is largely driven by growth of the underlying germline cluster. However, studies of mutants suggest that follicle cells play a critical role in regulating the growth of the germline cluster: egg chambers with AMPKa mutant follicle cells are disproportionately larger than more posterior, hence older egg chambers. Furthermore, while our previous work has quantified the spatiotemporal growth pattern of the germline cluster, thus revealing the allometric growth of its cells, it is unclear how this growth is coordinated with that of the overlying soma. To address this problem, we have developed experimental approaches for the collection and analysis (segmentation and single cell counting of epithelia) of 3D images of growing egg chambers. We will be using a recently developed computational framework, the 3D Dynamical Vertex Model, to model the evolving cell packing on curved surfaces. In addition to distinguishing between the two scenarios outlined above, the mathematical model will have utility beyond the chosen experimental system in addressing how mechanical forces interplay with genetically predetermined developmental pathways.

76 Repression of a CDK1 - Myb - Aurora B network remodels mitotic cycles into polyploid endocycles. M. Rotelli1, S. Chen1, R. Policastro1, G. Zentner1, C. Walczak2,3, M. Lilly4, B. Calvi1,3 1) Dept of Biology, Indiana University, Bloomington, IN; 2) Medical Science, Indiana University, Bloomington, IN; 3) Melvin and Bren Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN; 4) National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

The endocycle is a cell cycle variant that entails periodic duplication of the genome without cell division, which results in large polyploid cells. Specific cell types switch from mitotic cycles to endocycles during development. Cells also switch to endocycles during wound healing, in response to stress, and in cancer. It is still not well understood, however, how cell cycle remodeling regulates the switch from mitotic cycles to endocycles in development and disease.

To investigate how the cell cycle is remodeled, we created induced endocycling cells (iECs) by knockdown of the mitotic Cyclin A (CycA) in S2 cells, and compared them to control mitotic cycling S2 cells. We found that Cyclin A knockdown iECs have dampened expression of genes that are required for mitosis and normally induced by the Myb transcription factor at G2/M. This result is similar to developmental endocycling cells in Drosophila tissues, which we previously showed also have a dampened Myb transcriptome. These findings suggest that inhibition of a CycA / CDK1 – Myb network promotes the transition from mitotic cycles to endocycles in both iECs and in development. In support of this model, knockdown of Myb in S2 cells and fly tissues repressed mitotic gene expression and induced endocycles. Moreover, RNA-Seq analysis showed that CycA and Myb knockdown iECs have in common a reduced expression of a large cadre of genes that are normally induced by Myb at G2/M and that are required for multiple steps of mitosis and cytokinesis. We found that knockdown of one Myb target, Aurora B (AurB), was sufficient to induce endocycles in both S2 cells and fly tissues.

Treating human cells with CDK and AurB kinase inhibitors also induced endocycles, suggesting that the status of a CDK1 - Myb - AurB mitotic network is relevant to the transition from mitotic cycles to endocycles in humans. Upon drug washout, these human iECs returned to a highly error prone mitosis, which resulted in genome instability and acquisition of resistance to chemotherapeutics. This result raises the possibility that CDK1 and AurB kinase inhibitors that are being used in the clinic may actually promote therapy resistance of some cancer cells. Altogether, our study has defined a CycA / CDK1 - Myb - Aurora B mitotic network, repression of which enforces the transition to endocycles in both development and disease.
77  **Variant cell cycles ensure a functional blood-brain barrier in Drosophila.**  J.R. Von Stetina¹, L.E. Frawley¹, Y. Unhavaithaya², T.L. Orr-Weaver¹  1) Orr-Weaver Lab, Whitehead Institute, Cambridge, MA; 2) Decision Resources Group, Boston, MA.

How different tissues coordinate growth during organogenesis is a poorly understood process. In many tissues, large cells are required, and this is achieved by increased ploidy. Previous studies from our laboratory demonstrated that subperineurial glia (SPG) use polyploidization to enlarge cell size to accommodate the increasing underlying neuronal mass during brain growth, thus maintaining the integrity of the BBB, without disruption of septate junctions by cell division and cytokinesis [1]. We also showed that the SPG are unique cells, as they increase their size via two variant cell cycles, the endocycle that produces polyploid cells with one nucleus and endomitosis, which results in multinucleate cells. By exploring why both the endocycle and endomitosis are used by the SPG we have found that: 1) the transition into endomitosis is developmentally controlled; 2) Notch signaling and Cdc25/String phosphatase activity are required for the proper endocycle to endomitosis ratio in SPG and a functional BBB; and 3) SPG in the brain exploit endomitosis to boost their cell size above that attainable by the endocycle in response to substantial brain growth during larval development. In addition, we have made the unexpected finding that nuclear number rather than ploidy alone also affects cell size. We are currently investigating how the multicellularity observed in endomitotic SPG leads to bigger cells by looking at global transcription in individual nuclei within multinucleate SPG and by altering the normal distribution of nuclei in these endomitotic cells. Understanding the regulation and function of ploidy in SPG will shed light not only into how the BBB-forming glia control the underlying neurons during normal brain development, but also into the fundamental differences between mononucleate versus multinucleate polyploid cells.


78  **JNK and Yorkie cooperate to drive tumor progression by generating polyploid giant cells in Drosophila.**  B.J. Cong, S. Ohsawa, T. Igaki  Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Kyoto, JP.

Epithelial cancer tissues often possess polyploid giant cells, which are thought to be highly oncogenic. However, the mechanisms by which polyploid giant cells are generated in tumor tissues and how such cells contribute to tumor progression in vivo remain elusive. Here, we found in Drosophila imaginal epithelium that clones of cells deficient for endocytic 'neoplastic tumor-suppressor' genes such as rab5, vps25, erupted/tsg101, or avalanche/synaptotagmin-7 induce polyploid giant cells. Our genetic analyses of Rab5-deficient cells revealed that cooperative activation of Eiger-JNK and Yorkie generates polyploid giant cells via endoreplication. Furthermore, we found that malignant tumors induced by Ras activation and cell polarity defect also include polyploid giant cells. Strikingly, elimination of polyploid giant cells from such malignant tumors by blocking endoreplication strongly suppressed tumor growth and metastatic behavior. The mechanism by which polyploid giant cells are generated in tumors will be presented.

79  **Investigating the interaction of inflammatory pathways in tumor microenvironment using Drosophila cancer models.**  K Snigdha¹, A Singh¹,²,³, M Kango-Singh¹,²,³  1) Department of Biology, University of Dayton; 2) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton; 3) Premedical Programs, University of Dayton.

Tumor microenvironment (TME) consists of the tumor cells and the surrounding normal cells and plays a critical role in tumor survival and progression. Previous studies have shown presence of inflammatory components in the TME but how these components affect tumor progression it is still not well understood. The core inflammatory pathways like TLR, TNF etc. produce these components and are conserved in Drosophila. Since 90% of tumors are epithelial in origin, we used the wing imaginal disc of Drosophila melanogaster to study the interaction of these key inflammatory pathways in the TME. To model the TME, we created FLP-out clones of co-activated oncogenic forms of Yki or Ras¹² in polarity deficient (scribble mutant) cells marked by GFP surrounded by normal cells. These mosaic clones allow us to test changes in intercellular and signaling interactions within the tumor, surrounding its microenvironment and in distant normal cells. This clonal system recapitulates the clonal origins of human cancer and provides a distinct advantage in analyzing intratumoral and other interactions that affect tumor growth. Using immunohistochemistry, we studied the activity of TLR, TNF and JNK pathway. TLR component, Cactus and activated form of JNK, p-JNK were induced in the tumor cells whereas Drosophila TNF ligand, Eiger was upregulated in the surrounding normal cells. We hypothesized that a crosstalk between these key pathways in the TME promotes tumor survival and progression. So we decided to study the genetic epistasis interaction between JNK and TNF pathways to assess the requirement and roles of these signals in the TME. Our preliminary data suggest specific and significant roles for the TNF and JNK pathways. We are currently testing if TLR, TNF and JNK pathway genetically regulate each other or independently affect the TME and growth of tumors. Our progress from these studies will be presented. Our research will help unravel the correlation between inflammatory pathways and tumor progression in an in vivo model.

80  **Deciphering the complexity of oncogenic Ras signaling.**  C. Chabu¹, Da-Ming Li², Tian Xu²  1) University of Missouri, Columbia, MO; 2) Yale University School of Medicine/HHMI, New Haven, CT.

Oncogenic Ras signaling is complex and involves multiple interacting signals that concertedly drive disease progression in
ways we do not understand. Animal models have played an instrumental role in the dissection of complex signaling dynamics. We took advantage of our Drosophila Ras tumor model to address two puzzling observations related to oncogenic Ras signaling: on the one hand, oncogenic Ras signaling requires its upstream receptor, epidermal growth factor receptor (EGFR) in some cancers, which cannot be explained with our understanding of canonical EGFR/Ras signaling. On the other hand, oncogenic EGFR/Ras signaling cooperates with hedgehog (Hh) signaling in cancers. The underlying mechanisms for both of these observations are not well understood. We found that oncogenic Ras stimulates the transcription and secretion of EGF to recruit EGFR function. Rather than signaling via the known canonical EGFR signaling pathway, EGFR acts via the small G-protein and vesicle trafficking regulator ADP-Ribosylation Factor 6 (ARF6). ARF6 stabilizes Hh molecules on signaling competent endosomes, thereby stimulating Hh signaling in oncogenic Ras cells and establishing oncogenic synergy. Consistent with this mechanism, inhibition of EGFR or ARF6 causes Hh protein to be routed to degradation pathways. In addition, depleting EGF or blocking EGFR function suppresses oncogenic Ras tumor overgrowth. More importantly, ARF6 or Hh knockdown prevents oncogenic Ras-mediated overgrowth in both fly and human cancer cells. This work provides the first unifying mechanism that explains both, the surprising role for EGFR in oncogenic Ras-mediated overgrowth and the oncogenic cooperation between EGFR/Ras and Hh signaling. More broadly, the work illustrates how one oncogenic mutation can, independent of any additional genetic lesions, trigger and incorporate other oncogenic signals in cancer cells to promote robust tumor growth.

81 The CAF-1 complex couples Hippo pathway target expression and cell cycle progression. W.B. Yee1, P.M. Delaney1, P.J. Vanderzalm1, R.G. Fehon1 1) Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL; 2) Department of Biology, John Carroll University, University Heights, OH.

The Hippo signaling pathway regulates tissue growth and organ development in vertebrates and invertebrates. Mutations in Hippo pathway components lead to tumorigenic diseases. For example, mutation of the human Neurofibromatosis 2 gene (called Merlin in Drosophila) leads to Schwann cell-derived and other tumors. Upstream components including Merlin interact to regulate a kinase cascade that controls the localization and thus activity of the transcriptional co-activator Yorkie. Through a genetic screen, we find that depletion of a CAF-1 complex subunit, p180, leads to increased Hippo pathway target gene expression. The CAF-1 (Chromatin Assembly Factor-1) complex functions in replication-dependent histone deposition. Our subsequent experiments have shown that the CAF-1 complex preferentially regulates Hippo pathway target expression in a cell cycle and Yorkie-dependent manner. We propose that CAF-1 and Yorkie function antagonistically to interact with naked, post-replication DNA. In the case of low Yorkie activity, CAF-1 quickly re-establishes the histone landscape on newly synthesized DNA to prevent Yorkie interaction with target loci. Interaction between Yorkie and its target loci leads to not only increased Hippo pathway target transcription but also changes in the epigenetic landscape that further enhance transcription. These observations suggest a connection between cell cycle progression and Hippo pathway target expression, enhancing our understanding of Hippo pathway regulation. Since both the CAF-1 complex and Hippo signaling are highly conserved, we expect that understanding this mechanism in flies will lead to new insights into the mammalian Hippo pathway and its role in human tumorigenesis.

82 Genetic analysis of invasive pathways engaged by the ECR-coactivator protein Taiman reveals requirement of Toll/Imd pathways and systemic immune response. P.K. Byun, C Zhang, KH Moberg Department of Cell Biology, School of Medicine, Emory University, Atlanta, GA.

In developing tissues and metastatic cancers, cells often migrate as a heterogeneous group in response to internal and external cues. In the Drosophila ovary, the steroid receptor transcriptional co-activator taiman (taim) promotes collective migration of border cells (BCs) through the adjacent cluster of nurse cells in response to the hormone ecdysone, but the mechanisms underlying this invasive effect are not well defined. Here we introduce a pathogenic model of Taim-driven tissue invasion that allows for rapid genetic screening for elements of the Taim-induced invasive program. Overexpression of taim in non-motile pupal wing cells causes them to invade through adjacent thoracic cuticle and into internal tissues, leading to an easily scored phenotype of wing-tips embedded into the thorax. Using a coupled approach of a dominant-modifier screen and RNA-seq analysis, we identify the Toll and innate immunity pathways as necessary for Taim-driven wing invasion. Expression of Taim in wing cells leads to a systemic immune response in larvae and pupae. Multiple components of the Toll and IMD pathways are overexpressed in Taim-wing cells and alleles of Toll/IMD components, such as Spatzle ligands (spz), dl, myd88, dominantly suppress Taim-driven wing invasion. Taim-invasion is also suppressed by the H99 deletion, which removes the pro-apoptotic RHG genes; in parallel, thoracic cells abutting Taim-expressing cells show increased hid expression. These data suggest that Taim overexpression can convert non-motile wing epithelial cells into an invasive group of cells by activating the innate immune system. More detailed mechanistic insight into the role of Toll/Imd signaling in this Taim-driven invasive model will be presented.

83 Integrins act as mechanosensors to regulate cell survival in Drosophila wing imaginal disc. A. Valencia Expósito1, M.J. Gomez-Lamarc2, T.J. Widmann3, M.D. Martin-Bermudo1 1) Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide/CSIC, Sevilla, ES; 2) Department of Physiology, Development and Neuroscience, University of Cambridge, England,
than the pouch, indicating that destabilization of established patterns of selector gene expression may occur more readily in some

posterior clones. Downregulation and ectopic expression of Ci are more apparent in clones in the hinge of the wing disc

surrounding wild-type tissue. The posterior clones also express patterni

the anterior selector gene Cubitus Interruptus (Ci), and anterior Yki

outside their normal expression domains. Strikingly, a subset of Yki

disc. Clones of cells expressing a constitutively active form of Yorkie (Yki

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in filaments, via a variety of cytoskeletal linker proteins, integrins can sense and transmit contractile forces derived from the ECM or from inside the cell. This mechanosensing ability is key to regulate a wide range of signaling pathways and genetic programs to control cell survival, adhesion, migration, proliferation and differentiation. However, despite the implication of integrins in mechanosensing and mechanotransduction, we are still at the beginning of understanding its importance in development and in overall physiology. In this work we have used Drosophila

wing imaginal disc as a model system to better understand the role of integrins as tension sensors during epithelial morphogenesis. We show that reducing integrin levels in the wing disc results in caspase-dependent anoikis, cell extrusion and ectopic folding of the epithelium. Moreover, although previous experiments have suggested that cell death results in tissue shape changes in wing disc, we find that blocking cell death in integrin mutant cells does not prevent the appearance of ectopic folds and cell extrusion. These results suggest that integrins regulate epithelial architecture independent of cell death. In fact, we have seen that elimination of integrin function results in increased actomyosin activity. Likewise, we show that increasing myosin activity per se phenocopies loss of integrin function. Previous work has demonstrated that an increase in RhO1/Rok/Myosin activity results in JNK activation. In our work we show that removal of integrins also results in JNK activation. Furthermore, downregulation of JNK activity rescues all integrin phenotypes. Altogether these results support a role for integrins in balancing internal actomyosin forces necessary for the maintenance of the epithelial morphology through the regulation of JNK activity.

84  Ecdysone limits wing imaginal disc regeneration through Broad Z1.  Faith Karanja, Sara Weintraub, Subhashri Sahu, Adrian Halme Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

As tissues develop, their regenerative capacity is often diminished. For instance, Drosophila melanogaster imaginal discs lose regenerative capacity at the end of larval development. The timing of when discs lose regenerative capacity coincides with the rapid increase in systemic levels of the steroid hormone ecdysone, a key coordinator of Drosophila developmental progression. We have shown that increasing systemic ecdysone levels by feeding larvae ecdysone is sufficient to limit regeneration in their imaginal tissues. Based on these data, we hypothesized that ecdysone signaling promotes changes in the imaginal disc epithelium that interfere with activation of regeneration pathways. We find that the broad splice variant Z1 (br-Z1), an early transcriptional target of the pre-pupal ecdysone peak, begins to be expressed in the wing imaginal disc at the same time that discs lose regenerative capacity. The expression of Br-Z1 inversely correlates with the regenerative capacity of the wing imaginal disc. When regeneration competent imaginal discs are damaged, Br-Z1 expression is delayed, which is consistent with the extension of the regenerative period in these larvae. In contrast, feeding the larvae ecdysone, which limits regenerative capacity, produces premature Br-Z1 expression. To determine directly whether Br-Z1 limits regeneration we overexpressed Br-Z1 early in the imaginal disc development and found it is sufficient to cell-autonomously suppress the expression of Wingless (Wg) and Dilp8 after damage. Inhibition of broad expression in late third instar imaginal discs using broad RNAi allows tissues to activate regenerative pathways past the known regeneration restriction point. Our preliminary data suggests that Br-Z1 may be functioning downstream of the JNK signaling pathway to regulate Wg expression in damaged tissues. In summary, these results provide insight into how endocrine signals act on tissues to regulate their regenerative competence. We are currently exploring whether ecdysone signaling also functions to shape the regenerative response and determine its duration through differential expression of broad splice variants.

85 Regulation of the stability of selector gene expression by the Hippo pathway co-activator Yorkie.  J. Downes, I. Hariharan Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Differentiated cells that become cancerous do so by inappropriately altering gene expression to allow for proliferation, evasion of the immune system, and invasion of surrounding tissue. This can cause the cells to assume a more stem-like state, but can also lead to aberrant expression of developmental patterning genes normally never seen in that cell's lineage. How tumors alter patterning gene expression and what role these patterning genes play in tumor growth and progression remains unclear. Since alterations of the Hippo pathway are observed in cancers, we examined the impact of increasing the activity of Yorkie (Yki), the transcriptional co-activator downstream of the Hippo pathway on cell fate determination in the wing-imaginal disc. Clones of cells expressing a constitutively active form of Yorkie (YkiCA) ectopically activate specific patterning genes outside their normal expression domains. Strikingly, a subset of YkiCA clones in the posterior compartment begin to express the anterior selector gene Cubitus Interruptus (Ci), and anterior YkiCA clones appear to express even higher levels of Ci than surrounding wild-type tissue. The posterior clones also express patterned genes downstream of Ci (e.g. dpp), suggesting that YkiCA clones have non-cell-autonomous effects on patterning in the wing disc. We observe reduced expression of the posterior selector gene en (en) in posterior clones which can account for the ectopic expression of Ci at least in posterior clones. En downregulation and ectopic expression of Ci are more apparent in clones in the hinge of the wing disc than the pouch, indicating that destabilization of established patterns of selector gene expression occur more readily in some
regions of the wing disc than others. Interestingly, the hinge region has been previously postulated to be a “tumor hot spot” and is important for regeneration of the pouch, suggesting that increased cell-fate plasticity might underlie some of these properties. We also observe changes in regulators of chromatin in these clones that potentially explain the changes in En and Ci expression. Thus, while Yki is generally thought to affect tissue growth by regulating the transcription of specific regulators of growth and cell survival, our work links Yki to mechanisms that alter chromatin states and de-stabilize networks of selector gene expression.

86 The architectural balance of the Ventral Nerve Cord depends on the level of JNK signaling activity. K. Karkali1, G. Panayotou2, T. E. Saunders3, E. Martin-Blanco1 1) Instituto de Biologia molecular de Barcelona, CSIC, Barcelona, ES; 2) BSRC Alexander Fleming, 34 Fleming Street, 16672 Vari, Greece; 3) Mechanobiology Institute, 5 Engineering Drive 1, National University of Singapore, 117411 Singapore.

The segmented nervous system of bilaterians is organized in structural and functional modules. Modules share across species a robust structural stability. How this robustness is acquired during development is currently unknown. Here, we investigate the sequence of events involved in the establishment of the architectural balance of the nervous system. We demonstrate that a unique robustness pattern is common to the arthropods nervous system plan. This pattern depends on the fine control of the JNK signaling in a subset of early-specified pioneer neurons. JNK activity affects the level of expression of cell adhesion molecules (Fas 2), in part through the modulation of the transcription factor zfh1. A deficit in Fas 2 affects the fasciculation of the axons of primary neurons, leading to secondary bundling defects that result in a general reduction in spatial correlations. Failure to fasciculate affects both architectural robustness and tensional balance, ultimately impeding nervous system condensation.

87 Programmed cell senescence is required for sensory organ development in Drosophila. Y. Zang, M. Yoshimoto, T. Igaki Laboratory of Genetics, Kyoto University Graduate School of Biostudies.

Cellular senescence, a state of irreversible cell cycle arrest, is often associated with tumor suppression and aging. Despite the extensive studies on the mechanism of cellular senescence triggered by telomere shortening or oncogene activation in mammals, its physiological role has still been elusive. Here, we discovered that developmentally programmed cellular senescence occurs in Drosophila imaginal epithelium. In the wing discs of the 3rd instar larvae, a cluster of cells showed highly elevated senescence-associated β-galactosidase (SA-β-gal) activity, cell cycle arrest, and increased heterochromatinization, the hallmarks of cellular senescence. The cluster of cells also showed highly elevated Ras signaling as well as increased oxidative stress, both of which are known to trigger cellular senescence in mammals. Intriguingly, we found that these senescent cells co-localized with proneural clusters, which develop into the sensory organ ‘campaniform sensilla’ in the dorsal radius of adult wing. To understand the role of programmed cell senescence in wing development, we searched for cellular signaling that can block cellular senescence. Interestingly, we found that forced activation of wingless (Wg) signaling strongly suppressed SA-β-gal activity as well as cell cycle arrest in the senescent cell cluster. This suppression of programmed cell senescence resulted in morphological defects in the campaniform sensilla. Our data indicate that developmentally induced cellular senescence facilitates proper development of sensory organs in Drosophila wing.

88 Roles of tricellular junctions and spindle orientation in tissue morphogenesis. Eric van Leen, Isabelle Gaugué, Floris Bosveld, Yohanns Bellaiche Institut Curie, Paris, FR.

The orientation of cell division along the interphase cell long axis, the century old Hertwig's rule, has profound roles in tissue proliferation, morphogenesis, architecture and mechanics. In epithelial tissues, the shape of the interphase cell is influenced by cell adhesion, mechanical stress, neighbourhood topology, and planar polarity pathways. We have recently uncovered the mechanisms by which cells sense and memorize their interphase shape. We found that in Drosophila epithelia, tricellular junctions (TCJ) localize microtubule force generators, orienting cell division via the Dynein associated protein Mud (NuMa in vertebrates) independently of the classical Pins/Gai pathway. Moreover, as cells round up during mitosis, TCJs serve as spatial landmarks, encoding information about interphase cell shape anisotropy to orient division in the rounded mitotic cells. Our work revealed that, in addition to their function as epithelial barrier structures, TCJs serve as polarity cues promoting geometry and mechanical sensing in epithelial tissues (Bosveld et al., 2016). Yet it is still unclear how cell division orientation contributes to tissue scale morphogenesis. To address this question, we screened for proteins that recruit Mud to TCJ. We found that the TCJ protein Anakonda (Aka) interacts with Mud and is required for the localization of Mud at TCJs. Moreover, the intracellular domain of Aka mediates mitotic spindle orientation in S2 cells via Mud. Currently, we are evaluating how CRISPR/Cas9 generated mutants affect spindle orientation and the impact of cell division misorientation at the scale of the entire tissue undergoing morphogenesis.
89  **Cell Size and Nuclear Scaling Relationships in Multinucleated Muscle Fibers.**  S.E. Windner¹, A. Manhart², A. Brown¹, A. Mogilner², M.K. Baylies¹ ¹) Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, NY; 2) Courant Institute, New York University, New York, NY.

Optimal cell function depends on the size of a cell and the appropriate relative size, i.e. scaling, of its organelles. Specifically, scaling of the nucleus is characteristic for each cell type and reflects cell state and activity. Despite obvious correlations, we know very little about the intracellular mechanisms that regulate nuclear scaling to establish and maintain specific cell sizes. Cell size regulation is of particular interest for muscle biologists, as muscle size is directly linked to force production. However, individual skeletal muscle fibers are among the largest cells and can contain hundreds of nuclei, which poses challenges to investigating global nuclear scaling relationships.

Here we present a *Drosophila in vivo* system to analyze muscle cell size and nuclear scaling, genetically manipulate individual cellular components, and quantify muscle function. Our data show that the number of nuclei, as well as their cumulative DNA content increase linearly with muscle cell size. However, the cumulative size of all nuclei in the cell is the most precise global nuclear scaling parameter, indicating that size regulation is highly coordinated among all nuclei in a muscle syncytium. Advanced statistical analyses and mathematical simulations suggest that nuclei are positioned to equally distribute cytoplasmic space and adjust their size based on the distance to neighboring nuclei. However, the relationship between individual nuclei and their surrounding cytoplasmic domain (local size scaling) varies within each cell. Differences in nuclear size scaling correlate with changes in transcriptional/translational activity and are specific to cytoplasmic domains adjacent to the myotendinous and neuromuscular junctions. Thus, individual nuclei within a muscle syncytium distinctly regulate cell size.

We propose that muscle fibers are composed of a heterogeneous population of cytoplasmic domains, which are established by the integration of local signals and the coordination of size scaling with nuclear activity. Genetic manipulations to alter nuclear size and DNA content confirm these observations and further show that changes in muscle size or in nuclear size scaling correlate with reduced muscle function. Our study provides the first comprehensive approach to unraveling the intrinsic regulation of size and nuclear scaling in multinucleated muscle fibers. Ultimately, identifying the underlying molecular mechanisms will provide insights to how disruption of sub-cellular organization results in muscle disease.

90  **Quantitative analysis of cell organization in tracheal tubes reveals unexpected cell behaviors and suggests an alternative role for Src42 in tracheal morphogenesis.**  R. Yang¹, M. Mani², G.J. Beitel¹ ¹) Dept. of Molecular Biosciences, Northwestern University, Evanston, IL; 2) Dept. of Engineering Sciences and Applied Mathematics, Northwestern University, Evanston, IL.

The architecture of biological tubes must be tightly regulated for an organism's survival. Understanding the mechanisms of tube size control requires the ability to quantify many aspects of tube and cell geometry. Although programs to analyze planar epithelia exist, they are unable to process the more complex problem of cells forming highly curved 3D tubes. We therefore developed greatly improved computational tools for measuring cell and tube morphology in the *Drosophila* trachea. Whereas previous approaches measured tens of cells in several tracheal segments (Nelson et al., 2012; Forster et al., 2012), the new workflow measures hundreds of cells from entire tracheal tubes.

In this workflow, the tracheal dorsal trunk and the apical surfaces of tracheal cells are segmented from confocal images using Ilastik, a freely accessible image analysis platform. Segmentation data are then imported to Matlab, and a marching cubes algorithm is used to define the centerline along the dorsal trunk and detect branches. Cell boundaries are then mapped onto the tube surface, and cells are analyzed as objects in 3D directly and as projections in 2D by unrolling and flattening the tube into a plane. Importantly, the 2D projections are compatible with a broad array of analysis tools such as Voronoi modeling, which shows that unlike the regular hexagonal cells seen in planar epithelia, the apical surfaces of tracheal cells are primarily shaped as irregular pentagons.

Quantitative analysis of the expansion of trachea from a narrow diameter tube at stage 14 to a somewhat longer (20%) but much larger diameter (100%) tube at stage 16 revealed three unexpected findings. First, there is an anterior-to-posterior gradient of cell orientation along the dorsal trunk. Second, comparison of computationally modeled cell orientation changes based on tube expansion parameters to actual cell orientations in WT revealed that after expansion, cell orientation changed in the opposite direction from that predicted by passive expansion. In effect, WT cells rotate as if countering the radially expansive forces of luminal matrix, which could potentially redirect radial force to elongate the tube. Third, cell orientation in Src42 mutants was more similar to the predicted passive expansion than WT, suggesting that Src42 cells fail to reorient as much as WT cells. Thus, Src42 may be required for executing cell rearrangements rather than directing polarized growth in tracheal cells.

91  **Unc-4 governs the identity of cholinergic neurons in the ventral nerve cord.**  H. Lacin¹, J.W. Truman¹² ¹) HHMI Janelia Research Campus, Ashburn, VA; 2) Biology, University of Washington, Seattle, WA.

Every neuron in the nervous system is unique. Each synapses with a different set of neurons and express a unique combination of molecules. Ultimately, neurons of different fates come together to form functionally meaningful circuits,
which produce variety of behaviors. To investigate how such a complex system is assembled, we study the *Drosophila* ventral nerve cord (VNC). Like the mammalian spinal cord, the fly VNC is densely populated with neurons so studying its organization is challenging. To overcome this, we have taken a lineage based approach to divide the VNC into developmentally and functionally related units. Over the last decade, we have laid the groundwork: (i) We identified all the stem cell lineages that make up the nerve cord. (ii) We further divided each lineage into two hemilineages (A and B), which arise from the iterated asymmetric divisions of the stem cells. (iii) We identified transcription factors (TF) whose combinatorial expression identifies each hemilineage unambiguously. (iv) We generated genetic tools to visualize and manipulate neuronal classes within each hemilineage. (v) Lastly, we showed that hemilineages are major functional units of the VNC and responsible for a specific behavior. To further characterize functional organization of the VNC, we have mapped the neurotransmitter profile of mature adult neurons by using the genetic and molecular tools. Surprisingly, we found that neurotransmitter code in the VNC is simple: all neurons within a hemilineage use the same neurotransmitter. We identified 14 cholinergic, 12 GABAergic, and 7 glutamatergic hemilineages per thoracic ganglia (out of 33 major hemilineages). Moreover, we found that 7 of the 14 cholinergic hemilineages express Unc-4, an uncharacterized homeodomain-containing TF and its expression is restricted to these lineages. To investigate the Unc-4 function, we engineered the unc-4 locus and generated null and conditional mutants as well as a variety of reporter lines via CRISPR editing. Adult unc-4 mutant animals survive into adulthood but they are uncoordinated, flightless, and not able to jump. We analyzed morphology and neurotransmitter profile of each Unc-4+ hemilineage in the mutant background and found that loss of unc-4 function results in a switch from cholinergic to GABAergic fate and defects in axonal projections. We observed these phenotypes only in a few lineages. 7B, one of these lineages, fails project its axons to the leg neuropil in unc-4 mutant animals. To assay if defects in 7B are responsible for any of the behavioral phenotypes observed in null mutant animals, we removed unc-4 only in this lineage. The resultant animals showed jumping defects indicating 7B is required for jumping. Overall, our results demonstrate that Unc-4 function in cholinergic neurons is necessary for proper fate acquisition.

92 Phenotypic convergence in the brain: distinct transcription factors regulate common terminal neuronal characters. N. Konstantinides	extsuperscript{1}, K. Kapuralin	extsuperscript{2}, K. Kapuralin	extsuperscript{2}, L. Barbosa	extsuperscript{1,2}, R. Satija	extsuperscript{1,2}, C. Desplan	extsuperscript{1,2} 1) Department of Biology, New York University, New York, NY 10003, USA; 2) New York University Abu Dhabi, Saadiyat Island, Abu Dhabi, UAE; 3) New York Genome Center, New York, NY 10013, USA.

Transcription factors regulate the molecular, morphological, and physiological characters of neurons and are responsible for the generation of their impression cell type diversity. Neuronal diversity is generated along two temporal scales: a) by cell type diversification over hundreds of millions years of evolution and b) by implementing neuronal identity during embryonic development. Altering transcription factor expression can lead to the evolution of new cell types. Therefore, how transcription factors are recruited in diverse cell types to orchestrate their specification and differentiation is a key question in neurobiology. The Drosophila optic lobe is an ideal system to probe this process because of its large number of cell types that have been exhaustively described. We used Drop-seq, a large-scale single-cell mRNA sequencing technique, to characterize the extensive cellular diversity in the Drosophila optic lobes and identify general principles that govern how transcription factors regulate cell type diversity. We sequenced 55,000 single neurons and glia and pooled them in 52 clusters of transcriptionally distinct cells. We validated the clustering and annotated the clusters using RNA sequencing of FACs-sorted single cell types, as well as known and newly identified neuronal and glial markers. To identify transcription factors responsible for inducing specific terminal differentiation features, we used machine-learning to generate a random forest model. The predictive power of the model was confirmed by showing that two transcription factors expressed specifically in cholinergic (apterous) and glutamatergic (traffic-jam) neurons are necessary for the expression of ChAT and VGLUT in many, but not all, cholinergic or glutamatergic neurons, respectively. We used a transcriptome-wide approach to show that the same terminal characters, including but not restricted to neurotransmitter identity, can be regulated by different transcription factors in different cell types, arguing for extensive phenotypic convergence. Our data provide a deep understanding of the developmental and functional specification of a complex brain structure.

93 The mechanism controlling stochastic photoreceptor specification in the fly eye. Alexandra Neuhaus-Follini, Caitlin Anderson, Sang Tran, Mini Yuan, Robert J. Johnston, Jr. Department of Biology, Johns Hopkins University, Baltimore, MD. Stochastic gene expression diversifies cell fates in the nervous system and may contribute to individuality. Stochastic cell fate specification is best understood in bacteria, where noisy gene expression and autoregulatory feedback randomly and transiently generate distinct cell fates across genetically identical organisms. How this process is regulated in animals and what general mechanistic features are shared across species remain poorly understood. We study this question in the fly eye, where the random mosaic of color-detecting R7 photoreceptor subtypes is determined by the stochastic on/off expression of the transcription factor Spineless (Ss). We identified two enhancer elements that are required for Ss expression: an “early enhancer,” which drives expression in R7 precursor cells, and a “late enhancer,” which drives expression in terminally differentiated R7 cells. Deletion of the early enhancer causes a loss of both early and late Ss expression and modulating the strength of the early enhancer changes the on/off ratio of Ss expression in differentiated R7 cells. These data suggest that Ss
expression in R7 precursor cells is required for subsequent ss expression in terminal R7s and that the strength of ss expression from the early enhancer sets the ratio of Ss\(^{+}\):Ss\(^{-}\) R7s in the adult. In addition, both early and late ss expression are reduced in a ss protein-coding null mutant, implying that autoregulatory feedback from Ss protein is required for ss expression. Together, these data suggest that priming gene expression and autoregulatory protein feedback are common features of stochastic cell fate specification across species. We also identified two Polycomb response elements (PREs) that are required to repress ss expression and uncovered roles for chromatin modifiers in setting the on/off ratio of ss expression in R7 cells. Therefore, we propose a model in which 1) variation in expression from the early enhancer produces variable Polycomb spreading between the early and late periods of ss expression and 2) the extent of this spreading determines whether the late enhancer can re-activate ss expression in differentiated R7s. In this way, variation in early ss expression could be converted into stochastic, alternative R7 fates.

94 The Krebs cycle enzyme Isocitrate Dehydrogenase 3A couples mitochondrial metabolism to synaptic transmission. B Ugur\(^1\), H Bao\(^2\), M Stawarski\(^3\), LR Duraine\(^5\), Z Zuo\(^6\), YQ Lin\(^7\), G Neely\(^2\), GT Macleod\(^4\), ER Chapman\(^2\), HJ Bellen\(^1,5,6,8,9\) 1) Developmental Biology, Baylor College of Medicine, Houston, TX, USA, 77030; 2) Department of Neuroscience, University of Wisconsin-Madison, Madison, WI, USA, 53705; 3) Howard Hughes Medical Institute, University of Wisconsin, Madison, WI, USA, 53705; 4) Department of Biological Sciences and Wilkes Honors College, Florida Atlantic University, Jupiter, FL, USA, 33458; 5) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX, USA, 77030; 6) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA, 77030; 7) The Dr. John and Anne Chong Lab for Functional Genomics, Charles Perkins Centre and School of Life; 8) Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA, 77030; 9) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA, 77030.

The Krebs cycle is a conserved pathway that plays a pivotal role in energy production and metabolism. The main function of the Krebs cycle is to generate electron carriers for the electron transport chain. However, loss of function mutations in different Krebs cycle enzymes cause different phenotypes in humans. Very recently, a homozygous mutation in IDH3A has been associated with a severe encephalopathy and intractable epileptic infantile onset (Fattal-Valevski et al., 2017). Although the patient is clearly defined as a mitochondrial encephalopathy, the symptoms are confined to the nervous system and differ from canonical mitochondrial cases. Overall, the relationship between metabolism, Krebs cycle and neuronal function is poorly characterized.

In this study, we show that the loss of a core Krebs cycle enzyme isocitrate dehydrogenase 3a (idh3a), shares striking phenotypic similarities with loss of the synaptic Ca\(^{2+}\) sensor, synaptotagmin1 (syt1) in Drosophila synapses. Our study reveals that reduced levels of the idh3a product, alpha-ketoglutarate (αKG), causes synaptic transmission defects through an ATP-independent mechanism in flies. Synaptic transmission is a fast and tightly regulated Ca\(^{2+}\)-dependent process. Upon a Ca\(^{2+}\) influx, synaptotagmin1 (Syt1) promotes fusion of synaptic vesicle (SV) with the plasma membrane. Using an in vitro model based on mouse proteins to pinpoint the mechanism of action, we show that αKG promotes Syt1-stimulated membrane fusion by enhancing the interaction between Syt1 and membrane lipids. The data reveal a novel, conserved metabolic regulation of synaptic transmission via αKG and provide a link between mitochondrial dysfunction and some neuronal disorders.

95 Combinations of DIPs and Dprs control olfactory receptor neuron axon sorting in Drosophila. P.C. Volkan\(^1\), S. Barish\(^1\), S. Nuss\(^1\), I. Strunilin\(^1\), S. Mukherjee\(^3\), C.D. Jones\(^2\) 1) Duke University, Department of Biology, Durham NC; 2) University of North Carolina- Chapel Hill, Integrative Program for Biological & Genome Sciences, Chapel Hill, NC; 3) Duke University, Department of Mathematics, Durham NC.

In Drosophila, 50 classes of olfactory receptor neurons (ORNs) connect to 50 class-specific glomeruli in the antennal lobe. Despite identification of cell surface molecules regulating axon guidance, how ORN axons sort to form 50 stereotypical glomeruli remains unclear. Here we show that the heterophilic binding proteins, DIPs/Dprs, are expressed in ORNs as glomeruli start to form. Each ORN class expresses a unique combination of DIPs/Dprs, with neurons of the same class expressing ligand-receptor pairs. Mathematical analysis of DIP/Dpr expression clusters of ORN classes, which mimics their glomerular positioning in the antennal lobe, suggesting a role in ORN axon sorting. Perturbations of DIP/dpr gene function result in invasions of neighboring glomeruli. Our results suggest that context-dependent adhesion through DIP/Dpr combinations sort ORN axons into different glomeruli. Mammalian orthologues of DIPs/Dprs include KirreI2/3, which sort mammalian ORN axons into separate glomeruli, suggesting convergent mechanisms of glomerular formation in mammals and flies.


Glia cells in the peripheral nerve wrap axons to insulate them and cover the peripheral nerve to generate the blood-nerve barrier and structural integrity. Glia-extracellular matrix interactions need to occur to enable efficient glial sheathment, however not much is known about how glial cells adhere and communicate with each other or the ECM. The Drosophila larval
nerve system is surrounded by an outer layer of perineurial glia that are in turn covered by the neural lamella, which is comprised of extracellular matrix proteins such as laminin and perlecan. The function of perineurial glia and their interaction with the neural lamella is just beginning to be elucidated. We found that Basigin, a transmembrane Ig domain protein, is highly expressed in perineurial glia surrounding the central and peripheral nervous systems of third instar larvae. Much of the research on Basigin has focused on its role in cancer, however other studies have found that Basigin is also involved in a wide range of developmental processes. Here, we investigate a developmental role for Basigin in Drosophila glia. We show that Basigin is specifically expressed in perineurial glia and is found in close proximity with integrin based focal adhesions. Knock down of Basigin in perineurial glia using RNAi results in a significantly shorter ventral nerve cord and ruffles in the peripheral nervous system and results in disruption of larval locomotion. We examined the domains within Basigin that are required for association with integrin, the changes to glial morphology and larval locomotion. We also examined the effect of Basigin knockdown on integrin-associated proteins, the cytoskeleton and the effect on the basal lamina. Overall we have identified a clear role for Basigin in mediating perineurial glia function in the peripheral nervous system.

**97 dCORL expression and function in insulin producing cells reversibly influences adult longevity.** S.J. Newfeld, N.L. Tran, N.T. Takaesu, S.L. Goldsmith, E.F. Cornell Sch Life Sci, Arizona State Univ, Tempe, AZ.

CORL proteins (SKOR in mice and Fussel in humans) are a family of CNS-specific proteins related to Snx/Ski oncogenes. Their developmental and adult roles are largely unknown. We analyzed a series of dCORL (fussel in Flybase) reporter genes that revealed numerous stage-specific interactions between intertwined activators and repressors spread over 12kb. The most robust reporter AH.lacZ is expressed in all dILP2 insulin producing cells (IPCs) of the larval and adult pars intercerebralis (PI). The transcription factor Drifter (Drf) is also expressed in the PI, in a subset of dILP2 IPCs and in non-dILP2 cells. dCORL mutant adult brains display a 35% reduction in the number IPCs. A specific subset of cells is absent, the dILP2 cells that do not express Drf, as Drf expression in the PI is unaffected. dCORL mutant female virgins have a significantly shorter lifespan that wild type but his defect is completely reversed by mating (lifespan doubles). Taken together, our analysis of dCORL suggests a previously unknown connection between reproductive activity, insulin signaling and longevity. The CNS-specificity and sequence conservation of all CORL family members suggest that this connection may be conserved in mammals.

**98 Neto - the obligatory subunit of glutamate receptors, functions in both pre- and post-synaptic compartments to enable synapse development and homeostasis at the Drosophila neuromuscular junction.** TH Han¹, C Ramos², RVicidomini¹, SD Choudhury¹, M Jarmik¹, M Serpe¹ 1) NICHD/ NIH, Bethesda, MD; 2) Institut de Genomique Fonctionnelle de Lyon, France.

Synapse development is a highly orchestrated process coordinated by intercellular communication between the pre- and postsynaptic compartments, and by neuronal activity itself. Here we use the Drosophila NMJ to dissect the mechanisms of synapse assembly and homeostasis. We have recently discovered the obligatory auxiliary protein, Neto, essential for ionotropic glutamate receptors (iGluRs) clustering and NMJ functionality. Neto belongs to a family of highly conserved auxiliary proteins that regulate glutamatergic synapses. Drosophila neto encodes two isoforms, Neto-α and Neto-β, which share the extracellular and transmembrane domains, but have different cytoplasmic parts, generated by alternative splicing. Both intracellular domains are rich in putative phosphorylation motifs and docking sites. Our previous studies reveal that Neto isoforms engage in extracellular interactions that stabilize iGluRs at synaptic sites and trigger postsynaptic differentiation. In addition, Neto-β, the predominant isofom at the fly NMJ, mediates intracellular interactions that anchor postsynaptic density (PSD) components and sculpt iGluRs postsynaptic composition. In the absence of Neto-β, the NMJs are short, with enlarged boutons, and drastically reduced levels of P21 activated kinase (PAK), a PSD protein which stabilizes type-A iGluRs. Our current studies focus on identifying the Neto-β motifs important for the assembly and maintenance of PSDs; to this end we have generated an allelic series that progressively truncates its 350-residue intracellular domain. All neto-β mutants have normal evoked potentials due to a robust presynaptic compensatory response. In contrast, our recent studies indicate that loss of Neto-α (which represents Drosophila NMJ. In the absence of Neto-α, the glutamate receptors fields/PSDs appear enlarged and the sharp boundaries between PSDs and Dlg/PSD-95 are lost. Knockdown and rescue experiments indicate that presynaptic, and not postsynaptic, Neto-α modulates neurotransmitter release. In addition, neuronal Neto-α is required for synapse homeostasis. Chronic or acute/pharmacological reduction of postsynaptic iGluRs activities trigger increased presynaptic release in control, but not in neto-α null mutants, or in animals where Neto-α is perturbed in the presynaptic compartment. Our studies demonstrate that Neto proteins function in both motor neurons and muscles to enable synapse assembly and to coordinate synapse development and function.

**99 Examination of Perception and Performance in an Undergraduate Genetics Flipped Classroom.** J.L. Leatherman¹, L. Cleveland² 1) School of Biological Sciences, University of Northern Colorado, Greeley, CO; 2) MAST Institute, University of Northern Colorado, Greeley, CO.

Education literature clearly demonstrates the importance of active learning strategies for student success in undergraduate STEM courses. It is less clear, however, which type of active learning is most effective at promoting student learning. One educational innovation that is receiving much attention currently is the Flipped Classroom, where students learn the didactic
material outside of class, often in the form of online lectures, then the class time is used for application and discussion of the material. In this study, we compared undergraduate Genetics courses that both had active learning components: one (non-flipped course) had about 80% lecture with 20% of the class period used for active learning, while the other (the flipped course) had nearly 100% of the class time dedicated to active learning, and students watched videos of the material before class.

Comparison of exam scores between the active-learning non-flipped and flipped courses showed no significant differences between the two delivery methods. Thus, an increase in the amount of time spent on active learning did not provide any benefit to student performance beyond that observed with active learning. We also used a survey to collect data on students' perceptions of the flipped learning environment. Survey results revealed that 56% of the students were satisfied, and 39% were dissatisfied with the flipped learning environment (the remaining 5% were neutral). We found that students' perceptions of the flipped learning environment were not correlated to their performance on exams. Correlation analysis of survey responses to the satisfied versus dissatisfied student groups revealed that the clearest defining characteristic of dissatisfied students was not that they disliked classroom active-learning activities, but rather that they disliked and had trouble learning the course material from videos.

100 Inspiring Genetics Classroom Innovation with the Journal CourseSource. M. Smith¹, E. Vinson¹, J. Blum² ¹)
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Fostered by national reports such as Vision and Change, changes in the way colleges and universities are approaching their undergraduate STEM courses can be observed nationwide. Change has come in the form of initiatives dedicated to advancing evidence-based science education practices, research-based undergraduate courses, and other efforts that aim to provide educators with the tools and strategies needed to transform their classrooms.

One stumbling block in the process of this transformation is the time and energy commitment, which can be substantial, needed to produce evidence-based active-learning materials. In response to this need, and recommended in the Vision and Change report, a peer-reviewed, open access journal of student-centered biology education resources was created: CourseSource.

CourseSource is an open-access, online journal of peer-reviewed undergraduate biological teaching materials that: 1) incorporate student-centered, evidence-based pedagogy; 2) focus on professional society-developed learning goals including those developed by the Genetics Society of America; and 3) are organized and formatted so that use in other classrooms can easily occur.

We will familiarize the audience with CourseSource and provide examples of effective genetics instructional activities that can be used in the classroom. In addition, we will show how CourseSource provides authors with the opportunity to publish teaching materials in a high-quality, peer-reviewed format that documents their scholarly teaching efforts, accomplishments, and innovations. Often these activities are created by multiple authors; furthermore, publishing in CourseSource can foster new collaborations and provide opportunities to try student-centered instructional practices in the classroom.

101 The Genomics Education Partnership: Infusing Genomics into the Undergraduate Curriculum through Course-Based Research Experiences. J. DiAngelo¹, A. Arsham², J. Braverman³, A. Haberman⁴, M.L. Johnson⁵, C. Jones⁶, L. Kadlec⁷, J. Kagey⁸, J. Kennell⁹, S.C. Silver Key¹⁰, J. Leatherman¹¹, J. Mierisch¹², H. Mistry¹³, A. Nagengast¹⁴, D. Paetkau¹⁵, S. Parrish¹⁶, J. Sanford¹⁷, R. Spokony¹⁸, M. Wawersik¹⁹, L. Reed¹⁰, Faculty and Students of the Genomics Education Partnership 1) Penn State Berks, Reading, PA; 2) Bemidji State University, Bemidji, MN; 3) St. Joseph's University, Philadelphia, PA; 4) University of San Diego, San Diego, CA; 5) Notre Dame College, South Euclid, OH; 6) Moravian College, Bethlehem, PA; 7) Wilkes University, Wilkes-Barre, PA; 8) University of Detroit Mercy, Detroit, MI; 9) Vassar College, Poughkeepsie, NY; 10) North Carolina Central University, Durham, NC; 11) University of Northern Colorado, Greeley, CO; 12) Loyola University Chicago, Chicago, IL; 13) Widener University, Chester, PA; 14) St. Mary's College, Notre Dame, IN; 15) McDaniel College, Westminster, MD; 16) Ohio Northern University, Ada, OH; 17) Baruch College, CUNY, New York, NY; 18) College of William and Mary, Williamsburg, VA; 19) University of Alabama, Tuscaloosa, AL.

The Genomics Education Partnership (GEP; http://ggep.wustl.edu) is a consortium of faculty members from over 100 institutions who are involving students in Course-based Undergraduate Research Experiences (CUREs) and independent research courses in genomics. Currently students participate in improving the genome sequence and annotating the small, heterochromatic F element (dot chromosome) and a comparable portion of the euchromatic D element in a group of Drosophila species. Assessments show that GEP students from very diverse schools learn about genes and genomes, and gain an appreciation of the research process. To broaden the scope of scientific projects undertaken, GEP has partnered with Galaxy to produce G-OnRamp (https://gonramp.org), a suite of software and training materials that enables biologists to create UCSC Assembly Hubs or JBrowse Genome Browsers for annotation of any eukaryotic genome. GEP faculty are beginning to use the browsers produced by G-OnRamp to study the evolution of biochemical pathways such as triglyceride
production in parasitoid wasps. If interested in participating in the Summer 2018 G-OnRamp training workshops, sign up for mailings at http://gonramp.org/signup or contact S. Elgin (selgin@wustl.edu). Supported by NSF IUSE #1431407, NIH R25GM119157, and Washington University in St. Louis.

102 Frontiers for Young Minds - Fruit Fly Genetics in Science Communication. M.T. Juarez Biomedical Education, City College of New York, New York, NY.

Scientific writing is a communication tool for disseminating research and an important skill to develop for our trainees. The broader impacts of our research depend on effectively communicating our discoveries. In an effort to promote trainee communication skills, I made a student writing exercise into a pilot project to develop a revised version of my previously published research article on Drosophila genetics and wound repair. Frontiers for Young Minds serves as an open-access resource that not only creates science literature for a younger audience but also brings kids into the review process. Specifically, scientists write kid-friendly versions of their articles, which are then reviewed by young people in the target age range for the pieces (ages 8–15). Authors “translate” the main ideas in the articles through the use of keywords as well as a glossary section to define any scientific nomenclature. A science mentor – other than the authors – guides the young reviewer through the review process. In an online discussion forum curated by the Frontiers for Young Minds editors, the authors and mentors discuss the comments from the young reviewers and work together to identify components of the articles that spark the curiosity of the kids and concepts that need further clarification. Frontiers for Young Minds uses two article formats: 1) new discovery—to introduce a recent development in science by highlighting a previously published and peer-reviewed article; and 2) core concept—to provide a kid-friendly explanation of a fundamental scientific idea. The final product of our writing exercise serves, “How does a fruit fly say ouch”, as a resource to share with new students as they join the lab. My future goals are to translate this writing exercise into an undergraduate course through using small groups and discussions with invited speakers. Another application of this writing exercise is being incorporated into a graduate level seminar series in which a final report can include a section written in a Frontiers for Young Minds style. Upon reflection of this writing exercise, I encourage all research scientists to expand the impact of their discoveries and share their knowledge with society. Improving science communication will not only benefit scientific training but also promote scientific literacy within communities with limited access to STEM fields.

103 dOCRL maintains immune quiescence by regulating endosomal traffic. Sj. Del Signore1, S.A. Biber1, K.S. Lehmann1, S.R. Heimler1, B.H. Rosenfeld1, T.L. Eskin1, S.T. Sweeney2, A.A. Rodal1 1) Biology, Brandeis University, Waltham, MA; 2) Biology, University of York, York, UK.

Lowe Syndrome is a developmental disorder characterized by eye, kidney, and neurological pathologies, and is caused by mutations in the phosphatidylinositol-5-phosphatase OCRL. OCRL plays diverse roles in endocytic and endolysosomal trafficking, cytokinesis, and ciliogenesis, but it is unclear which of these cellular functions underlie specific patient symptoms. Here, we show that mutation of Drosophila OCRL causes aberrant activation of hemocytes, which are macrophage-like cells of the innate immune system. Tissue specific rescue of OCRL expression in either hemocytes or muscles, but not lymph gland or fat body, was sufficient to rescue immune-cell activation, suggesting that OCRL acts in multiple immune-relevant tissues. Here, we analyzed the direct role of OCRL on hemocyte physiology. We identified many cell biological defects consistent with other systems, including cytokinesis defects, aberrant actin assembly, and broad defects in endosomal structure and function. Specifically, we found defects in endocytosis and lysosomal trafficking, and accumulation of Rab7 and Lysotracker positive compartments. Among these many phenotypes, we pinpointed the cause of innate immune activation to reduced Rab11-dependent recycling traffic and concomitantly increased Rab7-dependent late endosome traffic. Specifically, we found that expression of Rab11C4 or Rab7C4 in hemocytes was sufficient to induce immune cell activation in otherwise normal larvae. Conversely, expression of Rab11C1 or Rab7D24 in hemocytes suppressed immune cell activation in OCRL mutant larvae. Notably, rescue with Rab7D24, but not Rab11C1, rescued the aberrant accumulation of F-actin and lysosomes, suggesting distinct mechanisms of rescue. Further, we found that loss of docrl amplifies multiple immune-relevant signals, including Toll, Jun kinase, and STAT, and leads to Rab11-sensitive mis-sorting and excessive secretion of the Toll ligand Spätzle. Thus, docrl regulation of endosomal traffic maintains hemocytes in a poised, but quiescent state, suggesting mechanisms by which endosomal misregulation of signaling may contribute to symptoms of Lowe syndrome.

104 A morphogenetic switch that reprograms membrane trafficking from recycling to degradation. S. Laiouar, V. Riechmann, N. Berns Department of Cell and Molecular Biology, Medical Faculty Mannheim, Heidelberg University, Mannheim, DE.

Maintenance of cell-cell adhesion in epithelial tissues involves constant endocytosis and recycling of adhesion proteins. The removal of these adhesion proteins is required for cell shape changes that occur during development and disease. How this removal is facilitated and how this process relates to membrane trafficking is unknown. Here we shed light on a mechanism that reprograms membrane trafficking during morphogenesis of the follicular epithelium. We show that RabX1, the V-ATPase proton pump and the Ser/Thr kinase Tao act in concert to redirect adhesion protein trafficking from recycling to lysosomal degradation.
Before morphogenesis, the adhesion proteins E-cadherin, Fasciclin2 and Fasciclin3 are endocytosed and recycled to maintain epithelial cell adhesion. We show that RabX1 orchestrates their recycling in an endosomal sorting compartment that connects the domains of Rab5, Rab11 and Rab7. Prior to morphogenesis, recycling ceases and adhesion proteins are degraded in lysosomes. This allows shrinking of the lateral membrane and leads to a transition from a cuboidal to a squamous epithelium. Our data indicate that morphogenesis is initiated by a switch in the RabX1 compartment, which redirects adhesion proteins into the endolysosomal pathway. The switch leads to the formation of dynamic RabX1 tubules that connect the sorting compartment with lysosomes. Interestingly, formation of these tubules is dependent on V-ATPase regulation within the RabX1 compartment. Interfering with this regulation by overexpressing its regulatory subunit Vha44 blocks RabX1 tubulation. This leads to a dramatic accumulation of E-cadherin, Fasciclin2 and Fasciclin3 within an enlarged RabX1 compartment, which reflects a block in lysosomal degradation. Intriguingly, co-overexpression of an activated form of Tao suppresses this phenotype completely. This suggests that Tao kinase controls the membrane trafficking switch. Thus, our data support a model in which a Tao induced V-ATPase activation leads to the formation of dynamic RabX1 tubules that redirect endosomal sorting from recycling to lysosomal degradation.

105 Self-organization of the actin cytoskeleton drives secretion in Drosophila salivary glands. E.D. Schèter, D. Segal, A. Zaritsky, D. Meyen, T. Roussos, B. Shilo 1) Department of Molecular Genetics, University of Toronto, ON, CA; 2) Cell Biology Program, The Hospital for Sick Children, Toronto, ON, CA.

Coordinated formation and disassembly of contractile actin-based structures has been shown to underlie diverse settings of tissue morphogenesis. Here we propose that such a mechanism mediates the contractile activity necessary for content release from large secretory vesicles. A common feature of these systems is formation of an actin coat around each vesicle following their fusion with the apical cell membrane and recruitment of myosin, which together mediate the forces necessary for vesicle contraction. Using live imaging of cultured Drosophila larval salivary glands, an established model for such secretory systems, we have followed the dynamics of actomyosin coat formation and content release from glycoprotein (“glue”)-filled vesicles into the gland lumen. We previously demonstrated critical regulatory roles for the Rho1 GTPase in both actin coat formation (via activation of the Formin-family protein Diaphanous) and Myosin II-based contractation (via Rho kinase) in this system. Surprisingly, we have now found that disassembly of the actin coat, which accompanies vesicle content release, is necessary for contraction of the actomyosin network. This process was monitored using secretion-arrested vesicles, and found to be dependent on Rho1 inactivation, mediated by a dedicated RhoGAP and branched-actin polymerization. The sequential temporal recruitment of active Rho and its inhibitors is evident by cycles of active Rho1 and actin coat accumulation and depletion in such vesicles, implying that a feedback-based mechanism regulates actin coat disassembly from the vesicle surface. Contraction-driven content release, the final step of this form of exocytosis, is therefore achieved by coordinating formation and disassembly of the contractile machinery.

106 Phosphatidylinositol 4,5-bisphosphate (PIP2) is essential for cilium assembly and function in Drosophila. J.A. Brill, A. Gupta 1) Department of Molecular Genetics, University of Toronto, ON, CA; 2) Cell Biology Program, The Hospital for Sick Children, Toronto, ON, CA.

Cilia are antenna-like sensory organelles whose malfunction underlies many genetic disorders in humans. Recent studies have found that dephosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) by the inositol 5-phosphatase INPP5E is necessary for cilium assembly and function. Here, we present evidence against a simplistic model for the PIP2 code of cilia. Specifically, we show that PIP2 is essential for both formation and proper function of chordotonal cilia in Drosophila melanogaster. We report the presence of a tightly localized pool of relatively high PIP2 levels within the chordotonal cilium, suggesting that the notion of cilia as organelles devoid of PIP2 should be reconsidered. We also observe that neuronal clones mutant for the phosphatidylinositol 4-phosphate 5-kinase ortholog Skittles (sktl) do not assemble cilium. Moreover, in contrast to overexpression of Sktl in chordotonal neurons [1], RNAi-mediated sktl knockdown does not affect localization of the Tubby domain protein Tulip. Instead, sktl RNAi induces defects in intraflagellar transport, which is unaffected in inpp5e mutants that contain high intraciliary levels of PIP2 [1]. We further report a novel role for PIP2 in transition zone length control: sktl RNAi induces aberrant elongation of transition zone-associated Cep290. This phenotype is mimicked by loss of PIP2 in the ciliated male germline of Drosophila. Our observations reveal unappreciated complexity in the functional relationship between PIP2 and cilia.

References

107 Coordinated contractility initiates cell dispersal at the onset of migration. Benjamin Lin Lin, Beatriz Garcia, Ruth Lehmann 1) Kimmel Center for Biology and Medicine at the Skirball Institute, NYU School of Medicine, New York, USA; 2) Howard Hughes Medical Institute.
External signals can shape tissue architecture during development by initiating single cell migration programs, which stimulate subsets of cells to individualize and migrate to defined locations. In adulthood, these programs can be re-engaged to aid in tissue repair, but chronic activation can contribute to fibrosis and improper activation can drive cancer metastasis. Thus understanding the molecular mechanisms initiating individual motility has widespread significance for a variety of human diseases. Drosophila primordial germ cells (PGCs) undergo a distinct, external cue driven migratory transition from an adhesive clustered population to individual migrating cells before their subsequent invasion of the endoderm, and thus serve as a model system for dissecting the signaling pathways driving cellular dispersal. Using live cell imaging, we show here that efficient PGC dispersal requires coordinated contractility between PGCs along the cluster periphery and central PGCs. Peripheral PGCs polarize a contractile signaling network, comprised of Rho1, Rock, F-actin, and non-muscle myosin II, at cell interfaces contacting central PGCs. This contractile signaling network is activated basally but its orientation is dependent on Tre1, a G-protein-coupled-receptor (GPCR) we previously identified as being essential for PGC scattering. Central PGCs resist deformations imposed by the local contraction of peripheral PGCs by generating a stiff, isotropic actomyosin cortex tied to mitotic rounding. Breaking the contraction symmetry between central and peripheral PGCs by removing Tre1 or by depleting maternal pebble protein, a Rho1 guanine exchange involved in mitotic rounding, causes PGC migration defects. Based on these observations, we propose that the resistance provided by the stiff isotropic actomyosin cortex surrounding central PGCs enables tugging peripheral PGCs to break away and individualize.

108 Multiple feedback mechanisms fine-tune Rho signaling to regulate morphogenetic outcomes. Katy Ong, Camille Collier, Stephen DiNardo University of Pennsylvania, Philadelphia, PA.

Rho signaling is a conserved mechanism for generating forces through activation of contractile actomyosin. How this pathway is tuned to produce different morphogenetic outputs is poorly understood. In the embryonic epithelium, we investigate how Rho signaling controls force asymmetries to drive morphogenesis in tissues. Specifically, we study a distinctive mid-embryogenesis event termed “alignment” in which coordinated cell shape changes result in a unique geometry of rectilinear cells separated by aligned cell-cell contacts. We found that this rearrangement is driven by contractility of actomyosin cables that elevates the local tension along select interfaces. Our data show that during alignment there is polarization of two branches of Rho signaling, Rho Kinase (ROK) and Diaphanous (Dia). Both of these branches cooperate in the formation of actomyosin cables. Constitutive activation of these Rho effectors caused aligning cells to instead invaginate. These observations support emerging evidence that Rho signaling needs to be moderated to achieve the proper morphogenetic outcome. We therefore tested for potential feedback regulation within the pathway. We discovered a mutual dependence between F-actin and Myo-II that is critical for enrichment of each of these cytoskeletal components to aligning interfaces. Furthermore, disruption of F-actin led to upregulation of Rho signaling, suggesting that F-actin polymerization results in negative feedback to Rho. Myo-II knockdown had no impact on Rho signaling activation or polarity, indicating that contractility does not initiate feedback. However, inhibiting ROK caused depletion of Dia from aligning interfaces. This indicates the ROK has a Myo-II independent interaction with Dia. Taken together, our work suggests that multiple feedback mechanisms factor in to determining Rho signaling outcomes and that the functional significance of feedback is context dependent.

109 The Role for Microtubules in Organizing Actomyosin Contractility during Ventral Furrow Formation. C. Ko, A. Martin Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

While the regulation and organization of actomyosin during cell shape change has been well studied in a variety of tissue morphogenetic processes, how the microtubule (MT) cytoskeleton regulates actomyosin behavior to promote cell shape change is less well understood. We sought to investigate the organization and function of the MT cytoskeleton and its associated proteins during ventral furrow formation in early Drosophila embryos, where cells undergo actomyosin-dependent constriction of cell apices in order to fold the tissue. We found that the localization of GFP-tagged Patronin, a MT minus end-binding protein, exhibited a dynamic pattern of localization in ventral cells. Patronin first localized to intercellular junctions at the end of cellularization, but later concentrated in the middle of the cell apex at the start of apical constriction. The transition in Patronin localization from the junctions to the middle of the cell apex was correlated with the appearance of apical myosin. In addition, this pattern of localization was abrogated upon injection of drugs that disrupt actomyosin contractility, suggesting that an intact actomyosin network is necessary for Patronin localization. In embryos depleted of Patronin, we observed a disruption of E-cadherin (Ecad) localization and abnormal myosin network dynamics. Because Patronin has two distinct localizations in the cell leading up to tissue folding, we suggest a model whereby the MT network may first be organized at the junctions to establish apically-polarized Ecad clusters and then in the middle of the cell to help regulate and/or organize the actomyosin network. Currently, we are testing this model and working to elucidate the molecular mechanism by which the MT network promotes apical constriction.

110 Role of lipid droplets in Drosophila models of kidney disease. A. Lubojemska1, M. Irina Stefana2, Alex. P Gould1 1) The Francis Crick Institute, London, GB; 2) The Wellcome Trust Centre for Human Genetics, Oxford, GB.

A common feature of kidney diseases such as diabetic nephropathy is the accumulation of lipid droplets (LDs). However, it
remains unclear how renal LDs are induced and whether their functions are harmful or beneficial. We have established several different Drosophila dietary models of impaired renal function, all of which are associated with the presence of ectopic LDs in nephrocytes (cells analogous to mammalian podocytes). Using these models, nephrocyte-specific knockdowns demonstrate that, in contrast to LD induction in other contexts, renal LD accumulation does not require ROS-induced JNK signalling. Instead, we find that manipulations that decrease triglyceride synthesis or enhance triglyceride catabolism can block renal LDs efficiently. Interestingly, however, depending upon whether these manipulations act primarily upstream or downstream of triglyceride synthesis, they either further deteriorate or enhance the ability of nephrocytes to endocytose extracellular cargo molecules. Together these findings provide evidence supporting the hypothesis that LDs can play a beneficial role in kidney disease, helping to protect nephrocytes from nutritional stress.


111 Interphase localization of Abnormal Spindle to the nucleus is important for proper brain size. Todd Schoborg, Lauren Smith, Samantha Smith, Carey Fagerstrom, Nasser Rusan Cell Biology & Physiology Center, Naitonal Heart, Lung and Blood Institute, NIH, Bethesda, MD.

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder characterized by reduced brain size and life span. While the clinical aspects of the disorder are well characterized, the molecular mechanism remains poorly understood. Previous models favored cell division defects induced by mitotic spindle errors as the cause of the disorder, leading to reduced neuron/glia numbers and a smaller brain. The most commonly mutated gene in human MCPH patients, Abnormal Spindle-Like, Microcephaly Associated (ASPM) is known to be important for proper centrosome and mitotic spindle function during mitosis. However, our recent analysis of the Drosophila melanogaster ortholog, Abnormal Spindle (Asp), showed that mitotic spindle & cell division defects are not the primary cause of MCPH in Asp mutant animals, suggesting the current model needs to be revised. We now provide evidence that Asp contributes to proper brain size through a novel role in the interphase nucleus. Using a combination of transgenic rescue assays and high resolution microcomputed tomography (micro-CT) of intact animals, we have identified the minimal fragment of Asp's N-terminus (Asp<sub>597</sub>, 597 aa) required for proper brain size and morphology. Subcellular localization of Asp<sub>597</sub> within the developing larval & adult brain revealed an unexpected localization to the interphase nucleus of distinct neural stem cell populations and mature neurons. This nuclear localization is controlled through a conserved cluster of nuclear localization sequences, whose deletion leads to cytoplasmic accumulation of Asp<sub>597</sub> during interphase and drives the full-length protein onto microtubules, leading to disruption of the microtubule cytoskeleton. Intriguingly, RNA-Seq analysis of Asp mutant brains revealed a significant downregulation of actin-related genes, including myosin heavy chain (Mhc) and the troponin complex member wupA, whose expression could be restored to wildtype levels in the AspN<sub>597</sub> background. Collectively, these data suggest two possible models for Asp's nuclear localization: either Asp has a direct nuclear role (i.e., transcriptional regulation) or is sequestered there to keep it from disrupting normal interphase function of the cytoskeleton. We are currently testing these two models. Together, our data highlights the first interphase role for Asp and suggests that other MCPH genes may contribute to the disorder through non-canonical pathways that funnel through the nucleus.

112 Notch induced tumorigenesis in a Drosophila transition zone model. S. Yang, W. Deng Department of Biological Science, Florida State University, Tallahassee, FL.

Transition zones are regions in the animal body where two types of epithelial tissue meet. Many transition zones are also high-risk sites for tumorigenesis. However, little is known on why transition zones are more susceptible for tumor formation. Here we report that the Drosophila salivary gland imaginal ring can be used as a model to study tumorigenesis in transition zones. We found that constitutive activation of Notch signaling in the imaginal ring during the third larval instar is sufficient to induce overproliferation and tumor formation. Interestingly, tumorigenesis always occurs at the posterior end of the imaginal ring, which is a transitional area between the polyploid giant cells and diploid imaginal ring cells. These Notch-induced tumors grow rapidly and metastasize once implanted in the abdomen of adult flies, suggesting that they have the characteristics of malignant neoplasm. Further studies revealed that the posterior end of the salivary gland imaginal ring has high Jak/Stat and JNK activities that are necessary for tumor growth, and JNK-activated MMP1 is required for tumor formation in this transition zone model. Furthermore, we found that ectopic MMP1 expression can transform the anterior end of the salivary gland imaginal ring into a tumor hotspot. Together, these studies reveal how JNK and Jak/STAT pathways promote Notch-induced neoplastic tumor formation at the Drosophila transitional zone model, and identify the salivary gland imaginal ring as an in vivo model for site-specific tumorigenesis.


Acute myeloid leukemia (AML) represents one third of blood cancers diagnosed each year and is associated with the lowest
rate of survival. Several chromosomal aberrations altering a large number of genes have been discovered in primary AML samples. Yet, a unified view of the underlying cause(s) leading to the disease is still unclear. Intriguingly, a significant number of translocations detected in AML involves the NUP98 gene, which is a core component of the nuclear pore complex. NUP98-HOXA9 (NA9) is the first translocation of this category to be discovered. It fuses the N-terminal part of NUP98 to the C-terminal DNA binding domain of HOXA9. How NUP98-HOXA9 expression in bone marrow progenitor cells causes leukemia is not understood. Since NUP98 and HOXA9 are conserved in Drosophila, we sought to use flies to identify key factors relevant to NA9 activity by conducting a dominant modifier screen in the eye. Human NA9 expression during eye development generates a dose-sensitive abnormal eye phenotype. Consistent with a specific HOX-mediated effect, the phenotype can be suppressed by depleting key HOX co-factors, namely, HTH (Meis ortholog) or EXD (PBX ortholog). Conversely, HTH overexpression strongly correlated with NA9 during eye development. We also found that NA9 activity depended on an intact PBX interacting motif and HOXA9 DNA-binding domain. Together, these findings recapitulate key features observed in mouse NA9 leukemia models. We next conducted the screen, which enabled us to isolate 102 recessive-lethal dominant modifiers that respectively fell into 16 and 11 complementation groups of suppressors and enhancers. Deficiencies, P-elements, and genomic sequencing were then used to map and identify the various groups. Interestingly, two genes encoding known physical interactors of NUP98, namely, Rae1 and emb, were identified as enhancers. Importantly, previous studies in mammals suggested that their homologs, Rae1 and Crm1/Xpo1, are contributing to NA9-mediated leukemogenesis. The screen also uncovered mutations in genes related to epigenetic regulation (U(Pc) and grappa), planar cell polarity (grh, stan and ed), as well as translation initiation (eIF3B and eIF3I). The characterization of these new genes will bring a new level of understanding as to how NA9 promote AML development in human.

114 Branchless mediates muscle wasting in obesity-enhanced tumorigenesis. H. Newton1,2, L. Camplessel1,2, S. Hirabayashi1,2 1) Metabolism and Cell Growth Group, MRC London Institute of Medical Sciences, London, UK; 2) Institute of Clinical Sciences, Imperial College London, London, UK.

Epidemiological studies have demonstrated that obesity increases the risk and progression of various cancers. Cancer induces cachexia, a multi-factorial wasting syndrome characterized by the progressive loss of skeletal muscle tissue with or without loss of adipose tissue. However, muscle wasting in cachexia is often hidden in the context of obesity. As a result, the molecular mechanisms underlying muscle wasting in the presence of both obesity and cancer remain poorly understood.

Our previous work demonstrates that co-activation of the oncoproteins Ras and Src in the eye epithelial tissue of Drosophila larvae raised on a control diet (CD) leads to benign tumor growth. In contrast, raising the same animal on a high sugar diet (HSD) is sufficient to induce obesity, and convert transformed tissue from benign growths into aggressive tumors. Strikingly, we also observe that even in the presence of obesity, HSD-enhanced tumorigenesis is accompanied by the progressive wasting of peripheral muscle tissue.

We have used this model of muscle wasting in obesity-enhanced tumorigenesis to identify tumor-secreted factors which may be responsible. We identify Branchless (Bnl; dFGF) as one of the most highly expressed tumor-secreted factors in HSD-enhanced Ras/Src tumors. Increased expression of Bnl was also observed in the fat body of HSD-induced obese animals. Tumor-autonomous knockdown of Bnl partially rescues muscle wasting. Conversely, tumor-autonomous overexpression of Bnl promotes muscle wasting in animals raised on CD, and HSD-induced obesity further enhances this effect. We propose Bnl-secreted from both the tumor and fat body as a mediator of muscle wasting in obesity-enhanced Ras/Src tumorigenesis.


115 Optogenetic Control of Drosophila Cardiac Function with Red-light Excitation. J. Men1, A. Wyzlic1, L. Gopfert1, A. Li2, R. Tanzi2, C. Zhou1,3,4 1) Department of Bioengineering, Lehigh University, Bethlehem, PA, USA; 2) Genetics and Aging Research Unit, Department of Neurology, Massachusetts General Institute for Neurodegenerative Diseases, Massachusetts General Hospital and Harvard Medical School, Boston, MA-02114, USA; 3) Department of Electrical and Computer Engineering and Bioengineering Program, Lehigh University, PA, USA; 4) Center for Photonics and Nanoelectronics, Lehigh University, Bethlehem, PA, USA.

Cardiac optogenetics is a promising alternative to traditional electrical stimulations in controlling activity of cardiac tissues non-invasively. In recent years, cardiac functions of animals such as rat, zebrafish, and fruit fly, have been controlled through excitation of opsins expressed cardiac tissues using patterned light. In this study, we expressed red-shifted excitatory and inhibitory opsins (ReaChR and halorhodopsin) in the heart of Drosophila melanogaster, and used red-light stimulation for deep penetration into the myocardial structures. M-mode images acquired with a custom optical coherence microscopy (OCM) system demonstrated controlled heart function in vivo and in real time throughout the Drosophila life cycle (larva, early pupa, late pupa, and adult). Fast kinetics, low stimulation power, and broad heart-rate adjustable range were demonstrated using red light pacing. Cardiac function inactivation was performed with low power red light excitation. Drosophila heart rate can be
reduced compared to its resting heart rate, and reversible long pause of heart function can be achieved at various developmental stages. The effects of extensive pacing at early developmental stages on the fly heart development were also studied. This study demonstrated non-invasive cardiac control through activating and inhibiting heart functions of an intact animal, which is promising for nondestructive study of cardiac diseases, such as congenital heart disease, posteriority bradycardia, tachycardia, and regional mechanical dys-synchrony.

116 The multifaceted mechanisms of mitochondrial DNA selective inheritance in Drosophila. Z. Chen, H. Xu  National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

Although mitochondrial DNA (mtDNA) is prone to mutation and few mtDNA repair mechanisms exist, deleterious mutations are exceedingly rare. The mechanisms of how the transmission of detrimental mtDNA mutation is restricted remain debatable. So far, mitochondrial genetic bottleneck, Balbiani body mediated purifying selection and selective replication of wild-type mtDNA have been the most prevailing mechanisms proposed for selective mtDNA inheritance. Recent development of Drosophila models of mitochondrial DNA deficiency has proved Drosophila a valuable tool for investigating mtDNA inheritance. Some mechanisms in the germline selecting against mtDNA mutations have been found conserved in metazoans, however, it is to be determined what selecting mechanisms dominate in Drosophila and how these mechanisms operate at a molecular level. In this study, we used the heteroplasmic fly containing both wild type and mtCal1300 mtDNA to address the above questions. We found that in Drosophila, an effective mitochondrial bottleneck caused by reduced mtDNA nucleoid in germline development is absent. However, bottleneck could be strengthened artificially by reducing the number of mitochondrial genetic segregation units. A purifying selection, which involves the selective transportation of healthy mitochondria to the structure of Balbiani body is existed, but play a limited role in mtDNA selection. We provided direct experimental evidences that selective propagation of functional mtDNA at germarium region 2B plays a major role in mtDNA selective inheritance in Drosophila oogenesis. At region 2A, the preceding region of region 2B, the mtDNA copy number per mitochondrion is reduced to prepare for effective selection on organelle level based on individual fitness. Selective replication of wild-type mtDNA and Balbiani body mediated purifying selection could act synergistically to secure the functional mtDNA being transmitted through Drosophila oogenesis.

117 Metazoan Nuclear Pores provide a scaffold for poised genes and stabilized induced Enhancer-Promoter contacts. Pau Pascual1,2, Shawn Little1, Maya Capelson1,2 1) Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA; 2) Epigenetics Institute, University of Pennsylvania, Philadelphia, PA.

Understanding how patterns of gene expression are established, maintained, and transmitted during cell division is crucial to decipher how cells initiate and preserve their developmental identity. A growing number of components of gene expression machinery are known to be non-randomly arranged in the nucleoplasm and located in distinct nuclear compartments and scaffolds. Interactions between individual chromosomal regions and these various nuclear scaffolds have been shown to contribute to the initiation or maintenance of gene expression programs. One of the most prominent nuclear scaffolds is the Nuclear Pore Complex (NPC), which is embedded in the Nuclear Envelope, and consists of approximately 30 different subunits, called Nucleoporins (Nups). This large protein complex mediates nucleo-cytoplasmic transport of macromolecules, but it has also been implicated in the regulation of gene expression via direct binding to the genome. In Drosophila culture cells and in isolated larval tissues, we have identified genome-wide presence of multiple Nups at regulatory DNA elements, including promoters, enhancers, and insulators. Strikingly, we discovered that the NPC component Nup98 facilitates the looping contacts between promoters and enhancers, revealing Nups as a new class of architectural proteins that organize the 3D architecture of the genome and influence gene regulation. Consistent with this idea, we characterized the role of Nups in developmental transcriptional memory, in which ec dysone hormone-induced genes are activated more robustly in cells that have previously experienced the presence of the hormone. Interestingly, loss of Nup98 does not affect transcription during initial induction, but results in slower activation during re-induction, demonstrating a loss of transcriptional memory. To further characterize this phenomenon, we used single-molecule RNA FISH to measure activation dynamics of ec dysone-induced genes during the state of initial induction versus during the primed state after the establishment of transcriptional memory. Our results suggest a functional relationship between priming of transcriptional states and stabilization of enhancer-promoter loops by nuclear pore proteins.

118 Pairing TADs (PairiTs) drive homologous chromosomes together to promote interchromosomal gene regulation. Kayla Viets, Michael Sauria, Sang Tran, Caitlin Anderson, Raghav Goyal, James Taylor, Robert J. Johnston Jr. Department of Biology, Johns Hopkins University, Baltimore, MD.

Homologous DNA elements communicate between chromosomes to regulate gene expression in processes including genetic imprinting and X-inactivation, but the mechanisms driving these interactions are poorly understood. In flies, homologous chromosomes pair throughout development, promoting an interchromosomal gene regulatory mechanism known as transvection. Despite over a century of study, the sequence and structural features that drive chromosome-wide pairing in Drosophila are unknown. We identify topologically associated domains (TADs) across the fly genome that drive pairing with their endogenous loci when inserted onto other chromosomes. Our data suggest that these specialized TADs,
which we call **Pairing TADs** (PairiTs) are interspersed along chromosomes to facilitate homologous pairing.

Focusing on a PairiT containing **spineless** (ss), a gene whose stochastic expression in the *Drosophila* retina is controlled by a transvection-like process, we find that the intact ss PairiT drives pairing from multiple genomic sites, whereas fragments of the ss PairiT do not drive pairing. We force pairing by inserting fragments of ss into genomic sites that naturally loop to endogenous ss and find that pairing is necessary but not sufficient for cross regulation between ss alleles. Pairing driven by the ss PairiT is cell-type-specific, allowing interchromosomal gene regulation in the retina but not in other tissues. Thus, strongly interacting TADs such as ss button chromosomes together to facilitate interchromosomal regulation in a cell-type-specific manner.

119  **Phase separation drives heterochromatin domain formation.**  A.R. Strom$^{1,2}$, A. Emelyanov$^3$, M. Mir$^1$, D. Fyodorov$^4$, X. Darzacq$^5$, G.H. Karpen$^{1,2}$  1) UC Berkeley, Berkeley, CA; 2) Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Albert Einstein College of Medicine, New York, NY.

Compartmentalization is a theme used throughout all kingdoms of biology to create functionally distinct units within a complex cellular environment. In addition to membrane-bound organelles, compartments can exist in the cell that are not membrane bound, yet are still physically distinct from surrounding space. The cell contains a number of these membraneless organelles (nucleoli, stress granules, PML bodies, etc.), which are thought to be formed by liquid-liquid phase separation. We find that in early *Drosophila* embryos, heterochromatin forms by nucleating multiple HP1α foci that grow individually, then fuse together into the final domain. This formation process is reminiscent of liquid-like fusion of nucleoli, which led us to comprehensively study whether heterochromatin could also be a phase-separated system within the nucleus. We utilized Fluorescence Correlation Spectroscopy (FCS) methods to investigate protein diffusion dynamics and determine if heterochromatin displays characteristics associated with phase separation. We find that, similar to other membraneless organelles, the heterochromatin domain is indeed capable of liquid-like fusion, is selectively permeable, and the heteroeuochromatic interface has surface tension. Additionally, *Drosophila* HP1α protein is capable of liquid demixing *in vitro* and mediates domain formation *in vivo*. This work is the first to demonstrate that the heterochromatin domain is subject to phase separation principles, which suggests that phase interaction, rather than static hindrance due to chromatin compaction, defines accessibility of heterochromatic areas. It has been suggested that phase separation could be a general organizing property for many membraneless organelles; therefore this model has broad implications for understanding the mechanism of nuclear organization.

120  **Small RNA and the epigenetics of X recognition.**  N. Deshpande, V. Meller  Biological Sciences, Wayne State University, Detroit, MI.

Eukaryotic genomes are organized into large domains of coordinated regulation. The role of small RNAs in formation of these domains is largely unexplored. An extraordinary example of domain-wide regulation is X chromosome compensation in *Drosophila melanogaster* males. This process occurs by hypertranscription of genes on the single male X chromosome. Extensive research in this field has shown that the Male Specific Lethal (MSL) complex binds X-linked genes and modifies chromatin to increase expression. The components of this complex, and their actions on chromatin, are well studied. In contrast, the mechanism that results in exclusive recruitment to the X chromosome is not understood. Prior studies have found that the siRNA pathway contributes to X-localization of the MSL complex. Interestingly, siRNAs generated from repetitive sequences on the X chromosome promote X recognition. Insertion of DNA from these repeats on an autosome induces compensation of nearby autosomal genes. These findings implicate the siRNA pathway in dosage compensation, but no evidence of a direct interaction between the siRNA and MSL complex has been detected. This suggests that siRNA influences X-recognition by an indirect, novel mechanism. For example, Ago2-containing complexes could bind nascent RNAs from the X chromosome and recruit activities that alter chromatin structure or interphase chromosome organization in a manner that facilitates MSL recruitment. To test this model, I performed a genetic screen that identified numerous Ago2 interactors, including Su(var)3-9, that participate in dosage compensation. This suggested the possibility that repeats on the X are enriched for H3K9me2 through a siRNA-dependent mechanism. Using Chromatin Immunoprecipitation (ChIP), I demonstrated that H3K9me2 enrichment at X-linked repeat sequences depends on Su(var)3-9, but not the H3K9 methyltransferases SetDB1 or G9a. Genetic manipulations of the siRNA pathway disrupt H3K9me2 at and around repeats on the X chromosome without changing the global levels of H3K9me2. Similar disruptions are observed in chromatin surrounding insertions of X-linked repeats on an autosome. My research suggests a mechanism through which simple repeats can exert a profound effect on chromosome behavior and gene expression. As repeats make up a large proportion of eukaryotic genomes, similar regulatory pathways may be widespread.

121  **The addition and removal of chrY in *D. melanogaster* females can alter expression of rDNA-associated sequences with phenotypic consequences.**  K. Silkaitis$^1$, A.T. Branco$^{1,2}$, B. Lemos$^1$  1) Molecular and Integrative Physiological Sciences Program, Dept of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA; 2) Universidade Federal do Espirito Santo, Vitoria, Espirito Santo, Brazil.
The *Drosophila melanogaster* X and Y chromosomes are about the same size at ~40Mb, yet the X chromosome has more than 2,000 genes while chY has only ~15. Despite this paucity of genes, the Y chromosome affects gene expression and regulation in trans. Here, we further investigate the regulatory role of the Y chromosome by introgressing a free chY into males and females carrying attached sex chromosomes (X^XY and X^X) in an isogenic background. We find that X^XY females show increased fertility compared to X^X females. Yet, when the free chY from X^XY females is removed via outcrossing, we find the loss-of-Y female offspring display reduced viability and fertility compared to both the X^XY maternal strain and X^X progenitor females. These loss-of-Y females also display other characteristics typical of the rRNA-deficiency bobbed phenotype. Further indicating a causal role for the rDNA locus, we find rDNA-associated sequences are dysregulated between these three genotypes. When we expand our observations to additional X^XY strains, we find similar physiological and gene-expression responses in F1 females that have lost the free chY in comparison to their mother. Together, our data indicate that a Y chromosome in females can alter the structure and expression of the X-chromosome rDNA locus in trans.

### 122 rDNA-specific retrotransposons maintain rDNA copy number in the *Drosophila* male germline.

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Transposable elements (TEs) are considered genetic parasites that exploit hosts' genomes for their own propagation. Suppression of TE activity is thus believed to be paramount for protecting against accumulated genetic disruptions created by TE insertion, particularly in the germline. Despite this notion, here we provide evidence that activity of the highly conserved R1 and R2 retrotransposons in the *Drosophila* male germline is critical for maintaining germ cells during aging. Although unrelated by sequence homology, R1 and R2 share a common feature to exclusively insert within ribosomal DNA (rDNA) in a sequence-specific manner. The rDNA gene encodes the RNA components of the ribosome, and is tandemly-repeated hundreds of times to comprise the rDNA loci. rDNA is inherently unstable due to the potential for intra-chromosomal exchange, and we have recently shown that rDNA copies are progressively lost during aging in *Drosophila* male germ line stem cells (GSCs). Surprisingly, we find that inhibiting R1 or R2 expression by RNAi exacerbated rDNA copy loss during aging, and found a concomitant decrease in GSC numbers. We show that R1 and R2 expression is normally suppressed in the GSCs of young animals with robust rDNA copy number, but their expression is derepressed in GSCs both during aging and when rDNA copies are reduced. We had previously characterized that GSCs with reduced rDNA content are capable of restoring the lost rDNA abundance, similar to the phenomenon of rDNA magnification. Based on these observations, we hypothesize that R1 and R2 expression in these cells may contribute to the expansion of rDNA copies in GSCs required to normally maintain rDNA copy redundancy and GSC viability during aging. rDNA copy number is thought to be increased by unequal sister chromatid exchange (USE) of misaligned rDNA copies during the restoration of double-stranded DNA breaks (DSBs) in rDNA by homologous recombination-mediated repair. Our preliminary results indicate that R1 and R2 may be required to create DSBs that initiate USE at rDNA in GSCs with reduced rDNA copies, perhaps through their own endonuclease activity, to promote rDNA copy expansion and maintain GSC rDNA copy number. Together, we propose that R1 and R2, and potentially other highly conserved TEs, are domesticated to serve the host, and thus should be investigated as symbionts rather than genetic parasites.

### 123 Inaccessible chromatin and transcriptional repression cooperate to promote late replication during S phase.

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How origins of DNA replication are specified and activated in the context of an intact metazoan genome remains poorly understood. In contrast to *Saccharomyces cerevisiae*, replication initiation in metazoan genomes is not directed by well-defined sequence motifs. Rather, local chromatin environments have emerged as critical regulators of replication, resulting in asynchronous replication initiation that yields early and late replicating regions of the genome. Transcriptionally active, accessible euchromatin typically replicates early during S phase, whereas transcriptionally repressive, inaccessible heterochromatin typically replicates late. Current models of replication posit a stochastic process in which a higher density of specified origins in accessible euchromatin compared to inaccessible heterochromatin increases the probability of replication initiation, resulting in the earlier replication of euchromatin relative to heterochromatin. Despite strong genome-wide correlations between replication and chromatin, a true causal relationship between the two has yet to be determined.

We are determining how chromatin organization impacts replication in *Drosophila* using our genetic platform in which endogenous histone genes are replaced with transgenic histone genes encoding mutations that prevent modification of specific histone residues. Using this platform, we demonstrated that H3K9R mutation disrupts constitutive heterochromatin organization. To determine whether perturbation of heterochromatin affects replication initiation, we implemented a whole-
genome sequencing method to produce genome-wide replication timing profiles from wild type and H3K9R mutant diploid wing imaginal discs. We identified regions of the genome with altered replication in H3K9R mutants, including earlier replication of the normally late replicating pericentromeric heterochromatin. Our data indicate that open chromatin is necessary but not sufficient for early replication in animal cells. Furthermore, we provide evidence that transcription stimulates early replication in regions of open chromatin. Together, our data support a model in which inaccessible heterochromatin and transcriptional repression cooperate to promote late replication of pericentromeric heterochromatin.

124 Store-operated calcium entry functions downstream of Oamb in mature follicle cells for Drosophila ovulation. L. Deady1, A. Beard1, T. Tucker1, J. Sun1,2 1) Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

Octopamine mushroom body receptor (Oamb), an alpha adrenergic-like GPCR, is an octopamine (OA) receptor and is involved in many aspects of Drosophila behavior, including learning and memory, reward behavior, sleep-wake cycles, courtship behavior, oviduct contraction, and ovulation. Oamb can induce Ca2+ increase and cAMP production in heterologous cell systems; however, the signal transduction in vivo has not been characterized. Our recent work has demonstrated that OA activates Oamb on mature follicle cells to increase intracellular calcium and stimulate follicle rupture/ovulation. In the current study, we use genetic/pharmacological tools and calcium imaging to demonstrate the signal transduction pathway downstream of Oamb in mature follicle cells. We identified the Go protein transducing the Oamb signal as Gqq, whose activation is sufficient to induce Ca2+ increase and follicle rupture. In addition, we showed that IP3 receptor (encoded by CG1063) and Stromal interaction molecule (Stim, encoded by CG9126), which mediates store-operated calcium entry (SOCE), functions downstream of Oamb and Gqq to mediate Ca2+ increase and follicle rupture. Our results suggest that OA/Oamb in mature follicle cells activates the Gqq-coupled and SOCE-mediated pathway to induce follicle rupture/ovulation, and this signal transduction may be used in other Oamb-mediated cellular processes. It is interesting to note that Gqq has also been implicated in mammalian follicle rupture with no clear molecular mechanism. Our work suggests a similar adrenergic signal coupled with Gqq may function in mammalian ovulation.

125 Emei Regulates ER Cal2+ Storage to Drive Hippo-mediated Tumorigenesis. X. Ma1, J. Lu1, A. Moraru2, A. Teleman2, T. Xu1 1) Genetics, Yale School of Medicine, New Haven, CT; 2) German Cancer Research Center (DKFZ), Heidelberg, Germany.

Calcium iron (Ca2+) is a versatile second messenger that regulates various cellular and physiological functions, including contraction, secretion, gene expression and proliferation. However, the in vivo molecular mechanisms by which Ca2+ alternation contributes to tumorigenesis remain poorly understood. Here we show in Drosophila eye imaginal epithelial that loss of Emei, a novel ER localized novel tumor suppressor, synergizes with RasV12 to induce tumor progression via JNK-mediated Hippo inactivation. Emei disruption reduces ER Ca2+ storage and subsequently activates JNK signaling by increasing cytosolic Ca2+ level. Importantly, genetically increasing cytosolic Ca2+ concentration cooperates with RasV12 to drive tumorigenesis via inactivating the Hippo pathway. Our findings provide a mechanistic basis for Ca2+ signaling in regulating tumor progression, and further highlight the power of Drosophila as a model system to address human cancer biology questions.

126 A Yorkie-inhibitory checkpoint downstream of the tumor suppressor Fbw7. J. Wardwell-Ozgo1, J. Kupsc2, K. Moberg1 1) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA; 2) Woodruff Health Sciences Center, Emory University, Atlanta, GA.

The Hippo signaling pathway is a central regulator of organ growth and tissue homeostasis and is highly conserved between Drosophila and mammals. It controls growth by integrating intrinsic and extrinsic cues to limit nuclear import of the oncogenic transcriptional co-activator Yorkie. In the absence of inhibitory signals, Yorkie enters the nucleus and promotes expression of genes involved in cell proliferation and survival. The importance of this conserved pathway is highlighted by a growing body of evidence suggesting that dysregulation of the Hippo pathway underlies various human cancers. While the role of the Hippo pathway in growth control is fairly well defined, less is known regarding the extent to which other pathways and checkpoints also impinge on its regulation of Yorkie. Here we describe evidence of an anti-growth checkpoint linking Yorkie and the conserved tumor suppressor Fbw7 (archipelago). Fbw7 is the substrate adaptor of SCF-type E3 ubiquitin ligase that stimulates turnover of Cyclin E and dMyc. Thus, in most contexts, loss of Fbw7 leads to tissue hyperplasia caused by dMyc and Cyclin E hyper-accumulation. However, we find that loss of Fbw7 in a small group of cells in the developing wing epithelial sheets leads to paradoxical undergrowth that is associated with reduced Yorkie protein levels and activity. These effects are phenocopied by overexpression of Cyclin E, which appears to inhibit Yorkie by triggering its endolyssosomal routing and turnover. Additional genetic interactions between Fbw7 and Hippo components provide further support for a Yorkie-inhibitory checkpoint engaged in Fbw7-deficient cells that slows growth and elevates apoptosis. Insight into the functional relevance of this checkpoint could shift current research paradigms and offer potential therapeutic opportunities to the large cohort of Fbw7-deficient tumors in humans. Current work is focused on defining the relationship between Fbw7, Cyclin E, and Yorkie trafficking and turnover in wing disc cells.

Strict control mechanisms are needed to ensure the proper growth of an organ, yet the molecular nature of these mechanisms remains a fundamental question of biology. In an unbiased mosaic screen for genes involved in growth regulation, we identified a loss-of-function allele of the gene CtBP that conferred a growth advantage to homozygous mutant tissue. CtBP encodes a widely conserved transcriptional co-repressor with a critical function in development, yet its role in regulating tissue growth is unclear. To determine how CtBP might affect growth, we surveyed a range of reporters for known growth regulatory pathways for a response to CtBP manipulation. We find that expression of the growth-promoting microRNA bantam (ban) is distinctly sensitive to changes in CtBP activity. ban is a known target of the Hippo pathway effector Yorkie (Yki), yet the expression of two other Yki target genes, expanded and diap1, are not affected by CtBP, suggesting that CtBP is not a general modulator of the Hippo pathway. In fact, we observe that even a minimal enhancer reporter of ban is highly responsive to CtBP inactivation, and this effect can occur independent of Yki. Instead, this response appears to be expressively dependent on activity of the conserved Jun N-terminal Kinase (JNK) pathway. While JNK signaling has been broadly studied as a stress-response pathway, with key roles in regenerative and tumors growth, its function in developmental growth is less understood. We find that JNK activity is necessary and sufficient to drive expression of the minimal ban enhancer. Intriguingly, the Drosophila ortholog of Fos, a component of the downstream JNK pathway effector AP-1, contains a predicted CtBP-binding motif in its protein sequence, indicating that AP-1 may switch from a transcriptional activator to a repressor by recruiting CtBP. Taken together, our findings support a critical role for JNK signaling in promoting developmental growth by ensuring proper levels of ban during tissue development and that CtBP restricts growth by antagonizing ban expression.

128 Polyploid cell growth is required for wound repair to prevent mitotic induced cell death.  J. Grendler, V.P. Losick  MDI Biological Laboratory, Bar Harbor, ME.

Cells can either grow in size by becoming polyploid or divide during tissue repair. The molecular mechanism limiting cell division and promoting polyploid growth however remains poorly understood. Here we found that injury to adult fly epithelium causes cells to enter S phase through activation of Yki-dependent cell cycle genes: myc, cycE, and e2f1. Cells then fail to express mitotic cyclins and constitutively express the ubiquitin ligase Fzr, which targets the mitotic cyclins for proteolytic degradation. As a result, cells grow instead of dividing during wound repair similar to developmentally programmed polyploidy. The mitotic cell cycle program can be re-activated by simultaneously expressing the mitotic activator, Stg, while knocking down Fzr. However, forcing the mitotic cell cycle is detrimental to wound repair resulting in cell death and loss of epithelial integrity. In conclusion, Yki-dependent gene expression drives polyploid cell growth to enable wound repair when cell division is not a viable option.

129 Two distinct actin regulations are controlled by Annexins, RhoGEFs, and Rho family GTPases to orchestrate actomyosin ring dynamics in cell wound repair.  M. Nakamura, J.M. Verboon, A.N. Dominguez, S.M. Parkhurst  Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Single cells composing tissues and organs are subjected to damage caused by daily wear and tear and environmental/physiological stresses. To survive this damage and remain functional, cells have a robust repair mechanism comprised of rapid membrane resealing/remodeling and dynamic cytoskeletal repair at the cell cortex. Rho family GTPases are known to be one group of proteins that dynamically regulate the recruitment of actin and myosin to the wounds and their subsequent assembly into an actomyosin ring necessary for wound closure. For these processes, the activities and localization of Rho family GTPases are spatiotemporally regulated and dependent on the pre-patterning established by three RhoGEFs (RhoGEF2, RhoGEF3, and Pebble) that are known to be major upstream regulators of these GTPases. We find that AnnexinB9 (AnxB9) responds to a calcium influx and mediates actin stabilization required for RhoGEF recruitment to wounds. Thus, our results suggest that two distinct actin regulations mediated by AnxB9 and RhoGEFs/Rho family GTPases are required for cell wound repair: actin stabilization mediated by AnxB9 allows RhoGEFs to establish pre-patterns and then Rho family GTPases contribute to the assembly and translocation of the actomyosin ring. To further elucidate the mechanisms of early actin regulation and how RhoGEF3 and Pbl patterns are established, we examined the roles of the other two fly annexins: AnxB10 and AnxB11. We find that all three Anxs are required for cell wound repair and accumulate around the wounds in discrete localization patterns such that they form a pre-pattern to which the RhoGEFs respond. Surprisingly, this pre-pre-pattern for Rho family GTPase functions is established in less than 10 seconds. We are currently investigating the role of calcium and other early cell wound repair factors on Anxs spatiotemporal patterning dynamics.

130 The regulation of E-cadherin endocytosis by p120-catenin is dependent on RhoA and Arf1.  J. Greig, N. Bulgakova  Biomedical Science, University of Sheffield, Sheffield, South Yorkshire, GB.

Adhesions between cells are vital for the formation of multicellular structures and cell-cell communication. E-cadherin is the principal transmembrane protein which provides such adhesions between cells in the epithelium. The correct regulation of E-
cadherin localization, in space and time, is indispensable for proper development. Similarly, the dysfunction of E-cadherin is implicated in multiple disease processes, particularly cancer metastasis. E-cadherin is a highly dynamic protein which is constantly being turned over at the plasma membrane by endocytosis and recycling. The p120-catenin protein (p120ctn) has been identified as the key regulator of E-cadherin presentation and stability at the membrane, with emerging evidence that p120ctn can both prevent and promote E-cadherin internalization via the endocytotic system. However, the precise mechanism of how p120ctn regulates E-cadherin turnover has yet to be elucidated. To investigate the mechanism, we examined the effect that the loss of p120ctn has on clathrin. Under such a condition we found that clathrin forms larger and less mobile puncta at sites of cell adhesion. Suggesting that p120ctn activity is required for the abscission of the lattice from the membrane during endocytosis. Through a screen of candidate intermediaries between p120ctn and clathrin, we discovered that the loss of p120ctn leads to the downregulation of two GTPases: RhoA (measured through the localization and amounts of Rho-kinase, and cortical non-muscle myosin II) and Arf1. Arf1 is known to promote abscission of clathrin vesicles through local remodelling of actin cytoskeleton, whereas RhoA regulates many aspects of actin dynamics. We have further discovered that suppression or perturbation of either GTPase results in a significant reduction of E-cadherin amounts at cell membranes.

Our results support a model whereby p120ctn has a dual function in the regulation of the endocytotic turnover of E-cadherin, by recruiting and activating RhoA and Arf1. Activation of Arf1 would facilitate the cytoskeletal rearrangements required during invagination of the membrane and the formation of vesicles. At the same time, activation of RhoA would increase cortical tension through activation of non-muscle myosin II, thus counteracting membrane deformation.

131  Defining the solution space for the even-skipped expression pattern suggests regulatory plasticity in Drosophila. Ben Vincent¹, Meghan Bradgon¹, Garth Isley², Tara Lydiard-Martin¹, Clarissa Scholes¹, Zeba Wunderlich¹, Javier Estrada¹, Angela DePace¹ ¹) Department of Systems Biology, Harvard Medical School, Boston, MA; 2) Okinawa Institute of Science and Technology Graduate University, Onna, Okinawa 904-0495, Japan.

Developmental genes are regulated by multiple enhancer elements, each of which generates a portion of the total expression pattern necessary for correct cell type specification. Within enhancers, transcription factor binding sites can change over evolutionary time while maintaining enhancer function. Here, we investigate whether the function of individual enhancers can change while maintaining the expression pattern of the whole locus. We use the Drosophila gene even-skipped as a case study, which is expressed in seven stripes along the anterior-posterior axis in the blastoderm embryo. Are there different ways to partition the seven eve stripes among a set of modular enhancers? This question has been the subject of speculation since the eve locus was first dissected. Here, we define computational and experimental methods to address it directly. To predict alternative solutions for the seven-stripe eve pattern, we use computational models of enhancer function to predict how different eve stripe combinations can be generated from endogenous enhancers. We then validate these predictions by measuring the output of engineered enhancers in embryos. Our work suggests that there may be many ways to build a complex developmental expression pattern, which may influence how developmental genes evolve.

132  Nuclear microenvironments modulate transcription from low-affinity enhancers. J. Crocker¹, A. Tsai¹, A. Muthusamy², L.D. Lavis², R.H. Singer², D.L. Stern² ¹) EMBL, Heidelberg; 2) Janelia Research Camps, HHMI.

Transcription factors bind low-affinity DNA sequences for only short durations. It is not clear how brief, low-affinity interactions can drive efficient transcription. Here, we report that the transcription factor Ultrabithorax (Ubx) utilizes low-affinity binding sites in the Drosophila melanogaster shavenbaby (svb) locus and related enhancers in nuclear microenvironments of high Ubx concentrations. Related enhancers colocalize to the same microenvironments independently of their chromosomal location, suggesting that microenvironments are highly differentiated transcription domains. Manipulating the affinity of svb enhancers revealed an inverse relationship between enhancer affinity and Ubx concentration required for transcriptional activation. The Ubx cofactor, Homothorax (Hth), was co-enriched with Ubx near enhancers that require Hth, even though Ubx and Hth did not co-localize throughout the nucleus. Thus, microenvironments of high local transcription factor and cofactor concentrations could help low-affinity sites overcome their kinetic inefficiency. We further discuss ongoing effects to understand the underlying mechanisms of microenvironments. Mechanisms that generate these microenvironments could be a general feature of eukaryotic transcriptional regulation.

133  Assessing the role of Hox-cofactor interactions in vivo. Siqian Feng¹, Chaitanya Rastogi², Harmen Bussemaker², Richard S. Mann¹ ¹) Jerome L. Greene Science Center, Columbia University, New York, NY; 2) Department of Biological Sciences, Columbia University, New York, NY.

Hox proteins determine segmental identities along the anterior posterior body axis in metazoa. How Hox proteins specify the morphology of different body segments presents a paradox: Different Hox proteins display specific in vivo functions, yet have very similar DNA binding profiles as monomers in vitro. One hypothesis to explain this paradox is that Hox proteins interact with cofactors, and different Hox-cofactor complexes bind to unique DNA sequences. Indeed, it has been shown previously that Extradenticle (Exd) is a common cofactor for all 8 Drosophila Hox proteins, and different Hox-Exd
heterodimers show distinct DNA binding preferences in vitro using a mechanism known as latent specificity. However, the extent to which Hox DNA binding and function is modulated by Exd in vivo is still unknown.

To address this question, we have engineered several endogenous Hox loci to create 3xFLAG tagged WT alleles and 3xFLAG tagged (YPWM-AAAA) alleles, which have the canonical Exd interaction motif mutated. For the Hox gene Sex combs reduced (Scr), we characterized the phenotypes of the YPWM-mutated allele, which is embryonic lethal as a homozygote, in embryos, imaginal discs and adults. We find that mutating the YPWM motif affects some, but not all Scr functions. We next compared the DNA binding profiles of wild type and YPWM mutant Scr using ChIP-seq in T1 leg discs, and identified hundreds of YPWM-dependent Scr peaks, which we hypothesize are cofactor-dependent binding events. Consistently, the centers of these peaks show a strong enrichment for Hox-Exd heterodimer DNA motifs. Interestingly, AnTChiP experiments show that this Hox protein is depleted from these cofactor-dependent Scr peaks in both T1 and T2 leg discs, suggesting that many of the Scr cofactor-dependent binding events are specific to this Hox protein. To further test this idea, we analyzed a subset of these cofactor-dependent Scr binding regions in lacZ reporter gene assays. In many cases, we find that Hox-Exd binding site predictions correlate with expression specificity in vivo. Taken together, these results represent an important step towards understanding how cofactors modulate the binding specificities and functions of Hox proteins in vivo.

Changes in a Hox gene and its downstream regulatory network drive microevolution. Y. Liu1, M. Ramos-Womack2, K. LaRue2, W. Rogers3, T. Williams1, P. Andolfatto1,2, D. Stern3, M. Rebeiz1 1) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 2) Department of Ecology Evolution and Behavior, Princeton University, Princeton NJ; 3) Department of Biology, University of Dayton, Dayton, OH; 4) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 5) Janelia Farms Research Campus, Howard Hughes Medical Institute, Ashburn, VA.

A major objective of evolutionary biology is to derive a realistic understanding of how traits arose in the distant past through the genetic examination of recent phenotypic change. Hox genes play highly conserved roles in the organization of animal body plans, yet it has been difficult to measure their relative contribution to the evolution of body plan phenotypes. Here, we describe how mutations in a Hox gene and its downstream network generated differences in abdominal pigmentation between Drosophila yakuba and D. santomea. In D. santomea, regulatory mutations in the Hox gene Abd-B shifted its expression along the body axis. In parallel, regulatory changes at pdm3, encoding a pigment-repressing transcription factor, temporally extended its expression during pupal development. In two pigment-producing enzymes, we attribute the loss of yellow primarily to upstream changes, while the gain of ebony resulted from a transposon insertion that disabled a silencer element. We have confirmed the contributions of these loci by CRISPR-Cas9 induced mutations in hybrid animals. Our results highlight how the dissection of a microevolutionary trait regulated by these body plan genes uncovers a much more complex genetic composition of Hox genes and their downstream networks. Thus, by extension we envision that our findings set a precedent for anticipating such complexity when small changes are compounded over hundreds of millions of years.

Transcriptional bursting of segmentation gene expression in living Drosophila embryos. B. Lim1,2, T. Fukaya1,3, M. Levine1 1) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 2) Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA; 3) Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan.

Past studies on gene regulation in development have emphasized the spatial limits of gene expression. Newly developed quantitative live imaging methods offer the first opportunity to explore the temporal limits of gene expression. We have characterized the transcriptional dynamics of even-skipped (eve) and fushi tarazu (ftz) expression in living Drosophila embryos. The endogenous loci were tagged with MS2 and PP7 stem-loop RNAs using genome editing methods. Highly dynamic patterning of eve and ftz is observed, whereby nascent transcripts are initially expressed throughout the embryo and rapidly refined into sequential stripes. Surprisingly, the strongest early sites of ftz expression correspond to the inter-stripe regions of the mature pattern, suggesting a substantial reorganization in expression. Moreover, different stripes show distinct transcriptional kinetics, such that posterior nuclei within a stripe produce more mRNAs than anterior nuclei. Both eve and ftz display transcriptional bursting, and spatial refinement appears to occur by silencing gene expression during the refractory period between bursts. This implies that transcriptional bursting can be used as a mechanism to facilitate dynamic repression of gene expression. In summary, we propose that transcriptional bursting facilitates dynamic gene control in development.

Mechanisms regulating Grainy head activity during development. M. Nevi1, K. Schulz, C. Bartolotti 1) Department of Biomolecular Chemistry, University of Wisconsin - Madison, Madison, WI.

Metazoan development requires dramatic changes in cell identity to generate the diversity of cells that comprise the adult organism. These changes are orchestrated largely by shifts in gene expression, which have been proposed to be controlled by temporally dynamic transcription-factor binding. To further investigate the dynamics of transcription-factor binding, we focused on the highly conserved, master regulator of epithelial cell fate, Grainy head (GRH). Drosophila melanogaster possesses
a single grh gene—the founding member of the GRH family. Using a genomics approach, we determined both where GRH binds in the genome and the effects of GRH depletion on gene expression through embryonic development. Our results showed that GRH DNA occupancy remains largely unchanged through embryonic and larval development, but that DNA binding is not predictive of whether GRH is required for target gene expression at any single developmental time point. Thus, temporally dynamic binding is not responsible for stage specific GRH activity, and events subsequent to DNA binding are responsible for the regulation of GRH target gene expression. Previous studies have suggested several mechanisms that may function together or independently to regulate GRH activity, including variation in cofactor interactions, post-translational modifications, and changes in chromatin accessibility. We are investigating the roles of these various pathways in controlling GRH function during development. From our genomic analysis of GRH, we proposed that GRH might modulate chromatin accessibility to define cis-regulatory regions. Preliminary data support this hypothesis at a subset of loci. This GRH-mediated accessibility might be controlled through interactions with chromatin remodelers. Thus, we are currently investigating the hypothesis that GRH activity is modulated through the cofactors it recruits. In addition, phosphorylation regulates GRH activity during wound healing. To determine the role of this modification in development, we identified a novel site of phosphorylation and are characterizing its functional significance. To better understand the function of this conserved transcription factor in development and disease, we have demonstrated that GRH activity is not regulated at the level of DNA binding and are currently determining the pathways that control the activity of this essential factor during development.

137 The Ecdysone Hormone Receptor directs genome-wide changes in gene expression and chromatin accessibility during wing morphogenesis. C.M. Uyehara1,2,4, D.J. McKay1,2,4 1) Department of Biology; 2) Department of Genetics; 3) Curriculum in Genetics and Molecular Biology; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

A remarkable feature of insect development is the complete transformation of the body during metamorphosis. Decades of research have established the central role that pulses of the steroid hormone ecdysone play in promoting this process. At the genetic level, ecdysone acts through its receptor, ECR, which has been shown to induce a diverse array of transcriptional responses that vary between tissues and over time. However, although it has been known for many years that ECR promotes changes in gene expression, our understanding of how it effects these changes genome-wide in vivo remains incomplete. To investigate this ability, this project focuses on how ECR directs gene expression changes in the developing wing during the late-larval ecdysone pulse, which triggers the transition from the larval to pre-pupal stage. To determine ECR's role in promoting wing morphogenesis, we used a tissue-specific RNAi driver to knockdown ECR throughout wing development. We find that while ECR is not required for patterning the wing prior to the late-larval ecdysone pulse, it is essential to promote the majority of gene expression changes that follow this pulse. To identify direct targets of ECR in the developing wing, we used the recently developed technique CUT&RUN to generate genome-wide DNA binding profiles just prior to the late-larval ecdysone pulse and 6hrs following this pulse. We find that ECR binds to many thousands of sites genome-wide, including near many genes that have not been previously identified as direct targets of ECR. We also find that ECR binding is highly dynamic—a subset of its binding sites are unique to each time point. Lastly, to determine whether ECR promotes changes in the accessibility of DNA regulatory elements, we performed FAIREseq in WT and ECR knockdown wings. Surprisingly, we find that there are thousands of sites that change accessibility in WT wings during this time period, and nearly all of these fail-to-occur in ECR knockdown wings. Overall, these findings indicate that ECR shapes the response to hormone through multiple mechanisms, including by directly acting at thousands of DNA regulatory elements across the genome.

138 Protein valuation modulates aging under a complex nutritional environment. Y. Lyu1, H. Shaukat2, M. Plumhoff2, J. Eagy2, K. Wei2, S. Pletcher1 1) Department of Molecular and Integrative Physiology and Geriatrics Center, Biomedical Sciences and Research Building, University of Michigan, Ann Arbor, MI, USA; 2) College of Literature, Science, and the Arts, University of Michigan, Ann Arbor, MI, USA.

Reduced caloric intake had long been considered a powerful method for lifespan extension. This traditional view has recently been challenged, however, as accumulating evidence suggests that the levels of each macronutrient is important in modulating aging. Using Drosophila melanogaster, we discovered that when animals are presented with a diet in which macronutrients (sugar and yeast) have been separated (i.e., a choice diet) mortality rate and physiology are significantly impacted within 12 hours compared to siblings housed on a fixed diet of equivalent caloric and nutrient composition. In short, when flies are presented with a choice diet and allowed to direct their own nutrient intake, their reproductive rate is increased, lifespan is decreased, and internal fat and carbohydrate stores are rapidly, and significantly, altered. Importantly, flies given a choice diet do not consume different amounts of protein, and our data establish that a small set of neurons in the brain which mediate hunger and satiety are sufficient to modulate lifespan and physiology by influencing environmental macronutrient sensing. Flies that cannot synthesize serotonin, lack signaling through serotonergic neurons, or have loss of function in a single serotonin receptor, are immune to the effects of a choice diet and live nearly twice as long as wild-type. Our data suggest that specific neuronal assemblies, which evaluate internal and external nutrient availability and initiate physiological changes associated with neurological states such as hunger and satiety, play important roles in the modulation of lifespan. We hypothesize that protein valuation regulates lifespan by potentiating a protein hunger-satiety cycle, which
induces neuronal states reflective of the dynamics of both external and internal protein availability. These dynamics require serotonin signaling as well as other neuronal substrates. Understanding the neuronal mechanisms of aging in this short-lived model organism will likely provide new ideas for anti-aging interventions and illuminate how mechanisms of aging are influenced by motivational drives that are common to nearly all organisms.

139 Regulation of lifespan by dSirt6 in Drosophila melanogaster.  Jackson Taylor1, Jason Wood2, Chengyi Chang3, Matthew Finn4, Julianna Liu5, Stephen Helfand6 1) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 2) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 3) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 4) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 5) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 6) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI.

Sirtuins (SIRT1-7 in mammals) are a family of NAD+ dependent deacylases which regulate multiple cellular pathways involved in the aging process. SIRT6, in particular, has emerged as a key regulator of longevity, with roles in DNA repair, metabolism, and chromatin modification. Sirt6 knockout mice are short-lived, while overexpression of SIRT6 extends lifespan in male mice. Despite its involvement in many age-related pathways, the precise molecular mechanisms by which SIRT6 extends lifespan are not known. Here, we use the powerful genetic tools of Drosophila melanogaster to investigate the role of SIRT6 (dSirt6 in Drosophila) in regulating lifespan. We find whole-body overexpression of dSirt6 extends lifespan in flies, up to 38% in males and 40% in females. This lifespan-extending effect is repeatable in multiple overexpression systems, including both constitutive and inducible driver lines. dSirt6 OE flies exhibit reduced expression of H3K9ac and H3K56ac histone modifications at both young and old ages. Sirt6 overexpression also extends survival of flies treated with paraquat, an inducer of DNA damage, in young and aged flies. Together, these data suggest that dSirt6 overexpression robustly extends lifespan in Drosophila, and may promote longevity via both epigenetic mechanisms and through improvement in DNA repair.

140 The translation inhibitor 4E-BP regulates the effect of ambient temperature on Drosophila metabolism and lifespan.  I. Drago1,2, G.B Carvalho1,2, S. Hoxha1,2, R. Yamada1,2, O. Mahneva1, K.D. Bruce1,2, A. Soto Obando1,2, B. Conti1,2,4, W.W. Ja1,2 1) Department of Neuroscience, The Scripps Research Institute, Jupiter, Florida, USA; 2) Center on Aging, The Scripps Research Institute, Jupiter, Florida, USA; 3) Department of Biological Sciences, Florida Atlantic University, Boca Raton, Florida, USA; 4) Department of Molecular Medicine, The Scripps Research Institute, La Jolla, California, USA.

Studies on the biology of aging have revealed genetic and environmental factors that affect lifespan in different experimental models. Genetic factors include the insulin-signaling pathway, the GTPase Ras, and the histone deacetylase Sir2. Environmental factors include ambient temperature and nutrition. Temperature is the most potent environmental factor identified thus far: in Drosophila, a drop in ambient temperature from 25 °C to 18 °C doubles lifespan. While thermodynamic effects likely contribute to longevity in poikilotherms, physiological thermoresponsive mechanisms also play a role. It was recently discovered that the thermosensitive channel TRPA-1 is required upstream of DAF-16/FOXO to extend C. elegans lifespan at lower temperatures. Here, we show that in Drosophila, although lower ambient temperature decreases global protein translation, cold activates the elf4E binding protein 4E-BP, a genetically conserved translation inhibitor, to selectively maintain mitochondrial protein synthesis and activity. Using both genetic and pharmacological manipulations, we demonstrate that higher levels of active 4E-BP increase mitochondrial activity and blunt cold-mediated lifespan extension. Conversely, downregulating 4E-BP dampens metabolic responses to cold and extends life at cooler temperatures. 4E-BP is a nutrient sensor that has been implicated in lifespan extension by dietary restriction. Although previous studies have suggested that diet and temperature affect Drosophila aging through independent mechanisms, our results instead suggest that 4E-BP acts as a central regulator of lifespan and metabolism in response to environmental conditions.

141 A genetic program for germline quiescence.  Ethan Greenblatt, Allan Spradling  Department of Embryology, Carnegie Institution for Science, Baltimore, MD.

Germ cells alternate between periods of proliferation and quiescence in many animals, and some germ cells, such as those found in primordial follicles in humans, can be maintained in an arrested state for up to five decades prior to activation. How germ cells maintain their viability during extended periods of quiescence while remaining capable of responding rapidly to external cues is unclear. We established Drosophila as a model system for understanding the genetic requirements of extended cellular quiescence and identified dFmr1, a homolog of Fragile X Mental Retardation Protein 1 – a gene linked to a prominent human disorder resulting in premature reproductive failure – as a factor essential for the maintenance of arrested Drosophila oocytes. While arrested oocytes remain viable for up to several weeks following their production, those lacking dFmr1 become rapidly inviable, producing embryos with severe neuronal defects. We found that dFmr1 functions as a translational activator of a specific subset of genes during oocyte arrest, including the N-recognition Purity of Essence (Poe), as well as a number of genes with known neuronal functions. Poe is required for sustained oocyte arrest, and its expression is induced in an Fmr1-dependent manner starting in the final stages of oocyte maturation. These data indicate that the process of germline arrest is far more active than previously thought, requiring a set of "pilot light" genes whose continual translation...
is essential for the maintenance of viability. Some pilot light genes, such as Fmr1, orchestrate the expression of factors that are especially important for embryonic neural development.

142 **A Virus-acquired host cytokine controls systemic aging by antagonizing apoptosis.** M. Mlih, M. Khericha, J. Karpac

Aging is characterized by degeneration of unique tissues. However, tissues do not age in isolation, and dissecting the interconnectedness of tissue aging remains a challenge. Here, we employ a muscle-specific DNA damage model in *Drosophila* to reveal secreted factors that influence systemic aging in distal tissues. Utilizing this model, we uncovered a cytokine, Diedel, that when secreted from muscle or adipose can attenuate age-related intestinal tissue degeneration by promoting proliferative homeostasis of stem cells. Diedel is both necessary and sufficient to limit tissue degeneration and extend lifespan. Secreted homologs of Diedel are also found in viruses, having been acquired from host genomes. Focusing on potential mechanistic overlap between cellular aging and viral-host cell interactions and, we found that Diedel is a functionally conserved inhibitor of apoptosis and thus acts a systemic rheostat to limit apoptosis-induced degeneration of the aging intestine. These results highlight a key role for secreted antagonists of apoptosis, distinctively those acquired by viral genomes, in the systemic coordination of tissue aging.

143 **Increasing glucose uptake prevents age-dependent reductions in local ATP levels in brain neurons and suppresses declines in locomotor functions in *Drosophila*.** M. Oka1, E. Suzuki2,3, S. Hisanaga1, KM. Iijima4,5, K Ando1

Aging is associated with metabolic changes at system and cellular levels. Neurons are highly energy-demanding cells and require local management of metabolism due to their polarized structures. Disruption of local energy homeostasis in neurons has been associated with neurodegenerative diseases. However, whether and how ATP levels changes in different parts of the neuron in the brain during normal aging, and whether it plays a causative role in the age-associated decline in neuronal functions, are not fully understood.

In this study, we analyzed the age-dependent changes in ATP levels in the cell body and axon of brain neurons in *Drosophila* by using a genetically encoded fluorescent ATP biosensor. We found that ATP levels reduced in the cell body, but not in the axon, during aging. To gain insight into underlying mechanisms, we analyzed the processes of ATP production in young and aged brain neurons. Ultrastructural analyses revealed that the number of mitochondria was not reduced, while mitochondria without normal cristae were more often observed, in the cell body in the aged brain neurons. We also found that mRNA levels of glycolytic enzymes were reduced during aging. Furthermore, mRNA levels of neuronal glucose transporter were reduced during aging. Flies with suppression of glycolysis by knockdown of a glycolytic enzyme did not show age-dependent changes in ATP levels in the cell body, suggesting that reduction in expression of glycolytic enzymes contributes to reduction in ATP in the cell body during aging. Interestingly, enhancement of glucose uptake by overexpression of glucose transporter in neurons suppressed age-dependent reduction in the ATP levels, suggesting that intracellular glucose levels may limit ATP production in neurons in the aged brain. Importantly, overexpression of glucose transporter rescued the age-dependent decline in locomotor functions, suggesting that reduction in ATP levels in neurons may contribute to decline in neuronal functions during aging.

In summary, our results indicate that ATP levels decrease in the cell body of brain neurons during aging and that changes in neuronal glucose uptake may contribute to this process. These findings may help understanding mechanisms underlying decline of brain functions during aging and the pathogenesis of age-associated neurodegenerative diseases.

144 **An ABC transporter regulates aging-induced intestinal stem cell dysplasia in the midgut of Drosophila.** A. Sasaki1, T. Nishimura2, S. Yoo1

How homeostasis of stem cells becomes disrupted during aging is a fundamental and unresolved question. Intestinal stem cells (ISCs) overproliferate during aging, leading to tissue dysplasia in Drosophila. The ISC overproliferation was proposed to be a key mechanism for aging-induced deterioration of health and its regulation is different between males and females. Here we identify an ABC transporter that regulates the aging-induced intestinal stem cell proliferation in the midgut of Drosophila. The ABC transporter mutation completely suppresses the aging-induced ISC overproliferation and also regulates ISC responses to oxidative stress, one of the important processes that occur during aging. The ABC transporter is expressed strongly in the specific compartments of the midgut. A metabolomics analysis of the ABC transporter mutant indicates that there are defects in tryptophan and riboflavin metabolisms in this mutant. We also found that the ABC transporter's binding partner also regulates the aging-induced ISC proliferation and metabolic profiles in a similar manner to the ABC transporter. Furthermore, we elucidated that this ABC transporter is critically involved in the recently found sex difference of the intestinal
stem cell behavior during aging. Our findings provide unexpected, new insights into stem cell and aging biology. This is the first observation, to our knowledge, of an ABC transporter's role in the aging-induced ISC proliferation.

145 Role of the adherens junction protein α-Catenin in the regulation of tissue growth. Lidia Kazakova, Victoria Yan, Ritu Sarpal, Ulrich Tepass  Cell and Systems Biology, University of Toronto, Toronto, Ontario, CA.
Epithelial cells are connected by intercellular adhesion complexes called adherens junctions (AJs), which couple the cytoskeletons of neighboring cells. We analyze α-Catenin, an essential AJ component that links F-actin to the E-cadherin/β-catenin complex, both directly and indirectly through other actin-binding proteins. α-Catenin acts as a tumour suppressor in flies and mammals through the inhibition of oncogenic pathways including Hippo/Yap and JNK. We have further investigated α-Catenin's function in growth regulation of the Drosophila wing disc epithelium. Notably, analysis of a phenotypic series for α-Catenin shows that α-Catenin has genetically separable functions in growth regulation and epithelial integrity. (i) Moderate loss of α-Catenin causes a cell-autonomous tissue overgrowth while wing discs retain junctional and epithelial integrity, and an activation of Hippo/Yap and JNK pathways. (ii) A stronger loss of α-Catenin in conjunction with a suppression of cell death causes the formation of solid multilayered tumors where cells do not exhibit overt protrusive activity. Finally, (iii) cells of the wing disc that completely lack α-Catenin die. If cell death is suppressed, α-Catenin null cells are found below the epithelium and exhibit long protrusions suggesting that they have undergone an epithelial-mesenchymal transition. A similar phenotypic series can be seen for loss of DE-cadherin function. This, together with the finding that a DE-cadherin-α-Catenin fusion protein can fully rescue the α-Catenin mutant defects, suggests that α-Catenin regulates growth as an AJ proteins rather than as a cytosolic factor as was proposed by a number of studies. We are currently exploring the roles of different α-Catenin domains, the ability of α-Catenin to act as a mechanosensor, and how α-Catenin binding partners contribute to growth regulation.


Intracellular trafficking is known to control epithelial cell polarity and vice versa, but the molecular mechanisms underlying this interdependence remain poorly understood. The Class III phosphatidylinositol 3-kinase (PI3K-III) produces phosphatidylinositol 3-phosphate (PI3P) on endosomal and autophagic membranes. We show that PI3K-III inactivation disrupts epithelial organisation, causing dysplasia-like growth and invasive behaviour provoked by activation of the JNK pathway. Liver Kinase B1 (LKB1), a regulator of cellular metabolism and polarity, is found on plasma- and endosomal-membranes. Upon PI3K-III inactivation LKB1 localisation is disturbed and its activity increased. Co-depletion of LKB1 and PI3K-III suppressed JNK activation and restored epithelial integrity. Furthermore, PI3K-III inactivation cooperated with RasV12 to promote tumour growth in vivo in an Lkb1-dependent manner. The endosomal, but not autophagic, function of PI3K-III is required for regulated LKB1 activity. Screening for conserved PI3K-III effector proteins (containing PX or FYVE domains that bind PI3P) that recapitulated the PI3K-III phenotypes identified the endosomal protein WDFY2. We define WDFY2 as an LKB1 regulator that directs epithelial organisation in both Drosophila tissues and human organoids. Thus, we define a novel Class III PI3K regulated endosomal signalling platform of LKB1, the dysregulation of which ends LKB1 with tumour-promoting properties. Available online https://www.nature.com/articles/ncb3631

147 Characterization of a putative prion-like phosphatase, CG5830, in Drosophila melanogaster. Zelina Nii1,2, Kausik S1,2 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Prions are toxic self-perpetuating protein aggregates known to cause several neurodegenerative diseases in mammals. In yeast and more recently in multicellular eukaryotes proteins with prion-like (PrL) domains, which act as regulatory switches controlling normal physiological functions such as metabolism, immune response and memory, have been identified. The fact that non-pathogenic PrL proteins are distributed across multiple phyla raises the possibility that proteins with self-sustaining conformational states are part of an evolutionarily conserved regulatory mechanism involved in normal physiological functions. In a systematic screen for novel PrL proteins in Drosophila, we identified CG5830, a putative phosphatase. CG5830 encodes a 329 amino-acid protein with a PrL domain at N terminal end. We find N-terminal domain of CG5830 is important for its oligomerization and membrane localization. We have identified substrates of CG5830 phosphatase using proteomics. Using one of these substrates, discontinuous actin hexagon (Dah) and purified CG5830, we find oligomerization enhances phosphatase activity. In cells, the PrL domain is important for phosphatase activity in addition to membrane localization and oligomerization. We also find CG5830 is expressed ubiquitously throughout embryonic development. In early embryos CG5830 is diffused in the membrane and in late segmented embryos it becomes punctate. The punctate appearance of CG5830 coincides with dephosphorylation of Dah and in CG5830 mutant embryos development does not proceed after segmentation. We postulate that regulated higher-order assembly of CG5830 via the PrL domain controls its phosphatase
activity and embryonic development.

148 Extracellular adenosine as a cytoprotectant and growth regulator. Michal Zurovec1,2, Lucie Kucerova1, Roman Sidorov1, Vladav Broz1 1) Dept Physiology, Biology Centre, Inst Entomology, Ceske Budejovic, CZ; 2) University of South Bohemia, Ceske Budejovice, CZ.

Extracellular adenosine (Ado) is a signaling molecule that is central to the maintenance of the metabolic balance of cells within a tissue. We previously showed that mutations in the Drosophila Ado receptor (AdoR) or silencing of the equilibrative Ado transporter 2 (Ent2) by RNAi cause a severe reduction in the frequency of hyperplastic wts and dco epithelial outgrowths (Sidorov et al 2015, doi: 10.1007/s11302-014-9435-2).

Here we performed detailed clonal analysis and followed the flipase-induced GFP-labeled mosaic clones during larval development. We observed that the AdoR null clones (with or without the additional wts mutation) initially appear at the same rate as the control clones. The null clones have difficulties with survival in a heterozygous background and are eliminated by apoptosis.

We demonstrate that not only the hyperplastic wts1 mutant clones but also the control neutral clones require functional AdoR; otherwise, they are gradually eliminated. The elimination of wts1 AdoR double mutant clones, however, is more effective, so the cytoprotective effect of AdoR on wts1 cells is more obvious than on wt cells. Our results suggest that Ado signaling differentially affects populations of cells with different genotypes and might be involved in the regulation of mutant cell susceptibility to apoptotic stimuli.

149 Activation-induced substrate engagement in ERK signaling. S. Paul1, L. Yang1,2, H. Mattingly2, Y. Goyal2, S. Shvartsman3, A. Veraksa1 1) Department of Biology, University of Massachusetts Boston, Boston, MA; 2) Department of Chemical and Biological Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

The extracellular signal regulated kinase (ERK) pathway is an essential component of developmental signaling in multicellular organisms. As a final element of the highly conserved Raf-MEK-ERK kinase cascade, ERK phosphorylates multiple substrates, including transcription factors, resulting in a fine-tuned control of target gene expression. The dissociation of activated dually phosphorylated ERK (dpERK) from MEK, a kinase that phosphorylates ERK, and other cytoplasmic anchors has been suggested as a primary regulatory mechanism that allows access of ERK to its substrates. Here, we report that the interaction of ERK with its substrate Capicua (Cic) is also controlled at the level of ERK phosphorylation, where Cic has preferential binding affinity for dpERK, compared to unphosphorylated ERK. Cic-dpERK interaction forms a positive regulatory feedback loop with the mechanism of substrate competition, in which binding to Cic shields dpERK from the action of phosphatases. Mathematical modeling suggests that preferential association of Cic with dpERK serves two functions: first, it prevents unproductive competition of Cic with the unphosphorylated form of ERK, when Cic, ERK and dpERK are localized in the same cellular compartment; second, it contributes to signal amplification and allows for efficient signal propagation when only a small proportion of ERK is converted to dpERK. We propose that high-affinity interactions between activated ERK and its substrates are an important component of this signal transduction cascade. These activation-dependent interactions contribute to robust pathway output along with the induced dissociation of dpERK from MEK and other anchors.

150 Ras-dependent control of tissue morphogenesis. H.E. Johnson1, S.Y. Shvartsman2,3, J.E. Toettcher1 1) Molecular Biology, Princeton University, Princeton, NJ; 2) Chemical and Biological Engineering, Princeton University, Princeton, NJ; 3) The Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

The direct role played by Ras/Erk signaling in cell growth and differentiation has long been appreciated. In contrast, the relationship between Ras signaling, cell motility and tissue-level morphogenesis is complex and has remained poorly defined. Here, we set out to precisely map how the Ras/Erk pathway contributes to one morphogenetic event: collective cell movement during gastrulation in the early Drosophila embryo. Using precise optogenetic control of Ras we found that its pathway is sufficient to induce cells to adopt a contractile cell fate at nearly any illuminated location within the embryo, and where contraction is precisely timed to the onset of gastrulation. Light-stimulated tissue mimics the gene expression and physical organization of the posterior midgut (PMG), a tissue normally patterned by the Torso receptor tyrosine kinase. We define the transcriptional network by which Erk programs PMG cell fate, leading to the accumulation of apical myosin and tissue contraction at gastrulation. By systematically varying the timing and duration of Erk activity, we found that PMG cell fate is determined by the cumulative load of Erk activity delivered over a two hour window in early embryogenesis, revealing a previously unknown long-term memory of signaling that spans multiple nuclear division cycles. Our work mechanistically defines an Erk-dependent cell fate choice and establishes a model system for interrogating how signaling pathway activity can program large-scale changes in tissue organization in vivo.

151 Dynamic 3D tissue architecture directs BMP morphogen signaling during Drosophila wing morphogenesis. Martin Kracklauer1, Jinghua Gui1, Daniel Toddie-Moore1, Yunxian Huang1, Kenji Kikushima1, Stephanie Nix2,
Yukitaka Ishimoto², Osamu Shimm³ 1) Biotechnikian Instituutti, Helsingin Yliopisto, Helsinki, FI; 2) Department of Machine Intelligence and Systems Engineering, Akita Prefectural University, Akita, Japan.

At the level of organogenesis, tissue morphogenesis drives developmental processes in animals, often involving the formation of complex three-dimensional tissue architectures from simpler two-dimensional structures. These processes can be directed by growth factor signaling pathways. However, little is known about how morphological changes affect the spatiotemporal distribution of growth factor signaling. Using live and fixed tissue imaging and in algorithmo modeling approaches in the Drosophila pupal wing, we address how Decapentaplegic (Dpp) / Bone Morphogenetic Protein (BMP) signaling and three-dimensional wing morphogenesis are linked. Our data indicate that Dpp, expressed in the longitudinal veins (LVs) of the pupal wing, spreads laterally throughout both dorsal and ventral epithelia during wing inflation to regulate wing size. Thereafter, the Dpp signal becomes refined to the LVs within each epithelium, and actively trafficks between the two epithelia to further refine vein patterning when the two epithelia appose. Our data suggest that the three-dimensional architecture of the wing epithelia directs the switch from lateral to interplanar Dpp signaling, providing novel insights into how three-dimensional morphogenesis occurs during organ development.

152 ‘Tethered’ Wingless (Nrt-Wg) is released and signals at a distance, consistent with Wg function as a morphogen. S. Petshow, M. Wehrli Integrative Biosciences, Oregon Health and Science University, Portland, OR.

Cell-cell communication by the Wnt signaling pathway is critical during development, stem cell maintenance, and pathogenesis. A key question about how Wnts function is whether Wnt-secreting cells only signal to their immediate neighbors or whether in some instances Wnt must signal at a distance. Alexandre et al. (Nature 505, 180-185) used membrane-tethered Wingless (Nrt-Wg) inserted into the wg locus to address this question. Surprisingly, with Nrt-Wg as the only source of Wg, animals were reportedly fully rescued. The authors concluded that long-range signaling by diffusible Wg was not required at any stage of development. However, for this conclusion to be correct, Nrt-Wg expressing cells must not release any fragments of Nrt-Wg or, if released, Wg must not be functional. As neither of these questions was stringently explored, we examined Nrt-Wg expressed from the endogenous locus, as well as expressed ectopically. Immunostained imaginal discs were cryosectioned and subjected to high-resolution imaging to reveal the protein distribution across the entire depth of the epithelium. As reporters for signaling, we used the Wg-target genes Distal-less (Dll) and Senseless (Sens). We also utilized bi-molecular fluorescence complementation (BiFC) as a more sensitive and rapid indicator for Wg pathway activation developed in the lab, wherein inactivation of the Armadillo/β-catenin destruction complex induces fluorescence.

We find that Nrt-Wg induces Sens and Dll in adjacent cells but not beyond, consistent a previous report (Zecca et al. Cell 87, 833-844). We then tested for release of Wg by Nrt-Wg expressing cells. Extracellular Wg was detected, as well as intracellular Wg, which co-stained for Wg (antibody 4D4) and the HA-tag contained in Nrt-Wg. Importantly, our BiFC reporter indicates that Wg pathway activation occurs away from Nrt-Wg expressing cells. We conclude that Nrt-Wg is not strictly confined to expressing cells but functional Wg is released allowing for long-range signaling. Nrt-Wg ‘rescue’ flies display aberrant patterning, reduced viability and sterility, indicating that normal development requires higher levels of secreted Wg and supports the notion that Wg/Wnts function as morphogens.

153 Damage-Activated Regeneration Enhancers (DAREs) permit control of regenerative capacity independent of the developmental program in imaginal discs. R. Harris¹, I.K. Hariharan² 1) School of Life Sciences, Arizona State University, Tempe, AZ; 2) University of California, Berkeley, CA.

Many organisms lose the ability to regenerate as they mature. Damaged Drosophila imaginal discs regenerate efficiently early in the third larval instar (L3), but progressively lose this ability as they proceed through development. This loss of regenerative capacity correlates with reduced damage-responsive expression of multiple genes, including those regulating the regrowth and re-patterning of the tissue, such as Myc, MMP1, and WNT signaling. These genes also have essential roles in the development of the imaginal disc, and so until recently it was unclear how their regenerative response can be selectively inhibited without compromising their normal developmental expression in undamaged discs. Our previous work found that the expression of two WNT genes, wg and WNT6, during regeneration requires a bipartite Damage-Activated Regeneration Enhancer, or DARE, whose activity declines during L3 as a result of highly localized epigenetic silencing at the enhancer, leading to a progressive loss of regenerative WNT expression. As the silencing is localized specifically to the DARE, this mechanism limits their activation solely in the context of regeneration, without affecting WNT expression directed by normal developmental signals.

Here we extend these findings, revealing that MMP1 is a key regeneration factor that is regulated by an equivalent DARE. This enhancer has a comparable bipartite structure and is inactivated by a similar localized loss of chromatin accessibility in mature tissue. Using a novel ablation system that allows direct genetic manipulation specifically of blastema cells, we show that alteration of MMP1 levels can augment the regenerative response, while knockdown of certain epigenetic silencing regulators in regenerating cells significantly improves regeneration of mature tissues. Analyzing whole-genome chromatin accessibility and transcriptional changes using ATAC-seq and RNA-seq we further show that potential DAREs are widespread.
throughout the genome, and likely regulate multiple factors that, similar to MMP1 and the WNT genes, have essential roles in both development and regeneration.

Thus, we propose a model in which the expression of multiple genes under the control of their cognate DAREs enables an organism to activate a coordinated program of gene expression that leads to regeneration. A mechanism of epigenetic silencing that is localized to the DAREs, and therefore does not affect coding regions and developmentally-regulated enhancers, enables an organism to shut off the capacity to regenerate while preserving the functions of those same genes in normal development.

154 M1BP, a master regulator of housekeeping genes, functions with TRF2 and a coactivator that has glutathione S transferase activity. D.S. Gilmour, D.G. Baumann Biochemistry and Molecular Biology, Penn State University, University Park, PA.

M1BP is a transcriptional activator that associates with core promoter region of approximately 3000 actively transcribed genes in Drosophila. The majority of these genes are ubiquitously expressed throughout development and have elevated levels of paused Pol II in the promoter proximal region. In addition, their gene bodies are highly occupied by an ordered array of nucleosomes. Given that these genes account for almost 40% of the active genes in Drosophila, metagene analyses that fail to take into account the unique features of M1BP-associated genes could lead to erroneous conclusions. To gain insight into mechanisms by which M1BP activates transcription, we have identified and characterized proteins that interact with M1BP. This lead to the discovery that M1BP functions at ribosomal protein genes to recruit the TBP-related factor, TRF2, to the promoters. Moreover, ChIP-exo analysis reveals that TAF1, the largest subunit of TFIID, co-occupies ribosomal promoters with TRF2 suggesting that TRF2 might substitute for TBP in a TFIID-related complex. We have also discovered an M1BP coactivator called GFZF. GFZF has been detected as a hit in numerous genetic and RNAi-mediated screens. ChIP-exo analysis reveals that GFZF binds the promoter region of over one thousand actively expressed genes and that almost all of these genes are co-occupied by M1BP. Reporter gene assays and chromatin immunoprecipitation (ChIP) experiments following RNAi-mediated depletion of GFZF reveal that GFZF functions as a transcriptional co-activator. In addition, we demonstrate that GFZF is a glutathione S-transferase with affinity for glutathione at a physiologically relevant level. GFZF is the first transcriptional co-activator with intrinsic GST activity to be identified, and its identification as a transcriptional co-activator provides an explanation for its role in numerous biological processes. GFZF might also provide means by which the cell can link the oxidation state of the cell to expression of housekeeping genes such as the ribosomal protein genes.

155 Promoter proximal pausing as a possible mechanism for transcriptional regulation by HP1 paralogs. S. John, N.C. Riddle Biology, University of Alabama-Birmingham, Birmingham, AL.

Developing a comprehensive understanding of the components of heterochromatin is key in appreciating inheritance of chromatin states. The protein HP1a is central in the formation of heterochromatin through its role in the propagation of H3K9 methylation and establishing a phase-separated environment between heterochromatin and euchromatin. However, HP1a and its paralogs are also observed to have transcription regulating properties and are known to bind transcription start sites. These additional functions of HP1 proteins likely contribute to the differential expression in a subset of genes between wild type and HP1 paralog knockout mutant Drosophila strains. Here, we investigate one possible mechanism for how HP1 proteins regulate transcription by studying their relationship with RNA polymerase II. We found significantly increased promoter proximal pausing in transcripts bound by HP1 paralogs in wild type third instar larvae and S2 cells using available ChIP-Seq, ChIP-chip and GRO-Seq data. Furthermore, transcripts bound by HP1 paralogs had diminished pausing in relevant knockout strains when compared to wild type, suggesting HP1 paralogs may interact with RNA polymerase II to regulate gene expression. These results help to broaden current understanding of the function of HP1 paralogs through characterizing their activity at transcription start sites.

156 Context matters: chromatin context effects Polycomb domain formation and function. Sandip De, Yuzhong Cheng, Ming-an Sun, Judith Kassis NICHD, NIH, BETHESDA, MD.

Polycomb group proteins (PcGs) are critical for genome organization, development and stem cell regeneration. PcG proteins were first identified in Drosophila as repressors of homeotic genes and form large repressed chromatin domains marked with H3K27me3. Polycomb group response elements (PREs) are DNA-elements that recruit Polycomb proteins (PcG) to chromatin and are thought to initiate PcG domain formation. One big question in the chromatin field is how large domains are formed, especially when proteins regulate transcription by studying their relationship with RNA polymerase I. We have identified and characterized proteins that interact with M1BP. This lead to the discovery that M1BP functions at ribosomal protein genes to recruit the TBP-related factor, TRF2, to the promoters. Moreover, ChIP-exo analysis reveals that TAF1, the largest subunit of TFIID, co-occupies ribosomal promoters with TRF2 suggesting that TRF2 might substitute for TBP in a TFIID-related complex. We have also discovered an M1BP coactivator called GFZF. GFZF has been detected as a hit in numerous genetic and RNAi-mediated screens. ChIP-exo analysis reveals that GFZF binds the promoter region of over one thousand actively expressed genes and that almost all of these genes are co-occupied by M1BP. Reporter gene assays and chromatin immunoprecipitation (ChIP) experiments following RNAi-mediated depletion of GFZF reveal that GFZF functions as a transcriptional co-activator. In addition, we demonstrate that GFZF is a glutathione S-transferase with affinity for glutathione at a physiologically relevant level. GFZF is the first transcriptional co-activator with intrinsic GST activity to be identified, and its identification as a transcriptional co-activator provides an explanation for its role in numerous biological processes. GFZF might also provide means by which the cell can link the oxidation state of the cell to expression of housekeeping genes such as the ribosomal protein genes.

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the strong PREs; the H3K27me3 level was reduced when the strong PREs were deleted from the transgene. We also observed severe abdominal defect in the transgenic line in the absence of the en strong PREs and due to the tissue specific mis-expression of en. In the presence of strong PREs, the 79kb-inv-en transgene was able to set up a 3D PcG domain similar to endogenous inv-en PcG domain. In the endogenous locus, spreading of the H3K27me3 mark is stopped by the ubiquitous transcription of flanking reporter genes. In contrast, the transgenic H3K27me3 repressive mark spreads over flanking genes, except where there is active transcription. Further, unlike in the endogenous locus, genes flanking the inv-en DNA come to be regulated like en. Our data show that gene organization plays a key role in regulating gene expression in development.

157  Multiple roles for insulator complex LBC in regulating gene expression in Drosophila at the local and global levels.  A. Kuribidaeva1,2, E. Schedl1,2 1) Princeton University, Princeton, NJ; 2) Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia.

Chromatin structure influences gene expression on a local level by regulating the expression patterns of individual genes, and on a global level by changing the activity of large gene domains or even entire chromosomes. Local regulation depends upon pairing interactions between boundary elements (insulators) that modulate the topology of individual chromatin domains to either prevent or facilitate regulatory interactions. Globally, multi-gene complexes or even entire chromosomes can experience coordinated epigenetic changes. Examples of global regulation include repression of the fly homoeotic gene complexes by PcG silencing and the upregulation X-chromosome gene expression by MSL complexes. Like the formation of local chromatin domains, these global mechanisms depend upon chromosome architectural elements.

MSL complexes exploit the pre-existing X-chromosome topological organization. This organization depends special elements called Chromatin Entry Sites (CES) that are distributed along the X-chromosome. While CES were initially shown to orchestrate MSL X-chromosome recruitment, recent studies indicate that CES are boundaries of topological domains. Likewise, a large network of contacts between Polycomb response elements (PREs) in different PcG target loci has been discovered in genome-wide studies. Like the CES, this 3D architecture is thought to facilitate the recruitment and/or functioning of PcG proteins. Thus, the MSL and PcG chromatin modifying complexes would appear to share a similar dependence upon chromosome architecture and chromosome architectural elements.

While it has been proposed that pairing interactions between CES or PREs determines the higher-order organization of their regulatory targets, the mechanism mediating the pairing of these elements is unknown. In fact, the core MSL and PcG proteins don't have architectural functions, and don't even bind directly to DNA. For this reason we sought to identify the factor(s) that confers the architectural functions of CES and PREs. We find that a large 1000 kD complex, called the LBC, is responsible for the architectural functions of CES and PREs. The LBC has previously been shown to be required for the architectural functions of two BX-C boundaries, Fab-7 and Fab-8. The LBC binds to a subclass of CES that have multiple Msr Recognition Sequences (MREs). Mutations that disrupt LBC binding in vitro abrogate CES function in vivo. T The LBC also binds to PREs from BX-C, engrailed and eve, and mutations which disrupt LBC binding in vitro abrogate PcG dependent silencing in vivo. We propose that the architectural functions of LBC provide a scaffold for the efficient recruitment and subsequent spreading of the MSL and PcG complexes, facilitating the epigenetic regulation of transcription.

158  A DNA/RNA dual activity topoisomerase regulates transcription through distinct mechanisms.  S. Lee1, Y. Xue1, W. Shen1, Y. Zhang1, Y. Joo1, S. Su1, M. Chinen2, W. Ku3, Y. Ding3, K. Becker1, E. Lei2, K. Zhao3, A. Sharov1, W. Wang1 1) National Institute on Aging, NIH, Baltimore, MD; 2) National Institute of Diabetes and Digestive Kidney Diseases, Bethesda, MD; 3) National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD.

Topoisomerases are essential enzymes that resolve topological stress during DNA metabolism. We have recently demonstrated that many Type IA topoisomerases from all three domains of life are dual-activity topoisomerases that can catalyze reactions on not only DNA, but also RNA. In animals, one of the two Type IA topoisomerases, Top3β, possesses dual activities. Top3β is the major mRNA-binding topoisomerase, and it stably associates with mRNA translation machinery. Current evidence suggests that Top3β forms a complex with TDRD3 (Tudor domain containing 3); and this complex can facilitate DNA transcription by reducing formation of R-loops, as well as regulate mRNA translation by interacting with mRNA-binding proteins, FMRP (Fragile X Mental Retardation syndrome Protein) and exon-junction complex. Mutation of Top3β has been linked to schizophrenia, autism, and intellectual disorders. However, how Top3β acts in DNA or RNA metabolic reactions remains largely unclear. Here we analyzed Top3β function in Drosophila, and found that it regulates transcription through distinct mechanisms.

In the first mechanism, we found that Top3β biochemically and genetically interacts with the RNAi-induced silencing complex (RISC). Position effect variegation assay (PEV) shows that Top3β and RISC mutants are both defective in heterochromatin formation and transcriptional silencing; and this defect is largely suppressed in their double mutants. Moreover, ChIP-seq assay shows that both Top3β and RISC single mutant flies exhibit reduced levels of heterochromatin protein HP1 in pericentric and telomeric heterochromatin; and the reduction in pericentric heterochromatin is also suppressed in their double mutant. Furthermore, expression of several genes and transposable elements within telomeric heterochromatin is increased in the Top3β mutant. Our data demonstrate that a dual-activity topoisomerase can work with siRNA machinery to promote heterochromatin formation and transcriptional silencing. In the second mechanism, our ChIP-seq assay revealed
that Top3β directly bind specific genes in euchromatin, and its binding sites often overlap with those of FMRP and RNA Polymerase II (PolII). Notably, many genes co-bound by Top3β and FMRP in fly heads show reduced Pol II binding and lower levels of transcripts in Top3β mutant flies, indicating that Top3β enhances transcription of these genes. Together, our data demonstrate that a dual-activity topoisomerase can regulate transcription through two distinct mechanisms: to silence gene expression by interacting with the siRNA machinery to promote heterochromatin formation, and to enhance gene expression by increasing amount of Pol II on DNA.

159 Activating and repressing stochastic gene expression between chromosomes. E. Urban, C. Chernoff, K. Viets, S. Tran, R. Johnston Biology, Johns Hopkins University, Baltimore, MD.

Cell fate specification during development is often thought of as a highly reproducible process, such that tight regulation of gene expression determines precise cell fates. Cellular diversity can also arise from stochastic gene expression, as in the specification of bacterial competence states, visual and olfactory receptors, motor neuron subtypes, immune cells and stem cells. In flies, a stochastic fate choice in the R7 photoreceptors of the retina is controlled by the transcription factor Senseless (Ss). Stochastic expression of ss is controlled by a complex cis-regulatory logic including a promoter, two activating enhancers, and two repressing silencers, resulting in a SsON state in 65% of R7s. Homologous chromosome pairing in flies enables interchromosomal communication (InterCom), in which alleles of ss act between chromosomes to upregulate or downregulate ss expression frequency. However, the mechanisms that regulate gene expression between chromosomes are poorly understood. Here we show that InterCom between natural variants of ss with high or low ss expression frequencies yields intermediate ratios of SsON to SsOFF cells, providing a biological role for transvection-like regulation in ensuring proper generation of the photoreceptor mosaic. Interchromosomal activation is mediated by an intact enhancer and promoter on the same chromosome, suggesting that looping in cis promotes gene activation in trans. In addition, the ss silencer represses endogenous ss expression when translocated to the X chromosome. This repression requires the presence of intact X and 3rd chromosomes, indicating that trans-repression is achieved through a chromosomal pairing mechanism. Our findings provide mechanistic insight into how activation and repression between chromosomes averages stochastic on/off gene expression between alleles.


Embryonic cells have their fates defined by transcription factors that are rapidly deployed, yet attempts to visualize these factors in vivo often fail because fluorescent protein fusions mature too slowly. To overcome this limitation we pioneered a protein tag, LlamaTag, which circumvents this maturation limit by binding already mature fluorescent proteins, making it possible to visualize transcription factor concentration dynamics in living embryos. Implementing this approach in Drosophila melanogaster, we discovered stochastic bursts in the concentration of the Fushi Tarazu protein that are caused by bursts in transcription. We further used LlamaTags to show that the concentration of protein in a given nucleus depends on transcription of that gene in neighboring nuclei; we speculate that this is an important mechanism for producing robust gene expression patterns in the fly embryo. Thus, LlamaTags now make it possible to quantify the flow of information along gene regulatory networks, at the single-cell level, in living embryos.

161 VEGF/Pvf1 mediated muscle-oenocyte communication regulates systemic lipid homeostasis. Arpan Ghosh, Norbert Perrimon Genetics, Harvard Medical School, Boston, MA.

Physical activity and improved skeletal muscle health can both prevent and ameliorate the pathophysiological effects of obesity. Somatic muscle (SM)-derived signaling molecules (myokines) are proposed to play a central role in mediating these beneficial effects. Yet the identity of many muscle-derived hormonal signals, their physiological roles and mechanistic details of their action(s) remain poorly understood. The vertebrate VEGF ligands VEGF-A/B encode for putative myokines. Both VEGF-A/B have been implicated in regulating lipid metabolism by affecting vascularization of white adipose tissue (WAT) and lipid transport across endothelial cells. However, whether muscle VEGFs can regulate lipid metabolism by directly signaling to metabolically active organs like the WAT and liver, or by mediating cross-talk between these organs is not known. Here we report that the Drosophila PDGF/VEGF orthologue Pvf1 is an activity-induced myokine that regulates systemic lipid homeostasis by signaling to the fly liver like cells called oenocytes. Muscle specific knock down of pvf1 causes increased accumulation of neutral lipids in the fly adipose tissue and oenocytes indicating a role for muscle-derived Pvf1 in systemic lipid homeostasis. Pvf signaling in flies is mediated by a single VEGFR ortholog, Pvr. Anti-Pvr immunostaining show that the protein is most abundantly present in the oenocytes. Interestingly, inhibiting Pvr signaling only in the oenocytes, and not in the adipose tissue or muscle, by expressing a dominant-negative Pvr (PvrDN) can phenocopy both the adipose tissue and oenocyte lipid accumulation phenotypes. These results show that muscle Pvf1 most possibly signals to the oenocytes to regulate systemic lipid homeostasis. Anti-Pvf1 staining of fly leg muscles show that Pvf1 is abundantly stored in the leg muscles and is localized at the H/Z bands. Such localization has been reported for transcription factors that can sense muscle activity and therefore indicates a role for muscle activity in Pvf1 release. Consistently, flies over-expressing pvf1 in the muscle showed increased sensitivity to exercise mediated loss of stored lipids, indicating that muscle Pvf1 is released in response to
activity and subsequently affects systemic lipid metabolism. These results, along with any new data further characterizing the muscle-oenocyte Pvf1 signaling axis and determining the mechanism by which oenocyte Pvf1 signaling regulates lipid metabolism, will be presented.

Eleftherianos Biological Sciences, George Washington University, Washington, DC.

Lipid droplets (LDs) are specialized organelles which are mainly involved in lipid metabolism. Recent evidence indicates that LDs perform additional functions through participating in certain interactions with pathogenic microbes. However, the exact nature of these interactions and the role of LDs in promoting host immune protection remain currently unexplored. Drosophila has served as an outstanding model for dissecting the molecular basis of innate immunity and lipid metabolism and analyzing the relationship between these important biological processes. Here we show that bacterial infection impairs lipid metabolism in Drosophila adult flies. The impairment is characterized by enlargement of LDs in the primary immune organ, the fat body, enhanced accumulation in the gut, and ectopic accumulation in the Malpighian tubules. The bacterial-induced accumulation of LDs can be mimicked by constitutive activation of the immune signaling pathways Toll, Imd, and Jak/Stat, which are responsible for the induction of antimicrobial peptides and other effector molecules upon infection. The enhanced accumulation of LDs is further linked to the misregulated expression of lipolytic machinery in both flies infected with bacteria and flies with genetically activated immune signaling. Finally, we show that flies with reduced lipid accumulation display increased bacterial burden, which is accompanied by enhanced susceptibility to bacterial infection. Our results show for the first time that the immune role of LDs is also conserved in Drosophila. Such findings generate novel insights into the capacity of the innate immune system and lead to the identification of previously unknown regulators of the host antibacterial immune response.

163 Mechanisms underlying peripheral insulin resistance and metabolic dysfunction caused by chronic immune activation. B.A. Martinez, R.G. Hoyle, M.L. Bland Pharmacology, University of Virginia, Charlottesville, VA.

Innate immune signaling through Toll receptors in the larval fat body inhibits insulin signaling and shifts metabolic pathways to decrease nutrient storage, perhaps to channel energy toward fighting infection. Insulin signaling promotes nutrient storage and whole-animal growth, while activation of Toll receptors during infection or genetic manipulation stimulates anti-microbial peptide synthesis. Chronic immune activation via expression of constitutively-active Toll10b receptors in the larval fat body decreases whole-animal growth and triglyceride levels while increasing fat body glycogen levels with no change in hemolymph trehalose or glucose. We measured transcript levels of genes that encode important lipogenic enzymes in fat bodies isolated from larvae with active fat body Toll signaling and GFP-expressing controls. Transcript levels of the master lipogenic regulator, Sterol Regulatory Element Binding Protein 1 (SREBP1), were doubled in fat bodies with active Toll signaling compared with controls. We detected similar levels of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) transcripts in both groups. Surprisingly, transcript levels of easily shocked (eas), an ethanolamine/choline kinase homolog, were increased six-fold in fat bodies with active Toll signaling. These data suggest that reduced triglyceride levels in larvae with Toll signaling are not due to impaired fatty acid synthesis, but that fatty acids are incorporated into phospholipids at the expense of triglyceride synthesis. Thin layer chromatography of isolated lipids from Toll10b- or GFP-expressing fat bodies show reduced triglyceride but elevated phosphatidylcholine in the Toll samples. We hypothesize that the reason for this metabolic switch leading to lipid incorporation into phospholipids rather than triglycerides is due to the demand of the endoplasmic reticulum (ER) to grow in size. The ER must produce antimicrobial peptides to mount an immune response for survival. This increase in ER size to accommodate the increase of inflammatory molecules is similar to mammalian B cells maturing into plasma cells to produce antibodies during infection. Due to the evolutionary conservation between the Toll and insulin signaling pathways in flies and mammals, it makes sense that the induction of ER biogenesis requires a high metabolic demand from the animal. In summary, our goal is to understand how chronic immune activation in the fat body can lead to metabolic dysfunction.

164 Non-canonical autophagy controls steroid hormone synthesis and developmental timing by regulating cholesterol trafficking. Xueyang Pan1,2, Michael O’Connor1 1) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 2) Molecular, Cellular, Developmental Biology and Genetics Program, University of Minnesota, Minneapolis, MN.

Endocrine system producing steroid hormones takes dominant control on the juvenile-to-adult transition like puberty in mammals and metamorphosis in insects. However, how nutritional status affects this process is not fully understood. Macroautophagy (hereafter autophagy), a common effector responding to systemic nutrient level, has been proved crucial for the development and function of endocrine organs, but no functioning mechanism was studied. Here we show that non-canonical autophagic process occurs in the major endocrine organ of Drosophila larva, the prothoracic gland (PG), and modulates metamorphic timing by regulating cholesterol trafficking. Autophagy in PG (APG) is stimulated by nutrient restriction (NR) at early L3 stage, but later turns uninducible in late L3 stage. Blocking APG causes precocious pumariation and higher systemic ecdysone (E) level during NR, while forced APG induction abrogates E production and delays pumariation in
well-fed larvae. This proves that APG disrupts E production and pupariation activity. To our surprise, the developmental defects caused by forced APG induction can be rescued by cholesterol feeding. Further, the autophagosomes contain cholesterol and colocalize with cholesterol trafficking effector NPC1a, suggesting that APG is involved in cholesterol metabolism. Using live imaging approach, we saw dynamic formation of Atg8 marked tubular network upon NR treatment, which is not commonly observed in autophagy process. Further, the autophagosomes overlap with Rab7-marked late endosomes but not LAMP-marked lysosomes, and blocking auto-endolysosome trafficking does not disrupt the acidification of autophagosomes, suggesting a non-canonical autophagic flux. In all, our findings uncover a novel form of autophagy that processes cholesterol metabolism in steroid hormone producing endocrine organ and thus modulates hormone synthesis and development.

165 Pigment-dispersing factor signalling functions in the Drosophila prothoracic gland to regulate body size and developmental timing. M.J. Saligari, M.A. Henstridge, T.K. Johnson, C.K. Mirth, C.G. Warr 1) School of Biological Sciences, Monash University, Clayton VIC, Australia; 2) Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton VIC, Australia.

The rate and duration of growth in animals is coordinated by endocrine systems which produce secreted peptide hormones and steroid hormones. In insects, developmental transitions are regulated by pulses of production of the steroid hormone ecdysone in the larval prothoracic gland (PG). Multiple environmental factors and signaling pathways regulate ecdysone production. To find new regulators of growth we used RNAi to knockdown a set of candidate neuropeptide and growth factor receptors in the PG using phantom-Gal4. We found that knocking down Pigment-dispersing factor receptor (PdfR) caused a severe developmental delay and increased adult weight. PdfR<null> mutants are also developmentally delayed and have increased body size, but these phenotypes are much less severe than with PG-specific PdfR loss. PdfR is the receptor for pigment-dispersing factor (Pdf), a neuropeptide well known as a regulator of fly circadian rhythms. Although it is known that the Pdf neurons innervate the PTTH neurons to supply circadian input into PTTH release, this does not explain the delay we observed, which suggests that PdfR has a separate role in the PG. We investigated whether PdfR function is required in the PG to regulate ecdysone biosynthesis by several methods. Firstly, the developmental delay of phm>PdfR<null> larvae is completely rescued by feeding larvae ecdysone (20E). In addition, ecdysone titres in phm>PdfR<null> larvae are significantly reduced, between 34 hr and 50 hr after L3 ecdysis (AL3E), when compared with both phm-Gal4 and UAS-PdfR<null> controls. Finally, the transcription of at least one of the ecdysone biosynthetic genes (dib) is perturbed in phm>PdfR<null> larvae compared with time-matched controls. Overall, these results reveal a novel function for PdfR in regulating ecdysone biosynthesis in Drosophila, which is independent of the well-characterised role for PdfR in regulating circadian rhythms.


166 Allatostatin-A promotes larval developmental progression. N.M. Romero, D. Deveci, F.A. Martin, P. Leopold 1) University Côte d’Azur, CNRS, Inserm, Institute of Biology Valrose, Nice, FR; 2) Cajal Institute, Madrid, Spain.

The onset of puberty occurs in response to the integration of many internal homeostatic and external signals. However, it remains largely unknown which internal sensory mechanisms are involved in coupling those signals. In mammals, onset of puberty is ass


167 The Drosophila mitochondrial citrate transporter regulates L-2-hydroxyglutarate accumulation by coupling the tricarboxylic acid cycle with glycolysis. Hongde Li, Alexander Hurlburt, Jason Tennessen Department of Biology, Indiana University, Bloomington, IN.

The two enantiomers of 2-hydroxyglutarate (2HG) have emerged as potent regulators of metabolism, chromatin modifications, cell differentiation, and immune cell fate. While these compounds are commonly associated with tumor metabolism and referred to as oncometabolites, neither D-2HG nor L-2HG is tumor specific and both molecules are also
synthesized under physiological conditions. The metabolic mechanisms that control 2HG accumulation in healthy cells, however, remain poorly understood and most studies focus on ectopic 2HG accumulation in cancer cells, where neomorphic mutations in isocitrate dehydrogenase 1/2 induce D-2HG accumulation. In order to study endogenous 2HG metabolism, we recently demonstrated that Drosophila larvae accumulate very high levels of L-2HG, thereby establishing the fly as a new genetic model for studying these oncometabolites. In this regard, our current studies are focused on an inborn error of metabolism known as the combined D- and L-2HG aciduria, which is caused by mutations in the mitochondrial citrate transporter (MCT). Patients with MCT mutations accumulate high levels of both D- and L-2HG, and exhibit severe developmental defects. The mechanism by which MCT controls 2HG metabolism, however, remains unknown. Here we used a targeted metabolomic approach to demonstrate that 2HG levels are also elevated in Drosophila MCT mutants, indicating that we can use the fly to study the mechanisms that underlie this disease. Indeed, our studies revealed that Drosophila MCT mainly regulates L-2HG accumulation by controlling glycolysis. MCT mutants exhibit enhanced glycolytic flux and lactate production. As a result, elevated lactate levels promote L-2HG synthesis and inhibit L-2HG degradation, leading to increased L-2HG accumulation. Thus, our findings reveal a novel mechanism that regulates L-2HG buildup and hints at a potential metabolic therapy for treating patients with 2HG aciduria caused by MCT deficiency.

**168 Regulation of Mitochondrial Complex I Biogenesis in Drosophila Flight Muscles.** C Garcia¹, J Khajeh¹, E Coulanges¹, E Chen², E Owusu-Ansah¹ⁿ ¹  Department of Physiology and Cellular Biophysics, Columbia University Medical Center; 2) Department of Pharmacology, Columbia University Medical Center; 3) The Robert N. Butler Columbia Aging Center, Columbia University Medical Center.

The flight muscles of Drosophila are highly enriched with mitochondria, but the mechanism by which Mitochondrial Complex I (CI), which has over 40 subunits, is assembled in this tissue has not been described. We report the mechanism of CI biogenesis in Drosophila flight muscles; and show that it proceeds via the formation of ~315-, ~370-, ~550-, and ~815 kDa CI assembly intermediates. Additionally, we define specific roles for several CI subunits. In particular, we show that NDUFS5 is required for converting a transient CI assembly intermediate (an ~700 kDa assembly intermediate) into the ~815 kDa assembly intermediate. Importantly, incorporation of NDUFS5 into CI is necessary to stabilize or promote incorporation of NDUF10 into the complex. Our findings highlight the potential of studies of CI biogenesis in Drosophila to uncover novel mechanisms of CI assembly, and establish Drosophila as a suitable model organism for addressing questions relevant to CI biogenesis in humans.

**169 Phosphorylation of septin protein Pnut is important during early stages of embryonic development in Drosophila.** K Akhmetsova¹², M. Balasov¹, I. Chesnokov¹ ¹ University of Alabama at Birmingham, Birmingham, AL; 2) The Institute of Cytology and Genetics, Novosibirsk, Russia.

Septins belong to a family of polymerizing GTP binding proteins that are essential for cytokinesis in many organisms and are recognized as important components of the cytoskeleton. Drosophila melanogaster has five septins, three of which, Pnut, Sep1 and Sep2, form a heteromeric complex. We found that septin Pnut is phosphorylated during the first two hours of Drosophila embryo development. Mass spectrometry has identified that the phosphorylation occurs at the extreme C-terminus of the protein on amino acids T509 and S517. Using the method of P-element excision we obtained new pnut null allele – pnut¹⁰⁰ – that allowed us to investigate the role of Pnut phosphorylation during embryogenesis. Drosophila strains carrying non-phosphorylatable (T509 and S517 are substituted to alanines) and phospho-mimetic (T509 and S517 are substituted to serines) pnut transgenes were established. Both transgenes were able to rescue the lethality associated with pnut¹⁰⁰ null allele. However, non-phosphorylatable mutant was semi-lethal, and immunostaining revealed abnormal localization of the protein in early Drosophila embryos. Phospho-mimetic pnut mutant showed survival rates and protein localization similar to wild type, suggesting the importance of Pnut phosphorylation during embryogenesis. Recombinant septin complexes containing wild type or phosphorylation mutations in Pnut subunit were reconstituted using baculovirus expression system. We found that phospho-mimetic mutant form of Pnut disrupted the assembly of a functional septin complex (as shown by pull-down experiments) and septin filament formation (as shown by electron microscopy) in vitro. Overall, our findings indicate that phosphorylation of Pnut plays an important role in regulating septin complex functions during organism development. We hypothesize that Pnut phosphorylation may facilitate septin structures disassembly during specific stages in Drosophila embryo development.

**170 Myosin regulation during Drosophila salivary gland invagination.** S. Chung¹², S. Kim², D.J. Andrew² ¹ Department of Biological Sciences, Louisiana State University, Baton Rouge, LA; 2) Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD.

The formation of three-dimensional tubes from flat epithelial sheets is a fundamental process in forming organs. To achieve this dramatic tissue shape change, cells must change their shapes and/or positions. A key molecule that generates the force to drive such changes is non-muscle myosin II (hereafter referred to as myosin). Using Drosophila embryonic salivary gland (SG) as a model system, we elucidate the mechanism for regulating apicomedial myosin during tissue invagination. Apicomedial myosin forms in the center region of the apical end of cells and generates force to drive apical constriction, a
major cell shape change during tube formation. We show that the FoxA transcription factor Fork head (Fkh) regulates SG expression of folded gastrulation (fog) to accumulate Rho-associated kinase (Rok) in the apicominal region of cells during SG invagination. Cells with high apicominal accumulation of Rok form a pulsatile apicominal myosin structure, which provides a contractile force for apical constriction of cells. Surprisingly, we demonstrate that neither loss of spatially coordinated apical constriction nor its complete blockage prevent internalization and tube formation, although such manipulations affect the geometry of invagination. When apical constriction is disrupted, compressing force generated by a tissue-level myosin cable contributes to SG invagination. We further show that fully elongated polarized SGs can form outside the embryo, suggesting that tube formation and elongation are intrinsic properties of the SG. We are currently addressing the question of how the invagination process is localized by exploring the spatial control of Fg activity.

171 Characterization of a novel actin regulator, HtsRC. J. Gerdes1, A. Hudson1, K. Mannix1, L. Cooley1,2,3 1) Genetics, Yale University School of Medicine, New Haven, CT; 2) Cell Biology, Yale University School of Medicine, New Haven, CT; 3) Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT.

Development of eggs and sperm occurs in clusters of sister cells that are formed through incomplete cytokinesis. These cells remain connected by intercellular bridges called ring canals (RCs), which replace the cytokinetic cleavage furrows. In the Drosophila melanogaster female germline, RCs acquire a robust filamentous actin cytoskeleton that supports the dramatic increase in RC lumen diameter over the course of egg chamber development. F-actin accumulation requires the Kelch-Cullin3RING ubiquitin ligase, which regulates protein turnover by the proteasome. We have recently demonstrated that HtsRC, one of the products of the hu-li tai shao gene, is the target of the Kelch-Cullin3RING ubiquitin ligase. To understand the role of HtsRC, we used CRISPR gene editing to create mutations in the exon encoding HtsRC and recovered nonsense mutations early in the coding sequence that cause premature termination. Homozygous mutant females have decreased fecundity and produce small, malformed eggs. RCs lack F-actin, demonstrating that HtsRC is essential for F-actin accumulation. RCs are smaller than normal and contain Filamin but not Kelch, consistent with our previous analysis of the cheerio gene that encodes Filamin: RCs in cher mutants lack HtsRC and Kelch. Ectopic expression of HtsRC in the somatic follicle cells using the UAS/Gal4 system results in accumulation of filamentous actin aggregates, which do not colocalize with the RCs present in this tissue. These results support the conclusion that HtsRC protein is both necessary and sufficient for recruiting F-actin assemblies.

The predicted HtsRC protein is conserved only in the Drosophila family and contains no known functional domains. In vivo structure-function analysis with transgenic fragments of HtsRC did not identify a subdomain of HtsRC sufficient for recruiting F-actin. As recombinant HtsRC is insoluble and therefore not amenable to in vitro F-actin biochemistry, we are leveraging the formation of HtsRC-actin aggregates to characterize the role of HtsRC in recruiting F-actin to RCs, and to probe the relationship of HtsRC with known actin regulatory proteins using a range of genetic tools. Using genetic mosaic analysis, we found that the ectopic F-actin aggregates do not require the Arp2/3 complex, ruling out a role in activating Arp2/3-dependent nucleation. Further analysis of HtsRC function will enhance our understanding of actin regulatory mechanisms and provide new insights into the regulation of F-actin structures.

172 The polarity protein kinase Par-1 promotes Diaphanos activity for cleavage furrow ingression in the syncytial Drosophila embryo. T. Jiang, T. Harris Cell and Systems Biology, University of Toronto, Toronto, CA.

Par-1 regulates a variety of processes during animal development by phosphorylating a range of substrates for cell polarization. Although cell polarization often involves the cytoskeleton, little is known about how Par-1 regulates the actin cytoskeleton. Drosophila early embryo cleavage provides an ideal model to study actin networks. Actin caps grow rapidly and form pseud-cleavage furrows with actomyosin networks at their base. We previously found that Par-1 is required for cleavage furrow formation for embryos examined at cellularization. In this study, we investigated Par-1 RNAi phenotypes in earlier syncytial embryos by live imaging cytoskeleton markers. Individual MT spindles seem to be intact at metaphase. However, ingressing furrows were partially lost. The earliest detected defect was reduced cap growth and failures to form furrows between the diminished caps. We found that Par-1 kinase activity is required for this process, as expressing full-length Par-1 can fully rescue Par-1 RNAi defects, while kinase-dead Par-1 can not. Analyses of known targets of Par-1 such as Mbs, Dlg, and Baz suggest they are not involved. Instead, we identified the actin nucleator Diaphanos (Dia) as a candidate effector of Par-1. Knocking down Dia showed similar phenotypes in both syncytial and cellularizing embryos. Reducing Par-1 by half in Dia RNAi embryos enhanced the actin cap and furrow defects. Reciprocally, expressing full-length Par-1 but not kinase-dead Par-1 can suppress Dia RNAi defects. Strikingly, in addition to MT and furrow localization, Par-1 also localizes to actin caps, where it overlaps with Dia in distinct patches within caps. Par-1 and Dia also colocalize at actin networks of the cap and furrows as the networks reorganize during the cleavage cycle. They also colocalize at furrows through cellularization. We propose a model of Par-1 kinase activity promoting Dia for actin polymerization, actin cap growth, and cleavage furrow ingression in the syncytial Drosophila embryo.
Microtubules and microtubule plus end-binding proteins EB1 and CLIP-190 are essential for the spatiotemporal regulation of actin cable initiation and for the organization of the actin cable array during oogenesis. A.E. Leslie, O. Molinar, R. Jaiswal, J. Henty-Ridilla, B. Goode, B.M. McCartney 1) Biological Science, Carnegie Mellon University, Pittsburgh, PA; 2) Biology, Brandeis University, Waltham, MA.

The actin and microtubule (MT) cytoskeletons contribute to a wide range of processes including wound healing, subcellular localization of organelles, and cell motility. Actin and MTs have mostly studied as independent networks. However, crosstalk between these networks clearly occurs, and a growing number of proteins that regulate one cytoskeletal network are being discovered to collaborate with the other as well. While many associations are known, the underlying molecular mechanisms that govern this crosstalk are poorly understood. We are using actin cable assembly in Drosophila nurse cells as a model for dissecting actin-MT interactions. At stage 10B of oogenesis, actin cables emerge from the cortex and elongate toward the nucleus just prior to the onset of cytoplasmic dumping. This enormous array of actin cables continues to elongate after nuclear contact, pushing the nucleus away from the ring canals to prevent their obstruction during dumping. We have shown that the formin Diaphanous (Dia) and the nucleation-promoting factor Adenomatous polyposis coli-1 (APC1) promote actin cable assembly. Furthermore, MTs co-align with actin cables and disruption of MTs using nocodazole strongly inhibits early stages of initiation of actin cable assembly. In addition, knockdown of MT plus-end binding proteins EB1 and CLIP-190 results in premature cable initiation and a highly disorganized array, arguing that MTs have multiple points of regulatory control over the actin cable network. Interestingly, we have shown that EB1 and CLIP-190 are potent regulators of Dia and APC1 in vitro: EB1 strongly inhibits Dia-APC1 mediated actin assembly, while CLIP-190 significantly enhances Dia’s elongation rate. To begin to probe the regulatory interactions between MTs, MT-binding proteins, actin cables, and their regulators we are employing an optogenetic form of EB1 to allow acute and reversible disruption of EB1 activity during cable initiation and cable elongation. We have shown that in the absence of blue light in S2 cells t-EB1 localizes to MT plus-tips as expected. When exposed to blue light, t-EB1 rapidly dissociates from MTs (t1/2=700 ms) and when the blue light is removed it rapidly recovers MT localization (t1/2=30 s). We are currently implementing this optogenetic technique in nurse cells, coupled with live imaging of the cytoskeleton, to dissect the regulatory interactions underlying actin-MT crosstalk during actin cable assembly.


Within a single cell, simple actin monomers assemble into structures with a remarkable range of geometries, dynamic properties, and subcellular distributions, yet how this is achieved is poorly understood. Accumulating evidence suggests that regulated assembly of diverse actin networks requires a variety of actin assembly factors and crosstalk with microtubules. We are using assembly of actin cables during Drosophila oogenesis as a model to dissect these mechanisms. One of the last events in oogenesis is cytoplasmic dumping where nurse cells contract to expel their contents into the developing oocyte. At stage 10B just prior to dumping, the assembly of an array of actin cables initiates at the cell cortex and elongates toward the nucleus in each nurse cell. Once nuclear contact is made, the cables continue to elongate, pushing the nuclei away from the ring canals to prevent obstruction during dumping. We have shown that the formin Diaphanous (Dia), and its interactors Adenomatous polyposis coli-1 (APC1) and APC2 are required for proper assembly of actin cable arrays. Interestingly, while APC2 is necessary to initiate cables, loss of APC2 also results in premature cable assembly that is dependent on Dia activity. This suggests that APC2 may play a dual role in actin assembly: inhibiting a Dia-APC1 complex before proper temporal cues are received, and promoting activity of that complex after those cues are received. Consistent with that model, we find that APC2 can inhibit APC1’s actin assembly activity in vitro, and that a phosphomimetic form of APC2 that mimics phosphorylation by GSK3β enhances this inhibition. Similar to loss of APC2, knockdown of GSK3β in nurse cells promotes premature cable assembly, without interfering with the normal cable initiation at stage 10B. In addition to the role that actin assembly factors play in regulating actin cable formation, we found that microtubules co-align with the cables and that nocodazole inhibition of MTs blocks actin cable assembly. MT plus-end binding proteins EB1 and CLIP190 are also necessary for the proper spatiotemporal pattern of cable initiation. Taken together, these results suggest a complex network of protein control the spatiotemporal regulation of actin cable assembly during oogenesis. Currently, we are developing new imaging paradigms for this system including multicolor live imaging, electron microscopy, and superresolution approaches. These approaches will help us to achieve a more detailed understanding of the interactions between the proteins and cytoskeletal networks that create this striking array of actin cables.

Novel concepts of microtubule regulation during neuronal growth, maintenance and degeneration. Ines Hahn, Yue Qu, André Voelzmann, Jill Parkin, Andreas Prokop Faculty of Biology, Medicine & Health, The University of Manchester, Manchester, GB.

Axons are cable-like processes of neurons electrically wiring the nervous system. They are key lesion sites in trauma, neurodegenerative diseases and ageing, but need to be maintained for decades. Parallel bundles of microtubules (MTs) form their structural backbones and life-sustaining transport highways, the formation/maintenance of which is a key factor of axon
development/longevity. The underlying mechanisms are little understood.

Using Drosophila primary neurons we have studied >50 actin and MT regulators, which has led to the hypothesis of "local axon homeostasis" explaining how different MT regulators act together to form and maintain organised MT bundles (Voelzmann, 2016, BrainResBulletin 126, 226ff.). We now identified a cortical MT collapse factor which provides an important check point, eliminating MTs that break bundle organisation by going off-track. Bundle maintenance also requires continued regulation of MT polymerisation, and we provide here new insights into complex machinery that drives this process, including cortical actin and the spectraplakin Shot (Qu, 2017, Mol Biol Cell, 28, 296ff.), a ménage-à-trois of Eb1, Msps and Tau, as well as the complex tubulin provision machinery with Stathmin as an important gate keeper.

Our systematic dissection of the regulatory networks that underpin axonaxonal MT bundles provide new concepts for axonal biology with important implications for axon formation, ageing, regeneration and degeneration.

Supported by BBSRC

176  Echinoid Negatively Regulates Actomyosin network during Epithelial Morphogenesis.  Rahul Rote, Arsida Nocka, Laura Nilson  Department of Biology, McGill University, Montreal, Quebec, Canada.

Echinoid (Ed) is a homophilic cell adhesion molecule required in normal epithelial morphogenesis during embryonic dorsal closure and ovarian follicular epithelium development. Both processes are characterized by loss of Ed from a defined group of cells, followed by formation of an actomyosin cable and smooth contour at the interface between Ed-negative and Ed-positive cells. The Ed-positive cells also lose Ed from this interface, due to the lack of homophilic interaction, generating a planar polarized distribution of Ed in these cells. To test whether the global loss of Ed or the planar polarization of Ed leads to elevated levels of actomyosin, we characterized the ectopic Ed-positive/Ed-negative interfaces generated by ed loss of function clones in the ovarian follicular epithelium. We observed significant enrichment of F-actin, Myosin-II and Rho-kinase at the smooth interfaces between wild type and ed mutant cells at the clone border, and also at the regular interfaces in the clone interior. These observations suggest that Ed functions as a negative regulator of actomyosin network. Consistent with this interpretation, using differentially labeled markers for actomyosin in WT and ed mutant cells, we also observed that actomyosin is increased in the Ed-positive cells at the ed clone border, at the interface with the adjacent ed mutant cell, generating a polarized distribution complementary to that of Ed. Unpolarizing Ed in these WT cells is sufficient to rescue the smooth contour of the ed mutant clones, but not the elevated levels of actomyosin network components in the ed mutant cells. These data show that abolishing Ed polarization in these cells non-autonomously affects the shape of the ed mutant neighbour, and imply that the enrichment of actomyosin network components/contractility markers at ed mutant interfaces does not contribute to the smooth clone border. These results suggest that the cell shape change leading to the smooth Ed-positive/Ed-negative interfaces is a result of planar polarization of Ed and, thus, of the contractile actomyosin network in the Ed-positive cells.

177  Proximity-dependent biotinylation as a tool to identify ring canal protein interactomes in the Drosophila ovary.  Rebecca Starble, Katelynn Mannix, Ronit Kaufman, Lynn Cooley  Yale University, New Haven, CT.

During Drosophila oogenesis, germline cells are connected through intercellular bridges called ring canals (RCs). RCs are composed of a robust actin cytoskeleton that enables these structures to grow in diameter throughout oogenesis to support the development of the oocyte. While multiple proteins have been identified to play a role in RC assembly, the interaction networks with which these proteins associate to regulate RC cytoskeleton organization require further elucidation. However, the identification of RC protein-protein interactions (PPIs) is limited by the fact that RCs are highly insoluble, thus making it impossible to identify RC protein interactomes with conventional methods. One technique that can be used to identify PPIs is proximity-dependent biotinylation, a method in which proteins of interest are fused to enzymes that produce highly reactive biotin molecules that covalently react with and biotinylate proximal proteins. In this study, we have utilized this technique to overcome the limitation of RC insolubility, since the extremely strong interaction between biotin and streptavidin enables RC proteins to be solubilized with denaturing lysis conditions and biotinylated proteins to be captured with streptavidin beads and identified via mass spectrometry. We have created constructs in which the RC proteins htsRC, Kelch, and Pav were fused to ascorbate peroxidase (APEX), an enzyme that produces reactive biotin molecules in the presence of biotin phenol and hydrogen peroxide. Through immunofluorescence and western blotting, we have shown that APEX fusion constructs localize to RCs and biotinylate RC proteins. Our mass spectrometry results indicate that individual RC proteins exhibit unique protein interactomes consisting of both known RC proteins and previously uncharacterized proteins, which we have validated through multiple approaches. We show that proximity-dependent biotinylation is a valuable tool for identifying RC protein interactomes and further understanding the mechanisms through which the RC cytoskeleton is maintained, including actin regulatory mechanisms and substrates of E3 ubiquitin ligases. Furthermore, this technique has enabled us to examine PPIs in previously inaccessible contexts and in live, developing tissue.

The complex body plan of multicellular organisms is shaped into tissues and organs through the directed migration of cells and coordinated cell shape changes. Dynamic rearrangements of the actin cytoskeleton, which underlies the cell membrane, are the driving force behind these intricate cell behaviors. Abl non-receptor tyrosine kinases are key regulators that promote and coordinate cytoskeletal remodeling and regulate cell migration during morphogenesis. Abl has been linked to several types of cancer, and inappropriate activation of Abl in cell culture has been found to promote invasiveness and lead to defects in actin structures and cell migration. The pathways that regulate and are regulated by Abl during normal development and by the activated version of Abl in the disease state have not been clearly defined, and thus it is critical to continue to identify new components of these complex signaling networks. We use Drosophila embryos that express Bcr-Abl, an activated version of Abl that has been linked to chronic myelogenous leukemia in humans, as a model system to dissect the pathways by which Abl regulates cell migration in vivo. Expression of this activated form of Abl in the embryonic epithelium is lethal, with defects in cell shape, actin dynamics, and cell migration. In order to identify novel components of the pathway by which Abl regulates cell migration, we are doing a screen to find genomic regions and genes that, when one copy is removed, genetically modify the phenotypes associated with expression of activated Abl in the epithelium during embryonic development. From this screen, we have identified several modifiers of activated Abl-dependent phenotypes. In order to extend these studies to normal Abl signaling pathways that regulate development in vivo, we are examining the effects of mutant alleles of these activated Abl-interacting genes on the phenotypes associated with overexpression of wild-type Abl in the epithelium. Mutant alleles of nearly all of these alleles modified Abl-dependent phenotypes, suggesting that the products encoded by these genes both function during morphogenesis and are mis-regulated in the presence of activated versions of Abl. Taken together, these genetic studies provide insights into the pathways by which Abl directs cell migration in vivo.

Zasp52 LIM domains mediate protein recruitment to the Z-discs. Y. Xiao, N. Gonzalez, F. Schoeck. Department of Biology, McGill University, Montreal, Quebec, CA.

Striated muscles are vital for movement. Muscle striation is caused by the serial arrangement of thousands of small contractile units, termed sarcomeres. The sarcomere consists of interdigitating myosin thick filaments and actin thin filaments, with actin filaments anchored to the Z-disc and myosin filaments anchored to the M-line. Most human myopathies are related to mutations in Z-disc proteins. However, the initial assembly and maintenance of Z-discs are still not well characterized. Drosophila indirect flight muscles are striated muscles and share many similarities with human skeletal muscles. Previous studies show that the first steps of Z-disc formation are orchestrated by the cooperative action of alpha-actinin and Zasp/Alp/Enigma family proteins. The main member of this family in Drosophila is Zasp52, which localizes to the Z-disc and is required for Z-disc formation. We hypothesized that Zasp52 is required for protein recruitment at the Z-disc. To test this, we performed protein co-evolution analysis to discover potential binding partners of Zasp52. One of those, Zasp66, we analyzed in more detail. We used quantitative confocal image analysis of Zasp52 mutants to show that Zasp66 localization depends on Zasp52. Then, we show that Zasp52 recruits Zasp66 through specific LIM domains using yeast-two-hybrid and immunoprecipitation assays. In addition, in vivo overexpression of certain LIM domains in indirect flight muscles induces aggregate formation and flightlessness. Based on the above results, we propose that specific LIM domains of the Drosophila Alp/Enigma family protein Zasp52 recruit some of its binding partners and thereby contribute to Z-disc assembly.

Collision of expanding actin caps with actomyosin borders for cortical buckling and mitotic rounding in a syncytium. Y. Zhang, T. Jiang, C.G. Yu, T. Harris. Cell and Systems Biology, University of Toronto, Toronto, CA.

The early Drosophila embryo is a large, single, syncytial cell. Its cortex is remodelled to form furrows that compartmentalize dividing nuclei. Before furrow ingression, Rac-induced Arp2/3 networks generate an apical actin cap and Rho-induced actomyosin networks encircle each cap. How these two networks transform a flat cortex into a honeycomb-like, compartmental array remains unclear. With centrifugal growth, each apical cap meets its actomyosin border and furrow ingression begins. Through genetics and live molecular imaging, the cap guanine nucleotide exchange factor Sponge, a Rac-GTP sensor, and Arp3 coated the forming cap and then spread down the lateral furrows, as part of a continuous actin network. The adjacent actomyosin zone maintained its segregation from the growing cap network, forming a thin circumferential border at the base of furrows. By increasing or decreasing myosin activity genetically, the actomyosin borders were found to restrict and organize cap growth. Inversely, actin cap perturbation experiments revealed that each growing cap seems to push physically against the actomyosin border and compress its components as the furrow ingression occurs. Overall, the growing actin caps appear to collide physically with actomyosin borders, buckle, and continue growing for furrow ingression. This mesoscale mechanism seems to bud small compartments from a large cell for mitotic cell rounding in a syncytium.
Endosomal vacuoles of the prepupal salivary glands of Drosophila play an essential role in the metabolic reallocation of iron. Jose Ortega, Sarah Beyeler, Blake Riggs

The generally accepted view of the developmental importance of the Drosophila salivary glands (SGs) is the one for which they are exceptionally well known: the production and subsequent release of a glycoprotein-secretory glue used to affix a newly formed puparia to a substrate. After pupariation, the SGs were considered to be doomed for programmed cell death (PCD) which culminates ~16 hr after puparium formation (APF). Their notoriety for these processes resulted in a gap in our understanding of any processes occurring between the time immediately after pupariation until the time in the early pupa when PCD is executed. Recently we demonstrated that a great deal is going on in the SGs in this interval: the early-to-mid prepupal SGs undergo extensive endocytosis with widespread vacuolation of the cytoplasm followed by massive apocrine secretion. Here we describe additional novel properties of these endosomes. Vital pH-sensitive probes provided confirmatory evidence that these endosomes have acidic contents and that the endocytosis in the prepupal glands are of two types. The SGs simultaneously generate mildly acidic, small, basally-derived endosomes and strongly acidic, large and apical endosomes. Staining of the large vacuoles with vital acidic probes was possible only after there was ambipolar fusion of both basal and apical endosomes. While iron was not detectable directly due to limited staining sensitivity, we found increasing fluorescence of the glutathione-sensitive probe Cell-Tracker-Blue CMAC in large vacuoles, which appeared to depend on the amount of iron released by ferrireductase. Small basal endosomes were uniquely recognized by PNA lectin, whereas apical large vacuoles bound DBA lectin. Experimental evidence shows that heterologous iron-bound transferrin is viably internalized to prepupal SG cells by endocytosis from the basal surface, and once internalized, it undergoes intracellular trafficking as might be expected of an Fe$^{3+}$-carrier protein. We used both the homozygous viable $MvI^{PP}$ loss-of-function allele of Malvolio encoding specific transmembrane-divalent-metal-ion transporters (DMT), and the $Mvl$ RNAi transgenic construct. Manipulation of $Mvl$ function caused a loss of Cell-Tracker-Blue CMAC fluorescence signal in the majority of vacuoles, and the presence of dark green signal in only a few vacuoles, which indicates that there was very low or no activity of endosomal ferrireductase enzyme due to the lack of systemic Fe$^{3+}$ iron.

Non-apoptotic function of the executioner caspase drICE is required for endosomal trafficking and tracheal elongation. S.S. McSharry, G.J. Beitel

Caspases, well known for their roles in cell death, can also participate in non-apoptotic processes in morphogenesis. Here we show that drICE, the homologue of executioner caspase-3, colocalizes with endocytic machinery and is required for normal endosomal trafficking of the tracheal size determinant Serpentine. We previously showed that drICE activity is required for tracheal elongation downstream of the Hippo Network, which raised the question: why does activation of drICE not cause tracheal apoptosis? We now show that tracheal cells are not immune to apoptosis; while some tracheae lacking DIAP1, an endogenous inhibitor of drICE, have elongated trachea, the majority of mutants exhibited large-scale tracheal death. Moreover, tracheal cells require drICE for apoptosis: overexpression of pro-apoptotic genes Grim is sufficient to induce apoptosis, and cell death caused by Grim or DIAP1 is blocked by loss of drICE. These results suggest that the roles of drICE in tracheal morphogenesis and apoptosis are not mutually exclusive. The co-existence of apoptotic and morphogenetic activities of drICE suggest that the proteolytic activity and/or physical compartmentalization of drICE is tightly regulated to allow normal tracheal elongation without triggering apoptosis. Consistent with compartmentalization of drICE, cleaved drICE colocalizes with markers of endocytic compartments including Clathrin (apical surface), Rab5 (early endosomes), and Rab11 marking (recycling endosomes) in the trachea. drICE also colocalizes at endocytic compartments with tracheal size determinants Crumbs, pSrc, and Uninflatable, which are known to be trafficked throughout tracheal morphogenesis. Critically, these determinants remain colocalized even when their localization is dramatically altered in embryos with a mutation affecting trafficking machinery including Vps32 (shrub), Vps35, or Rhoc1. Consistent with drICE participating in endocytic trafficking, the tracheal size determinant Serpentine accumulates in cytoplasmic punctae in drICE mutants, and luminal Serpentine levels are reduced by loss of Yorkie, the Hippo Network-regulated transcription factor that regulates DIAP1. Together, these results demonstrate that non-apoptotic executioner caspase activity can modify tracheal length via endocytic trafficking, which has broad implications for morphogenesis and cell communication.

Proper Endoplasmic Reticulum partitioning is necessary for mitotic progression in Drosophila neuroblast. Jose Ortega, Sarah Beyeler, Blake Riggs

The mechanism underlying the generation of cell diversity is asymmetric cell division, which relies on the correct partitioning of cell fate determinants. There are several unanswered questions involving asymmetric cell divisions including the
mechanism surrounding partitioning of these factors or other components during mitosis. The divisions of Drosophila neural stem cells, i.e. neuroblast, have served as an excellent model examining the mechanism involving asymmetric cell divisions. Recent studies have shown that the Endoplasmic Reticulum (ER) is partitioned asymmetrically in dividing Drosophila embryonic proneural cells and larval neuroblast populations. Here we show that the highly conserved ER integral membrane protein Jagunal (Jagn) is necessary for proper partitioning of ER during mitosis in Drosophila larval neuroblast. Expression of double-stranded (ds) RNA corresponding to Jagn displayed defects in mitotic ER partitioning and showed significant delays and defects in mitotic progression. Specifically, there was an increase in the mitotic index in dividing neuroblast populations expressing Jagn dsRNA. In addition, inhibition of Jagn displays minor defects in microtubule bundling but does not appear to disrupt mitotic spindle assembly. These results suggest the presence of an organelle surveillance mechanism involving the correct partitioning of the ER during mitosis.


The most frequent genetic lesions in glioblastoma (GB) include EGFR which show constitutive kinase activity and Ras signaling to drive cellular proliferation and migration. We use a GB model in Drosophila melanogaster based on the expression of constitutively active EGFR and PI3K in glial cells, this model reproduces the highly proliferative and invasive neoplastic cells that create transplantable tumor-like growths, mimicking human GB.

An in vivo genetic screen to identify new modulators of GB progression was performed. As a result a member of the secretory pathway was identified: Kish. Downregulation of kish rescues glioma formation, proliferation, neurodegeneration and viability of the animals. There is a kish human orthologue TMEM167A which is conserved. We found that TMEM167A is upregulated in human GB samples and TMEM167A interference inhibits human glioma cells (U87) growth. Our aim is to understand how Kish/TMEM167A modulate EGFR signaling during its intracellular trafficking. The majority of EGFR signaling occurs at the plasma membrane, but it is known that activated EGFR-mediated signals continue from endosomes. With this goal we have measured EGFR accumulation in specific endosomes comparing GB and GB brain samples upon kish downregulation. The results show that when the secretory pathway is altered by kish inhibition in a GB, EGFR accumulates in early endosomes and lysosomes where they are targeted to degradation. Moreover, kish inhibition in GB restored total EGFR protein to control levels.

Finally, we performed a biased drug screen using a collection of compounds that affect vesicular transport, looking for a drug that is able to rescue the lethality induced by the GB. We found an interesting candidate that we are currently analyzing. These and future results will provide basic information on the glioma mechanisms and may be key for the design of future therapeutic strategies against specific targets involving EGFR signaling.


Progressive reduction of cholinergic activity in the brain is suggested to trigger neurodegenerative disorders. Choline Acetyltransferase (ChAT), a soluble protein required for acetylcholine synthesis at the presynaptic compartment, is transported as a coherent bulk in axons and the heterotrimeric Kinesin-2 motor is required for this process. Axonal transport of soluble proteins is described as a constitutive process assisted by occasional, non-specific interactions with moving vesicles and motor proteins. Here, we report that a specific association between ChAT and Kinesin-2 during a certain developmental period increases the anterograde flow of the protein in the sensory neurons of intact Drosophila nervous system. Further experimental analysis showed that cholinergic activity is essential for the axonal entry of ChAT and its episodic interaction with Kinesin-2. Altogether, these observations highlighted a phenomenon of synaptic activity-dependent regulation of a soluble protein transport in vivo, which could potentially define the quantum of its pre-synaptic influx.

186 The V-ATPase V1 subunit A1 is required for rhodopsin anterograde trafficking in Drosophila. H. Zhao, J. Wang. 1) School of Life Sciences, Tsinghua University, Beijing, CN; 2) National Institute of Biological Sciences, Beijing, CN.

Synthesis and maturation of the light sensor, rhodopsin, is critical for the maintenance of light sensitivity and for photoreceptor homeostasis. In Drosophila, the main rhodopsin, Rh1, is synthesized in the endoplasmic reticulum and transported to the rhabdomere through the secretory pathway. In an unbiased genetic screen for factors involved in rhodopsin homeostasis, we identified mutations in vha68-1, which encodes V-ATPase catalytic subunit A isoform 1 of the V1 component. Loss of vha68-1 in photoreceptor cells disrupted post-Golgi anterograde trafficking of Rh1, reduced light sensitivity, increased secretory vesicle pH, and resulted in incomplete Rh1 deglycosylation. In addition, vha68-1 was required for activity-independent photoreceptor cell survival. Importantly, vha68-1 mutants exhibited phenotypes similar to those exhibited by mutations in the V0 component of V-ATPase, vha100-1. These data demonstrate that V1 and V0 components of
V-ATPase play key roles in post-Golgi trafficking of Rh1, and that Drosophila may represent an important animal model system for studying diseases associated with V-ATPase dysfunction.


Membrane trafficking must adapt to changing cellular demands, in part through the coordinated activity of successive phosphoinositide regulators. Previously, we showed that Drosophila Mtm PI3-phosphatase interacts in a conserved protein complex of phosphoinositide regulators that modulates endosomal dynamics and transit. We now show that an Mtm endosomal function inhibits autophagy at the level of autolysosome maturation and acts in coordination with the single Drosophila Class II PI3-kinase (PI3KC2, or PI3K68D). Strikingly, PI3KC2 encodes for two alternatively spliced, co-expressed isoforms - a full-length protein and a truncated, noncatalytic isoform. Null alleles for PI3KC2 “long” or “short” isoforms revealed opposite roles for each that normally inhibit or derepresses autophagy, respectively, in a shared pathway with Mtm. Both PI3KC2 protein isoforms physically interacted with each other as well as within differential complexes with Mtm. Together, our results demonstrate a central upstream role for a noncatalytic PI3KC2 isoform in directing both PI3KC2 and Mtm phosphoinositide functions with consequences on endosomal flux and autolysosomal maturation. Importantly, the balance between PI3KC2 isoforms in flies impacted animal longevity and survival in response to stress, pointing to the significance of coordination between phosphoinositide regulators in homeostasis.

188 Regulation of endosomal Microautophagy in Drosophila. A. Mesquita, A. Jenny Dev Mol Biol, Albert Einstein College of Medicine (Chanin 503), Bronx, NY.

Autophagy delivers cytosolic components to lysosomes for degradation and is thus essential for cellular homeostasis and to cope with different stressors. As such, autophagy counters various human diseases, and its reduction enhances aging like phenotypes. Macroautophagy (MA) can selectively degrade organelles or aggregated proteins, but selective degradation of single proteins has mostly been described for Chaperone-mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two selective autophagic pathways, originally described only in mammals, are specific for proteins containing KFERQ-related targeting motifs.

Using a KFERQ-tagged fluorescent biosensor, we have identified an eMI-like pathway in the genetically easily tractable model organism Drosophila melanogaster. Upon starvation, this biosensor localizes to late endosomes/lysosomes upon prolonged starvation in an Hsc70-4 and ESCRT machinery dependent manner. Currently, we are characterizing the physiological role of eMI in flies with a focus on the types of cellular stress that modulate this route and their respective activation mechanisms. Preliminary data suggest that oxidative stress and DNA damage, but not ER stress can elicit an eMI response, suggesting a selectivity of the process.

189 Stabilized Acinus manages cellular stress and extends life by elevating basal levels of Autophagy. Nilay Nandi1, Helmut Kramer1,2 1) Dept of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX; 2) Dept of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX.

Autophagy is a tightly regulated cellular process that supports survival during cellular stress. We have identified Acinus (Acn) as a key integrator of several cellular stress responses. Acn manages stress by regulating basal levels of autophagy in a TOR-independent pathway (1). In adjusting basal autophagy levels to different challenges, we found a critical role for phosphorylation of the conserved serine-437 residue of Acn. Physiological relevance for this modification was confirmed by a phospho-mimetic S437D mutation in vivo: stabilized AcnS437D enhanced basal autophagy and extended life span. From a targeted screen, we found that Cdk5 phosphorylates Acn at serine-437. Knocking down Cdk5, or its required cofactor p35, drastically reduces Acn phosphorylation at serine 437, whereas Cdk5 gain-of-function increases pS437-Acn levels. In p35 mutants, basal autophagy and lifespan are reduced, but restored to near wild-type levels in the presence of stabilized AcnS437D. Acn-S437 phosphorylation is highly dynamic in developing eyes and we have identified a metal-dependent protein phosphatase (CG6036) necessary for dephosphorylating serine-437. Stress-induced inhibition of this phosphatase may contribute to elevated pS437-Acn levels. Expression of aggregation-prone polyQ-containing proteins or the Amyloid β-42 peptide, but not alpha-Synuclein and Amyotrophic Lateral Sclerosis (ALS)-linked human SOD1, triggers Cdk5-dependent phosphorylation of S437-Acn. Moreover, phospho-mimetic AcnS437D with elevated basal autophagy yielded a reduction in polyQ accumulation. By contrast, basal autophagy is reduced in p35 mutants causing elevated polyQ protein levels. Not only neurodegenerative stress but also oxidative stress can trigger phosphorylation at serine-437, but in a Cdk5-p35 independent pathway. Our data indicate that phosphorylation of Acn is required to maintain the protective role of basal autophagy under several stress situations.


190 The role of Clueless in mitochondrial function. K.M. Sheard1,2, S.A. Thibault-Sennett1, R.T. Cox1,2 1) Biochemistry and Molecular Biology, Uniformed Services University, Bethesda, MD; 2) Molecular and Cell Biology Program, Uniformed Services
Mitochondrial DNA is inherited maternally and cannot be produced de novo. Consequently, mutations in mtDNA can expand clonally in the cell during mitochondrial biogenesis and lead to severe mitochondrial diseases. Mitochondrial diseases affect 1:5000 people and affect tissues that require large amounts of ATP, such as the heart, brain, and muscles. Since mitochondria are exclusively maternally inherited, it is imperative that females ensure properly functioning mitochondria be inherited by their offspring.

The gene clueless has been shown to be necessary for mitochondrial function. clu is a highly conserved gene with homologs in humans, Drosophila, and yeast, and Drosophila clu mutants have physically damaged mitochondria, increased mitochondrial oxidative damage, reduced ATP levels, and aberrantly mislocalized mitochondria which aggregate into clusters. These effects are direct. Clu binds to ribosomes at the mitochondrial outer membrane, mRNAs bound for import into the mitochondria, as well as to the mitochondrial outer membrane proteins TOM20 and Porin. In addition, Clu participates in mitochondrial quality control through association with PINK1 and Parkin, both mediators of mitophagy of unhealthy mitochondria. Clu protein is dispersed throughout the cell's cytoplasm and also exists in aggregates. We have found these protein aggregates are no longer visible in parkin and clu mutants, however, why they disperse and how this relates to Clu function is currently unknown. Our goal is to elucidate what these “particles” are and how they function in the cell. Using live-imaging and immunofluorescence, we will present our data on Clu particle dynamics and behavior in order to determine the composition and nature of Clu particles and how it impacts mitochondrial protein import.


Early Drosophila embryos are filled with maternally provided storage organelles, including yolk vesicles, glycogen granules (GGs), and lipid droplets (LDs). These organelles undergo large-scale intra-embryonic redistribution during cleavage and syncytial blastoderm stages; at cellularization, this distribution controls their allocation between the cells forming at the surface and the internal yolk cell. We find that in embryos lacking the LD protein Jabba lipid droplets are clustered around GGs and are even partially embedded into them; in the wild type, the two organelles are largely unassociated. LD-GG clustering is apparently quite robust; when intact embryos are centrifuged to separate contents by density, LDs and GGs accumulate at opposite ends of wild-type embryos; however, in jabba mutants, some LDs are displaced into the region enriched in GGs. Intermingling of LDs and GGs is already detectable in stage 12 oocytes and persists beyond cellularization. Jabba's best characterized function is as histone anchor on lipid droplets. Ongoing structure-function experiments, however, suggests that Jabba's histone binding ability is not required to keep LDs and GGs separate. In jabba mutants, the typical outward displacement of LDs during cleavage stages is impaired; in turn, GGs are shifted further outward than normal. Presumably, the association between LDs and GGs interferes with the independent trafficking of both organelles. As a consequence of this mistrafficking, LDs are highly enriched in the yolk cell and depleted from the newly formed diploid cells. Because later in embryogenesis, LDs persist at higher levels in jabba compared to wild-type embryos, we propose that either the tight association with GGs or the misallocation to the yolk cell impairs LD turnover. When we employed klar mutants to mislocalize LDs to the yolk cell in the absence of GG clustering, LD turnover was also delayed, indicating that misallocation-alone impairs LD breakdown. Preliminary evidence suggests that in jabba mutants glycogen breakdown is also abnormal. We conclude that preventing inappropriate interactions between storage organelles is critical for their proper trafficking and metabolism.

192 Tracking Centrosomes to Follow Endoplasmic Reticulum Inheritance in Drosophila Embryos. C. Brown, B. Riggs Cell and Molecular Biology, San Francisco State University, San Francisco, CA.

Asymmetric cell division (ACD) is the primary mechanism by which stem cells regenerate themselves, while also giving rise to more differentiated cell types. Research in Drosophila melanogaster neuroblasts suggests that ACD is achieved via both intracellular and extracellular cues, but these cues are often specific to certain cell types. Furthermore, the initial signaling cue that triggers both the intrinsic and extrinsic signaling cascades is poorly understood. To this effect, our laboratory has described an asymmetric partitioning of the endoplasmic reticulum (ER) that occurs just prior to cell fate selection in the developing Drosophila neuroectoderm. Within these neuronal progenitors, the ER is evenly distributed throughout the cell during prophase. As the cell cycle progresses, a significantly larger portion of the ER localizes toward one pole of the cell, while the smaller portion migrates toward the opposite pole, resulting in each daughter cell receiving differing amounts of ER. Defects in this ER partitioning pattern results in failure of proper mitotic spindle establishment and metaphase delay. To characterize the role of the asymmetric ER localization with respect to cell fate in these embryonic progenitor cells we focused on tracking centrosome inheritance during gastrulation. This will be done by using a photoconvertible centrosome marker, PACTd2-EosFP, to photo-label centrosomes and follow their movement during the corresponding cell cycle at the start of gastrulation. Using this technique, we can directly visualize the fate of mother and daughter cells with differing portions of the ER with confocal microscopy. This work will provide evidence that how organelles are inherited plays a crucial role in stem cell differentiation as well as define a novel role for the ER in cell fate selection.
Dynein associates the endoplasmic reticulum with centrosomal microtubules but is not required for mitotic partitioning of the organelle.  Darya S Karabashova, Jeremy T Smyth  Anatomy, Physiology & Genetics, Uniformed Services University, Bethesda, MD.

Mechanisms that ensure proper partitioning of the endoplasmic reticulum (ER) during cell division are largely unknown. We previously demonstrated that the ER associates extensively with astral microtubules (MTs) of the mitotic spindle in Drosophila cells in vivo as well as in mammalian tissue culture cells, suggesting that astral MT association may be an essential mechanism of ER partitioning. However, the specific molecular factors that link the ER with astral MTs remain elusive. To address this, we have developed a method to image live Drosophila spermatocytes that are simultaneously expressing fluorescently labeled MT and ER markers. We analyzed the spatial and temporal dynamics of ER-spindle MT association and saw that in control spermatocytes the ER was evenly distributed throughout the cytoplasm prior to meiosis onset, with a subset of ER membranes clearly organized around each centrosome. At the meiosis onset, as the centrosomes migrated to the nuclear envelope (NE), we saw a sudden sequestration of the ER onto centrosomal MTs before NE breakdown. This ER movement toward centrosomal MT minus ends suggested a role for the MT minus end motor dynein. Surprisingly, spermatocyte specific RNAi of dynein heavy chain (dhc64c) resulted in complete exclusion of the ER from centrosomal MTs prior to meiosis, suggesting a requirement for dynein in ER-MT association during interphase. The centrosomes then failed to migrate to the NE in dhc64c RNAi cells, as expected based on the known role for dynein in this process. However, we still observed the sequestration of the ER membranes toward the centrosomes at meiosis onset, which resulted in the ER completely associating with spindle MTs by NE breakdown. Thus, despite a role for dynein in ER-MT association during interphase, a dynein-independent mechanism links the ER with spindle MTs during cell division. We then tested the two other MT motors that generate centrosomal-directed forces in Drosophila spindles, Klp61f and Ncd (human Kinesin-5 and -14 orthologues, respectively). However, RNAi suppression of both of these factors had no effect on ER association with spindle microtubules. In conclusion, our results suggest that MT minus end motors are not required for ER partitioning in meiotic spermatocytes, though dynein is required for the organization of the ER around centrosomes during interphase.

Maintenance of visual neurotransmission during prolonged light requires AMPylation of BiP.  Andrew Moehlman1, Amanda Casey2, Kelly Servage2, Kim Orth2,3, Helmut Kramer1  1) Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX; 2) Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX; 3) Howard Hughes Medical Center, Dallas, TX.

To efficiently monitor the environment, visual systems must adapt to a wide range of short and long-term changes in light conditions. Here, we describe a novel role of a recently discovered post-translational protein modification, AMPylation, in the adaption to prolonged light exposure. AMPylation, the transfer of an adenosine monophosphate, is catalyzed by highly conserved Fic proteins. Drosophila null fic mutants are homozygous viable and fertile without developmental phenotypes. We have found that their visual system fails to adapt to a continuous 72-hour light exposure (LL). Instead, fic mutants, but not wild-type flies, show a severe disorganization of their rhabdomeres by electron microscopy, which is reflected in the loss of pseudopupil. In response to light pulses, the sustained negative potential (SNP) of photoreceptors is reduced and ON- and OFF-transient lamina responses are lost. Surprisingly, these defects are reversed following a 3-day recovery period under 12:12 hour light/dark cycle (LD). The first biochemically identified substrate of eukaryotic Fic was BiP (hsc70-3), an abundant and ubiquitous ER-resident chaperone. To test whether AMPylation of BiP contributes to the role of Fic in visual transmission, we generated transgenic flies in which endogenous BiP was replaced by wild-type BiP or an AMPylation-resistant BiP mutant. The vision defects observed in fic null flies were phenocopied by AMPylation-resistant BiP, consistent with BiP being a major target for Fic-mediated AMPylation in the visual system. BiP is a key regulator of the Unfolded Protein Response (UPR), suggesting that the physiological changes in fic and BiP point mutants reflects a reversible deregulation of a cellular stress-response. Consistent with this hypothesis, we observe under LL conditions, that markers for ER stress are elevated in the lamina of fic mutant animals, indicating a dysregulation of the UPR. These results demonstrate, for the first time, a physiological role for Fic-mediated AMPylation of BiP through the maintenance of visual neurotransmission, and indicate a role for the Unfolded Protein Response in regulating photoreceptor plasticity.

Roles of spastic paraplegia proteins in organising a dynamic axonal ER network.  Lu Zhao1, Belgin Yalcın2, Martin Stofanko1, Niamh O’Sullivan1, Anood Sohail1, Valentina Baena2, Mark Terasaki2, Cahir J O’Kane1  1) Department of Genetics, University of Cambridge, Cambridge, UK; 2) Department of Cell Biology, University of Connecticut Health Center, Farmington, CT.

Endoplasmic reticulum (ER) is unique among intracellular organelles by its physical continuity. In neurons, this apparent continuity throughout axons, dendrites and cell bodies potentially allows ER to be a channel for regional or long-distance communication within neurons that is independent of action potentials or physical transport along microtubules, comparable to a “neuron within a neuron”.

Axons contain a mainly smooth tubular ER network throughout their length, but the mechanisms that form and maintain this extended axonal network are still largely unknown. Some clues are emerging from mutations affecting reticulon or REEP
proteins, characterized by intramembrane hairpin domains that model ER membranes, that cause an axon degenerative disease, hereditary spastic paraplegia (HSP). To test whether these proteins have roles in the axonal ER network, we identified markers for axonal ER, and used these to show that loss of some Drosophila HSP hairpin proteins can cause partial loss of ER from distal motor axons. We also found that loss of both reticulon and REEP families leads to occasional discontinuities in axonal ER. Ultrastructural analysis reveals an extensive and mainly continuous ER network in axons, which shows larger and fewer tubules and less continuity in larvae that lack reticulon and REEP proteins, consistent with loss of membrane curvature. Live imaging and fluorescence recovery after photobleaching (FRAP) also show a physically continuous network of axonal ER that is interrupted in hairpin protein mutants, and in addition reveal remarkable dynamic features of the network. Therefore HSP hairpin-containing proteins are required for shaping and continuity of axonal ER, and the growing number of HSP genes may include others that affect axonal ER architecture. The occurrence of occasional gaps in HSP mutants provides a potential mechanism for susceptibility of longer axons for degeneration, and the mutant ER phenotypes that we recover allow us to test models of how ER can affect axon physiology and communication.

196 Investigating the role of nucleocytoplasmic transport in Inclusion Body Myositis.  
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Inclusion Body Myositis (IBM) is the most common myopathy in individuals over the age of 50. It is characterized by slowly progressive weakness in both proximal and distal muscles with histopathological features including rimmed vacuoles, protein inclusions, endomysial inflammation, and MHC I upregulation. Although IBM has been extensively studied, the cellular mechanism underlying the disease remains poorly understood, and there are no effective treatments available. Research in a variety of degenerative diseases has revealed several parallels between IBM and other neuromuscular degenerative diseases such as Amyotrophic lateral sclerosis (ALS), which supports the idea that there is likely an unappreciated commonality in their etiopathogenesis. Two hallmarks of degenerative diseases are protein mislocalization and abnormal protein aggregates in the nucleus and cytoplasm. In particular, TAR DNA-binding protein (TDP-43) is mislocalized from the nucleus and found aggregated in the cytoplasm in several diseases classified as TDP-43 proteinopathies, including ALS, frontotemporal lobar dementia (FTD), and IBM. Interestingly, it has been shown that cytoplasmic protein aggregates alone are sufficient to interfere with nucleocytoplasmic transport. Therefore, we hypothesize that nucleocytoplasmic transport may be a major pathway contributing to degeneration in IBM. We are using a Drosophila model of hereditary IBM to explore this question. This model consists of pathogenic mutations within Valosin-Containing Protein (VCP), which recapitulates the pathology of IBM associated with Paget’s disease of bone and frontotemporal lobar dementia (IBMPFD) when expressed in Drosophila muscle. In this model it was shown that VCP interacts genetically with TDP-43 and expression of mutant forms of VCP results in the mislocalization of TDP-43 to the cytoplasm. Using a shuttle GFP reporter we have preliminary data that indicates that nucleocytoplasmic transport is disrupted in this model. To further explore the role that nucleocytoplasmic transport could be playing in IBM pathogenesis we are performing a targeted genetic screen to elucidate interactions between our Drosophila model and components of the nucleocytoplasmic transport machinery. Initial data has revealed both genetic enhancers and suppressors of the mutant VCP phenotype. These data suggest that nucleocytoplasmic transport may be a promising therapeutic target to ameliorate degeneration in IBM.

197 Nuclear Wash functions in multiple nuclear complexes to affect nuclear morphology/events.  
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Wiskott Aldrich Syndrome (WAS) proteins are activated by upstream signals, usually from Rho family GTPases, to form new branched actin networks in association with the Arp2/3 complex. The WASH subfamily of WAS proteins has established cytoplasmic roles in oocyte and embryonic development, cell migrations, and endosome sorting and scission, where it functions as part of a WASH Regulatory Complex (SHRC) in a context-dependent manner. Wash also is found throughout the nucleus where it affects global nuclear organization and several nuclear events. In particular, Wash depletion in both cells and salivary gland nuclei causes a range of nuclear phenotypes including a wrinkled nucleus, increased DNA accessibility, disrupted chromosome organization, altered heterochromatin, and disruption of non-heterochromatin nuclear substructures. While we find that several of these phenotypes are attributable to a direct interaction with the Drosophila B-type lamin and/or its SHRC, mass spec and blue native (BN-) PAGE analyses show that nuclear Wash acts as part of multiple, separable complexes to affect its diverse set of nuclear properties/events. We are currently investigating the protein components and specific functions of each of these complexes to understand the mechanisms underlying each of Wash’s nuclear roles, and in particular, its previously unappreciated role in non-canonical nuclear export.

198 Dynamics of histone nuclear import in the early Drosophila embryo.  
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In Drosophila, a newly fertilized embryo undergoes thirteen rounds of rapid, synchronous, syncytial nuclear divisions to generate approximately 6,000 nuclei in the first two hours of embryogenesis. Remarkably, the embryo does not increase in volume during this period and all the materials required for the processes are maternally deposited as RNA and protein
during oogenesis. Therefore many nuclear proteins are supplied in the cytoplasm and must be rapidly partitioned into the exponentially increasing number of nuclei in order for proper development. Among these, histones are one of the most abundant proteins in the early embryo and must be rapidly transported into nuclei to chromatinize the thousands of newly synthesized genomes. However, regulation of the histone nuclear import and their dynamic properties in vivo is not yet well characterized in the early Drosophila embryo. Here, we constructed a novel endogenously-regulated histone H3 reporter tagged with a green-to-red photoswitchable fluorescent protein Dendra2. Using this system, we are able to measure in vivo parameters of histone dynamics in the embryo, including nuclear import rates, export rates, and the fraction of DNA-bound/unbound histones within the nuclei at various stages of development. We will discuss how these properties are related to the progression of nuclear cycles, the abundance of karyopherins, and the total DNA concentration in the embryo.

199 A New Twist in an Old Saga: an Essential Role of Drosophila RanGAP at the Nuclear Pore. Shane Chen, Maria Lyanguzova, Hangnoh Lee, Karen Plevock Haase, Ka Chun Yau, Alexei Arnaoutov, Mary Dasso National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Ran is a small GTPase that is critical for nuclear-cytoplasmic transport, nuclear envelope assembly, and mitotic spindle formation. These cellular processes are driven by a gradient of GTP-bound Ran (Ran-GTP) and GDP-bound Ran (Ran-GDP). For nuclear-cytoplasmic transport in interphase, Ran-GTP is maintained at a high level in the nucleus by the activity of RCC1, a nucleotide exchange factor. Ran-GDP is dominant in the cytoplasm due to the stimulation of GTP hydrolysis by RanGAP, Ran GTPase-activating protein. In vertebrates, RanGAP is constantly SUMOylated, facilitating its association to the nucleoporin RanBP2 at the cytoplasmic filaments of nuclear pore complex (NPC). During mitosis, the complex containing SUMOylated RanGAP and RanBP2 persists after nuclear envelope breakdown and localizes to mitotic spindles and to kinetochores. By contrast, yeast does not possess an obvious homologue to RanBP2, and the yeast RanGAP is not SUMOylated, rendering it dispersed in the cytoplasm.

To understand the regulation of RanGAP and the functional consequences of its association to the NPC, we examined the localization of RanGAP in Drosophila. Our results show that Drosophila RanGAP anchored at the nuclear envelope via association to RanBP2. The interaction between Drosophila RanGAP and RanBP2 is independent of SUMOylation, and occurs through direct binding of RanGAP to a non-conserved motif of RanBP2. The RanGAP binding motif of RanBP2 resides in an intron that is selectively retained in most tissues. Collectively, our overall goal is to better understand the biological significance of RanGAP-RanBP2 interaction by studying fly development, fertility, and viability.

200 Analysis of Gp210 function in Drosophila melanogaster. Brian Jenkins, Brad Darwin, Alex Chang, Cole Lambo, Sean Speese Department of Neurology, OHSU - Jungers Center for Neuroscience Research, Portland, OR.

Although Gp210 was the first nuclear pore complex (NPC) protein identified 35 years ago, a role in supporting nucleocytoplasmic transfer has remained elusive. Despite being one of the few transmembrane NPC proteins, a number of studies suggest that Gp210 is not required for assembly or localization of the NPC, consistent with observations that not all cells express Gp210. Recent inquiries into Gp210 function have uncovered roles in a variety of cellular processes including regulation of gene specific transcription in muscle cells, maintenance of ER homeostasis and in cellular differentiation. A particularly interesting and surprising finding is that some functions of Gp210 do not require the protein to be localized to the NPC.

Despite the intriguing nature of this protein, very few in-vivo studies have been carried out regarding its function.

We have undertaken an in-depth study of Gp210 function in Drosophila melanogaster, investigating gp210 mutant phenotypes in multiple tissues and cell types during various stages of the Drosophila life cycle. Our initial investigation takes advantage of a few existing P-element insertions into the gp210 locus which decrease transcript and protein levels to varying levels. Despite strong transcript and protein knock-down from two of these P-element insertions, both alleles are surprisingly viable and fertile as homozygotes and do not have a decreased lifespan. We are currently generating a null allele and tissue specific knockouts of Gp210 function using transgenic CRISPR/Cas9 approaches.

Our preliminary results have also led to the interesting discovery that gp210 mutants have an increase in Nuclear Envelope Budding (NEB) events, however, it is unclear if this phenotype is due to increasing or blocking of this process. During NEB, large granules containing RNA presumably bud through the nuclear envelope in a process akin to the nuclear egress of Herpesviridae nucleocapsids, but the cellular function of this pathway is still unknown. We are testing a number of possibilities to determine the mechanisms underlying the apparent increase in NEB events in gp210 mutants. In accordance with other studies, loss of GP210 function does not grossly alter the localization of the NPC but does lead to elevated levels of ER stress in some cell types, suggesting a possibly link between ER stress and the nuclear envelope budding pathway. Ultimately, these in-vivo studies will add to our understanding of this enigmatic NPC protein and hopefully begin to shed light onto the function of the NEB pathway.
202 Investigating the roles of Fascin in collective cell migration using Drosophila border cell migration. M. Lamb, K. Anliker, T. Tootle Anatomy and Cell Biology, University of Iowa, Iowa City, IA.

Fascin, an actin binding protein, is a regulator of many developmental processes and contributes to cancer aggressiveness. Functioning to bundle actin filaments, Fascin promotes cell motility, invasion, and adhesion through its canonical role of forming filopodia and invadopodia. Fascin controls cell migration during development such as, growth cone extension and dendrite formation. In addition, Fascin is highly upregulated in certain types of cancer, and elevated expression is associated with increased invasiveness, aggressiveness and mortality of these cancers. While Fascin's role in regulating cell migration has largely been attributed to its function as an actin bundler, Fascin has other functions including, interaction with mechanotransduction machinery, and nuclear localization, that may contribute to cell migration. While Fascin has been studied in the context of single cell migration and 2D migration, the role of Fascin in 3D collective cell migration has yet to be investigated. To study the role of Fascin in invasive, collective cellular migration in vivo we use Drosophila border cells as a model. Border cell migration occurs during Stage 9 of oogenesis in which a specified group of follicular epithelial cells cluster together and migrate posteriorly in between the nurse cells to the nurse cell - oocyte border. This process is crucial for oocyte development since aberrant or delayed border cell migration leads to female sterility. Fascin is highly expressed in the border cells and fascin-null flies are sterile. These and other findings led us to hypothesize that Fascin plays a critical role in promoting border cell migration during oogenesis. Contrary to prior reports, we find that follicles from young fascin-null flies display a significant delay in border cell migration. Cell-specific knockdown studies suggest that Fascin is required within the somatic and germline cells to mediate migration. Furthermore, rescue of Fascin expression in the germline cells, but not somatic cells, fails to rescue border cell migration. Together these findings implicate a cell-autonomous role for Fascin in collective cell migration in vivo during Drosophila oogenesis. Furthermore, these findings provide a system to investigate the actin bundling-independent functions of Fascin in invasive cellular migration. Overall, this research will lead to a more complete understanding of the function of Fascin in developmental cell migrations and cancer metastasis.

203 Balance of action by integrin-associated proteins revealed by myofibril attachment. H. Green, N.H. Brown Dept of Physiology, Development and Neuroscience, Univ Cambridge, Cambridge, GB.

We use the myotendinous junction of Drosophila indirect flight muscles to explore how the many intracellular proteins recruited by integrins coordinate their function to build complex actin structures. We discovered that myofibrill termini contain 3 distinct actin structures, marked by different actin regulators. We identify unique phenotypes caused by the absence of each integrin-associated-protein, permitting epistasis analysis. This revealed a balance between positive and negative activities, with Focal Adhesion Kinase inhibiting the elevation of integrin activity by tensin, and RSU1 keeping PINCH activity in check. Furthermore, vinculin facilitates mechanical opening of filamin, which can then work with Arp2/3 to build an actin buffer zone positioned between actin adjacent to the membrane and the first sarcomere. Thus, integration of integrin-associated protein activity builds the complex architecture of the myotendinous junction, linking the membrane anchor to the sarcomere via multiple actin-based structures.

204 Understanding how Hedgehog signaling regulates Cubitus Interruptus using the CRISPR/Cas9 gene editing system. Jamie Little, Elisa García, Amanda Sul, Hayon Kim, Daniel Kalderon Biological Sciences, Columbia University, White Plains, NY.

The Hedgehog protein (Hh) is a morphogen that is used in a variety of settings to regulate cell proliferation and patterning in flies and mammals. Hh signal transduction ultimately alters the activity of Cubitus Interruptus (Ci), the sole transcription factor of the pathway in flies (or Gli orthologs in mammals). When Hh is absent, Protein Kinase A (PKA) and Costal-2 (Cos2) promote proteolytic processing of to a transcriptional repressor form, Ci-75. When Hh is present, Ci-155 processing to Ci-75 is inhibited and Ci-155 is activated, leading to de-repression and activation of Hh target genes. To investigate how different facets of Ci regulation collaborate and uncover their underlying mechanisms we are creating designer ci alleles using the CRISPR-Cas9 gene editing technique. Importantly, this allows testing of Ci variants under physiological conditions. We aim to learn more about the necessity for regulated Ci-155 processing, whether PKA or Cos2 have processing-independent roles and to learn more about how Fused kinase activates Ci-155 and Suppressor of fused limits Ci-155 activation.

205 Examining the effectiveness of knocking down the Hedgehog signaling pathway using different RNAi lines in Drosophila. Julia Spear, Cheaney Seiler, Pavithra Vivekanand Susquehanna University, Selinsgrove, PA.

The Hedgehog (Hh) signaling pathway is a highly-conserved intercellular communication pathway that is required for cell differentiation and morphogenesis. In Drosophila melanogaster, the Hh signaling protein relieves suppression of the Smoothed (Smo) receptor to begin an intracellular cascade, ultimately activating Cubitus interruptus (Ci), a transcription factor that regulates the expression of important developmental genes. In previous work, RNA interference (RNAi) was used
to knockdown Pointed (Pnt), a transcription factor that functions downstream of the EGF RTK signaling pathway. Pnt is required for the differentiation of different cell types in multiple tissues. We demonstrated that the different RNAi lines behaved inconsistently with respect to knockdown efficiency in different tissues with the short-hairpin RNA eliciting the strongest loss-of-function phenotype. We wanted to examine whether we would observe similar variability in phenotypic severity with knockdown of Hh pathway members. Hh RNAi was expressed in different tissues using VDRC, Valium10, and Valium20 fly lines. VDRC and Valium10 produce long-hairpin RNA, while the Valium20 line generates short-hairpin RNA. In the wing, expression of Hh RNAi using the Valium10 line led to the strongest phenotype of crinkled wings in 76% of male and female flies. Wings from male flies expressing the VDRC and Valium20 lines exhibited either a partial or full loss of the anterior cross vein. The wings from female flies that expressed dsRNA from either the VDRC or the Valium20 RNAi lines were wild type. Experiments to test the effect of expressing dsRNA to target either hh or smo mRNA during eye and midline glia development are underway with each of the RNAi lines.

**206 Screening Candidates from a Genetic Screen to Identify Novel Regulators of Wingless Signaling.** A. Gutierrez, J. Kennell Biology, Vassar College, Poughkeepsie, NY.

Wingless (Wg) initiates a canonical signal transduction pathway in *Drosophila melanogaster* that influences gene expression during embryonic development. The protein Armadillo (Arm) is stabilized by Wg signaling and acts as a transcriptional co-activator of TCF, a transcription factor, to regulate gene expression. Wg signaling has been shown to contribute to tissue specific changes in gene expression, the mechanism by which is still unclear, but it is speculated that undiscovered regulators exist. Previously, the Kennell Lab conducted a small misexpression screen to identify novel regulators of Wg signaling. The misexpression screen was carried out using an Arm-dependent small-eye phenotype caused by activation of Wg signaling during eye development. The phenotype was activated using a modified form of Arm that cannot be degraded (Arm*). Using EP elements, or transposable elements with UAS enhancers that insert near promoters of genes, random genes were misexpressed along with Arm*. If any changes to the Arm-dependent phenotype occurred, those genes were considered possible regulators of Wg signaling. Candidate genes found to influence apoptosis independent of Wg signaling were discarded in a secondary screen. Fifteen genes remained as viable contenders. The two most promising candidate genes were chameau and Gbs 70E. They were selected based off of previously established functions. Chameau is an H4 histone acetyltransferase that co-activates transcription factors c-Jun and Fos. Rpd3, an antagonist of chameau, is recruited to inhibit Wg target gene expression. Gbs 70E is an inhibitory binding partner of PP1α96A, a phosphatase that positively regulates Arm stability. The two genes were subcloned into expression vectors and transfected into Kc167 cells to test Arm target gene expression. Future work will focus on conducting knockdowns of the candidate genes and monitoring their effect on Arm target genes and eye development.

**207 Emc regulates wingless signaling through Hippo-dependent non-apoptotic caspase signaling.** L. Nair1, N. Baker2 1) Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Graduate Institute of Life Sciences, National Defense Medical Center, Taiwan.

Transcription factors of the bHLH family of proteins, especially E and ID proteins play a crucial role in development and differentiation. We have previously shown that bHLH expression imbalance activates the Salvador-Warts-Hippo (SWH) pathway of tumor suppressors. In the absence of ID protein Extramacrochetae (Emc), E protein Daughterless (Da) activates the transcription of expanded (ex). In addition, Tyler and Baker (2007) reported that Ex is a negative regulator of wingless signaling. In this study, we have tested if Emc affects Wg signaling through Ex and SWH signaling. Using genetic epistasis experiments, we report here that: 1) Ex signals through the Hippo pathway and Yorkie to regulate bristle patterning; 2) Yorkie regulates neural patterning through Diap1, Dronc and non-apoptotic caspase activity; 3) Caspase cleavage of a particular Shaggy isoform inactivates Wingless signaling. Interestingly, Emc and Wg are both regulators of the morphogenetic furrow in the developing eye. Additional double-mutant combinations now establish that Emc regulates the morphogenetic furrow through wingless signaling, and apparently not through proneural genes.

**208 Drosophila Glioma models to study therapy resistance.** L. Roebke2, K. Snigdha3, M. Kango1,2,3 1) Department of Biology, University of Dayton; 2) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton; 3) Premedical Programs, University of Dayton.

Glioblastoma multiforme (GBM) is a devastating form of primary brain cancer with poor prognosis. Capitalizing on the mutations found in GBM patients and the similarities between mammalian and *Drosophila* genes involved in glial cell biology, *Drosophila* glioblastoma models have been established that show similarities to anaplastic glia from high-grade human glioma. High grade glioma is known to be recurrent and therapy resistant. These aspects of GBM lead us to ask how different molecular signals contribute to promoting glioma, and if interactions between glioma cells and the neighboring stromal cells play a role in the key aspects of disease presentation- the rapid growth, the therapy resistance, and the recurrent phenotype. We established a *Drosophila* glioma model where PI3K and EGFR/MAPK are coactivated using the GAL4-UAS system. The fly glioma (repoGAL4>UASptenRNAi +UASRas**V12**) model is especially advantageous due to conservation of cell-biological and signaling
mechanisms between flies and humans, and the ease of manipulating molecular signaling pathways in vivo due to the vast array of genetic tools available in flies. Next, using genetic and immunohistochemical approaches we tested the roles of key pathways and found that Wingless (Wg) and Hippo pathway may play an important role in glioma growth. We will present our findings from genetic interaction and epistasis studies that show the roles of Wg and Hippo signaling in glioma growth and therapy resistance. These studies will provide insights on molecular signals that promote gliomagenesis, and potential targets that may be evaluated for their therapeutic value in a whole organism in vivo glioma model.

209 Dally-like differentially regulates Wnt ligands in Drosophila gerarium to promote GSC maintenance and differentiation.  J. Waghamore1,2, X. Wang1,2, A. Page-McCaw1,2  1) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2) Program in Developmental Biology, Vanderbilt University, Nashville, TN.

The maintenance, proliferation, and survival of many cell types in Drosophila gerarium depend on coordinated activities of short and long-range signaling activities of secreted ligands such as Hedgehog, Decapentaplegic, Unpaired, and several members of the Wnt family. The extracellular spread of ligands has been explained by three mechanisms in the Drosophila gerarium. These include 1) cytoneme mediated spread of Hedgehog, 2) Dally mediated regulation of short-range Decapentaplegic ligand spread and activity to maintain germline stem cell niche, and 3) Dally-like mediated long-range spread of Wingless to follicle stem cells. Both Dally and Dally-like are cell-surface heparan sulfate proteoglycans that regulate the extracellular spread of Wingless, Hedgehog, Decapentaplegic, and Unpaired in other tissues. Dally-like acts as an exchange factor for Wingless and exhibits biphasic activity by promoting long-range signaling and suppressing short-range signaling. In this study, we investigated the role of Dally-like in regulation of different Wnt ligands in the gerarium. The Drosophila gerarium expresses Wingless, Wnt2, Wnt4 and Wnt6 ligands. Wingless and Wnt6 are required for maintenance of germline stem cell niche, whereas Wnt2 and Wnt4 maintain differentiation niche via several mechanisms. Mutations or genetic manipulation of Wnt signaling pathway components in the gerarium disrupts early oogenesis. Here, we show that overexpression of Dally-like in the differentiation niche results in loss of germline differentiation and loss of germline stem cells. Interestingly, while all four Wnts can bind to Dally-like in S2R+ cells, only Wingless and Wnt4 overexpression in the differentiation niche partially rescues the tumor phenotype, and only Wnt6 overexpression rescues the germline stem cell loss phenotype. Our results suggest that Dally-like has discrete Wnt ligand binding sites, and differentially regulates the spatial distribution and activity of Wnt ligands.

210 Wg signaling in vivo alters Axin-Sgg interactions to inhibit destruction complex activity.  M. Wehrli1, D.B Lybrand2, M. Naiman2, J.M. Laumann1, M. Boardman1, S. Petshow1, K. Hansen1, G. Scott1  1) Integrative Biosciences, Oregon Health and Science University, Portland, OR; 2) Reed College.

The central regulator of the Wnt/β-catenin pathway is the Axin/APC/GSK3β (Sgg) destruction complex, which in unstimulated conditions targets cytoplasmic β-catenin/Armadillo for degradation. How Wnt activation inhibits the destruction complex to permit β-catenin-dependent signaling remains controversial, in part because the destruction complex, and its regulation have never been observed in vivo. Using bimolecular fluorescence complementation methods, we have now analyzed the activity of the destruction complex under near-physiological conditions in Drosophila. By focusing on well-established patterns of Wg/Wnt signaling in the wing imaginal disc, we have defined the sequence of events by which activated Wg receptors induce a conformational change within the destruction complex, resulting in modified Axin-Sgg interactions that prevent Armadillo degradation. Surprisingly, the nucleus is surrounded by active destruction complexes, which principally control Armadillo's degradation and thereby nuclear access. These destruction complexes are inactivated and removed upon Wnt signal transduction. These results suggest a novel mechanistic model for dynamic Wg/Wnt signal transduction in vivo.

211 Length and Organization of Interfollicular Stalks is Critical for Oogenesis and Fecundity.  A.R. Mascaro1, A. Borensztejn2, K.A. Wharton1  1) Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 2) CBRC, Massachusetts General Hospital Research Institute/Harvard Medical School, Boston, MA.

The individualization of follicular egg chambers in Drosophila melanogaster ensures the proper development of an oocyte. As individual egg chambers, or follicles, are produced in the gerarium and then bud off, they are separated by a row of specialized cells called the interfollicular stalk. The stalk is initially present in a cluster, and then forms into a linear array of single cells. As oogenesis proceeds, we have found that the interfollicular stalk undergoes a reduction in cell number via apoptosis. Distinct from its role in fate specification, JAK/STAT signaling is required to restrict this apoptosis, with reduction in JAK/STAT signaling specifically in the stalk leading to excess cell death including complete loss of the stalk. This reduction in stalk length results in an overall decrease in fecundity, leading to fewer eggs being laid. Interestingly, hyperactivation of JAK/STAT signaling leads to longer than normal stalks and a similar decrease in fecundity, suggesting that a tight regulation of stalk length is critical to proper oogenesis. In addition to changes to cell number and cell death, we also find a disruption in the morphology and organization of the interfollicular stalks when manipulating JAK/STAT signaling as well as Bone Morphogenetic Protein (BMP) signaling. In contrast to the well characterized follicular epithelium surrounding each developing egg chamber within the Drosophila ovariole, little is currently known about organization structure of this unique
tissue. Questions remain regarding how the stalk cells rearrange to form a single line, what proteins are critical to establishment and maintenance of stalk organization, and why the stalk is so critical for oogenesis. We are examining and will present the wild-type properties of the stalk in this context, as well as changes that occur through manipulation of JAK/STAT or BMP signaling, which, based on preliminary findings, appear to alter proteins known to be involved in cell polarity and adhesion.

212 Muscle secreted Myoglianin regulates imaginal disc size. Ambuj Upadhyay, Michael O'Connor Dept. of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Organ size regulation is essential for proper function. Interestingly, the size of organs often scale proportionally with overall body size. The mechanism of how organs scale to body size remains largely unexplored. We are using the Drosophila melanogaster to identify such mechanism. Through a series of genetic analyses, we have identified myoglianin (myo), a TGF-β/Activin like growth factor, as an important regulator of wing growth. Myo mutant discs are significantly smaller, unlike other Activin like ligand mutants. Myo signaling in the discs require specific TGF-β receptors Babo-a, punt, and co-receptor plum. Surprisingly, myo is not expressed in the wing, rather it is secreted from the larval skeletal muscle. We propose myo signaling is a form of inter-organ communication which regulates scaling of wing size to the larval skeletal muscle serves as a proxy for overall body size.

213 Stem cell-niche interactions in the Drosophila ovarian germline. Scott Wilcockson, Hilary Ashe Faculty of Biology, Medicine and Health, University of Manchester, Manchester, GB.

Instructive signals secreted by niche cells ensure the localised maintenance of stable adult stem cell populations. In the Drosophila ovary, the somatic cells surrounding the germline employ multiple redundant mechanisms to restrict the range of the self-renewal signal, Dpp, to around a single cell diameter. This exquisite short-range signalling ensures the maintenance of a small number of germline stem cells whilst enabling cells to initiate cystoblast differentiation upon niche exit. We aim to identify mechanisms downstream of niche signalling that promote germline stem cell self-renewal. To this end we have performed RNA-seq of genetically expanded germline stem cell-like cells and cystoblasts to reveal novel networks regulating GSC self-renewal and differentiation. Here I will present evidence for a role for planar cell polarity and filopodia-like protrusions in the regulation of stem cell-niche interactions.

214 The Drosophila TGF-beta/Activin-like ligands Dawdle and Myoglianin modulate adult lifespan through regulation of 26S proteasome function in adult muscle. S. Langerak1, M. Kim2, H. Lamberg1, M. Godinez1, M. Main1, L. Winslow1, M.B. O’Connor2, C.C. Zhu1 1) Biological Sciences, Ferris State University, Big Rapids, Michigan 49307; 2) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota 55455.

The Drosophila Activin signaling pathway employs at least three separate ligands, Activin-β (ActB), Dawdle (Daw), and Myoglianin (Myo), to regulate several general aspects of fruit fly larval development including cell proliferation, neuronal remodeling, and metabolism. Here we provide experimental evidence showing that both Daw and Myo are anti-ageing factors in adult fruit flies. Knockdown of Myo or Daw in adult fruit flies reduced mean lifespan, while overexpression of either ligand in adult muscle tissues but not in adipose tissues enhanced mean lifespan. An examination of ubiquitinated protein aggregates in adult muscles revealed a strong inverse correlation between Myo or Daw initiated Activin signaling and the amount of ubiquitinated protein aggregates. We show that this correlation has important functional implications by demonstrating that overexpression of Daw or Myo in adult muscle tissues can partially rescue the reduced lifespan phenotype produced by knockdown of a 26S proteasome regulatory subunit Rpn1 in adult fly muscle, and that the prolonged lifespan caused by overexpression of Daw or Myo in adult muscle could be due to enhanced protein levels of the key subunits of 26S proteasome. Overall, our data suggest that Activin signaling initiated by Myo and Daw in adult Drosophila muscles influences lifespan, in part, by modulation of protein homeostasis through either direct or indirect regulation of the 26S proteasome levels. Since Myo is closely related to the vertebrate muscle mass regulator Myostatin (GDF8) and the Myostatin paralog GDF11, our observations may offer a new experimental model for probing the roles of GDF11/8 in ageing regulation in vertebrates.

215 Lgl regulates endosomal vesicle acidification and Notch signaling by promoting Vap33 interaction with the V-ATPase complex. M. Portela Esteban1, L. Yang1, S Paul1, A Veraksa2, L Parsons3, H Richardson2 1) Molecular, Cellular and Developmental Neurobiology, Cajal Institute, Madrid, Madrid, ES; 2) Department of Biochemistry & Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Australia; 3) Department of Biology, University of Massachusetts, Boston USA; 4) School of Biological Sciences, Monash University, Victoria, Australia.

The Drosophila melanogaster junctional neoplastic tumor suppressor, Lethal-2-giant larvae (Lgl), is a regulator of apico-
basal cell polarity and tissue growth. We have previously shown in the developing Drosophila eye epithelium that, without affecting cell polarity, depletion of Lgl results in ectopic cell proliferation and blockage of developmental cell death due to deregulation of the Hippo signaling pathway. Additionally we showed that Notch signaling is increased in lgl depleted eye tissue, independently of the function of Lgl in apico-basal cell polarity. Moreover, the deregulation of Notch signaling in lgl mutant tissue occurs by increased vesicle acidification, but the precise mechanism was unclear.

Here we investigate the mechanism by which Lgl regulates vesicle acidification. Our data shows that increased Vacuolar-ATPase (V-ATPase) activity in lgl mutant tissue is responsible for the elevated Notch signaling and tissue growth defects. We show that Lgl binds to Vap33 (Vamp-associated protein), which interacts with V-ATPase components. Vap33 physically and genetically interacts with Lgl and V-ATPase subunits in vivo and it represses V-ATPase activity and Notch signaling. Importantly, Lgl knockdown decreases binding of Vap33 to the V-ATPase component, Vha68-3. Thus, our results reveal a novel role for Lgl in promoting binding of Vap33 to the V-ATPase, thereby controlling V-ATPase activity, Notch signaling and tissue growth.

Altogether our data uncovers a novel mechanism by which Lgl regulates vesicle acidification through Vap33 and therefore the attenuation of ligand activated Notch signaling during Drosophila eye development. Our findings implicate the deregulation of Vap33 and V-ATPase activity in polarity-impaired epithelial cancers.

216 Molecular characterization of JAK/STAT regulation of spermatid differentiation. S. Dadkhah, Douglas Harrison, Jeramiah Smith University of Kentucky, Lexington, KY.

In addition to its role in testis stem cell maintenance, it has been found recently that the activation of the JAK/STAT pathway is required in the somatic cyst cells for later stages of spermiogenesis. A reporter of pathway activity is expressed in cyst cells encapsulating spermatids during elongation and disruption of pathway activity in cyst cells impairs individualization, the process in which the bundle of 64 interconnected spermatids is separated and their excess cytoplasm is removed. The aim of this project is to identify effectors regulated by JAK/STAT signaling that initiate individualization. Using RNA-seq, we have examined expression profiles from testes in which JAK/STAT signaling has been impaired prior to individualization by expressing the negative regulator Eye-transformer/Latran (ET/Lat) and compared to testes dissected from wild type flies. cDNA samples were sequenced using Illumina HiSeq 2500 for 100bp paired-end reads. These sequencing runs yielded 5.8 billion base pairs of sequence data from approximately 301 million separate reads in total from three biological replicates each across the controls and experimental data sets. These reads were mapped to the Drosophila reference sequence, and after annotating, RSEM was used to estimate expression level of genes from the RNA-Seq data. Next, EBseq was utilized to assess the differential expression levels of transcripts across control and experiments. The generated list of differentially expressed genes has been filtered for consistency across biological replicates and for the presence of an identified Stat92E binding site. From this analysis, we have identified significant differential expression (>2x log2) of 1508 transcripts represented 494 different genes. Of the genes showing differential expression, 311 were downregulated upon impairment of JAK/STAT signaling and 183 were upregulated. Down regulated genes were enriched for classes related to immune responses, proteases and apoptosis which can be interesting when comparing the cellular activities occurring during individualization.

217 Understanding the role of a nurse cell protein Cup, in border cell migration during Drosophila oogenesis. B. Saha, M. Prasad Biological Sciences, Indian Institute of Science Education and Research Kolkata, Kalyani, West Bengal, INDIA.

Though collective cell migration plays a critical role in several aspects of multicellular development, the underlying molecular mechanism regulating this process is still far from clear. We have employed the model of border cell (BC) migration during Drosophila oogenesis to understand how group cell movement is regulated in vivo. A fly ovary consists of a bunch of oval structures called the egg chambers, which develop through 14 stages of oogenesis to form the mature egg. An egg chamber consists of central germline cells (15 nurse cells & 1 oocyte) surrounded by a layer of 750 cuboidal follicle cells. At stage 8, group of 6-8 anterior follicle cells detach from the epithelial layer, invade the nearby nurse cells and migrate towards the oocyte. Failure of BCs to reach the oocyte results in female sterility. From a pilot screen of several female sterile lines, we identified mutation in cup gene impedes BC movement and also display defect in nurse cell organization. Cup is an mRNA binding protein that is specifically expressed in the nurse cells and is known to repress translation of some mRNAs including oskar. Our results suggest that Cup functions in the nurse cells to regulate JAK-STAT signaling in neighboring follicle cells. Since JAK-STAT signaling plays a critical role in BC fate specification, our results suggest that cup mutant egg chambers exhibit higher number of cells in the migrating cluster thus impeding BC movement. Further our results suggest that Cup regulates the transcripts of actin and microtubules in the nurse cells. As BCs adhere to nurse cell surface during movement, any defect in the nurse cell organization will impede BC movement. Hereby we propose that Cup maintains the nurse cell integrity and modulate the size of the border cell cluster to regulate the efficient border cell movement.

218 Mind bomb 2, a negative regulator of STAT activity, is required for normal border cell migration. S. Trivedi, M Starz-Gaiano Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD.
The JAK/STAT (Janus kinase/Signal transducer and activator of transcription) cascade provides signaling cues for many biological processes in animals, including stem cell maintenance and collective cell migration. Collective cell migration is very important for morphogenesis but is not as well characterized as single cell migration. Using Drosophila egg chambers, we study different layers of JAK/STAT regulation that are important for collective cell migration. The polar cells secrete Unpaired (Upd), which activates JAK/STAT signaling in the neighboring follicle cells. High JAK/STAT activation in the follicle cells specifies them as motile border cells. The border cells surround and carry the polar cells to the developing oocyte during oogenesis. We are characterizing an E3 ubiquitin ligase, Mind bomb 2 (Mib2), as a regulator of border cell migration and a possible component of JAK/STAT signaling. Earlier studies from our laboratory demonstrate that mib2 knockdown in the anterior follicle cells results in defective border cell migration, and work from other researchers has implicated Mib2 as a negative regulator of STAT activity in Drosophila cell culture. We are using a combined approach of RNA interference, overexpression, and mutant analysis to determine in which cells mib2 is required for border cell migration and to evaluate changes in STAT activity. As Mib2 has been shown to regulate cytoskeletal dynamics in Drosophila muscle maintenance, we are also examining its effect on actin cytoskeleton in the follicle cells. A better understanding of collective cell migration and JAK/STAT regulation can take us take a step further in the battle against diseases like rheumatoid arthritis, cancer metastasis, and neurodegenerative disorders.

219 Loss of the mucosal barrier alters the progenitor cell niche via JAK/STAT signaling. L. Zhang1, K. Ribbeck2, B. Turner1, K. Ten Hagen1 1) Developmental Glycobiology Section, NIDCR/NIH, Bethesda, MD; 2) Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

The mucosal barrier of our digestive tract is the first line of defense against pathogens and damage. Disruptions in this barrier are associated with diseases such as Crohn's disease, colitis and colon cancer, but mechanistic insights into these processes and diseases are limited. We have previously shown that loss of a conserved O-glycosyltransferase (PGANT4) in Drosophila results in aberrant secretion of components of the peritrophic/mucous membrane in the larval digestive tract. Here, we show that loss of PGANT4 disrupts the mucosal barrier, resulting in epithelial expression of the IL-6-like cytokine Upd3, leading to activation of JAK/STAT signaling, differentiation of cells that form the progenitor cell niche and abnormal proliferation of progenitor cells. This niche disruption could be recapitulated by overexpressing upd3 and rescued by deleting upd3, highlighting a crucial role for this cytokine. Moreover, niche integrity and cell proliferation in pgant4-deficient animals could be rescued by overexpression of the conserved cargo receptor Tango1 and partially rescued by supplementation with exogenous mucins or treatment with antibiotics. Our findings help elucidate the paracrine signaling events activated by a compromised mucosal barrier and provide a novel in vivo screening platform for mucin mimetics and other strategies to treat diseases of the oral mucosa and digestive tract.


Many developmental pathways are activated when a ligand binds to an extracellular receptor, leading to changes in the activity of intracellular signaling molecules such as kinases and transcriptional regulators, which in turn alter gene expression and cellular phenotype. Studying pathway activity in developmental models often relies on tissue-level assays of phosphorylation levels of kinases or changes in downstream gene expression. However, recent studies have shown that single-cell level differences in the kinetics of signaling molecules can influence the cell's response to pathway activation. Studies focusing on the transduction kinetics of pathways have suggested that there is an additional layer of information coded within the kinetics of signaling molecules that cannot easily be captured using tissue-level studies. It remains unclear whether cell-to-cell differences in signaling kinetics can contribute to developmental events in vivo. Drosophila provides an excellent system to investigate developmental questions, and here we report preliminary findings and designs of constructs to track kinetics of kinases downstream of Insulin signaling, Akt, at the single-cell level in Drosophila tissues. We designed a reporter for Akt, and found that the reporter responds to drug-induced stimulation and/or inhibition of Insulin signaling in S2 tissue culture cells. Transgenic animals carrying the reporters were generated, and fixed tissues of starved adults showed expected Akt activity patterns in different cell types. Live-imaging results of adult and larval tissues of transgenic animals, and data analysis methods will be discussed.

221 Identifying the molecular mechanism of the Jub/α-Catenin mechanosensitive interaction. H. Alegot, C. Markosian, K. Irvine Waksman Institute, Rutgers University, Piscataway, NJ.

Mechanical tension can regulate adult organ size by modulating the activity of the Hippo pathway. This regulation is mediated by the mechanosensitive recruitment of the Ajuba LIM domain protein Jub to the adherens junction (AJ). Under increased tension Jub is recruited to the AJ where it sequesters Warts, preventing it from inhibiting Yorkie nuclear localization and activity. Thus, increased tension leads to more proliferation. Here we investigate how tension regulates Jub/AJ interaction at the molecular and cellular level. Based on the model of mammalian α-Catenin/Vinculin interaction in vitro, we designed a structure function analysis of α-Catenin, combining in vitro and in vivo approaches. Our co-immunoprecipitation assays revealed that two different regions of α-Catenin contribute to Jub binding. In vivo we uncovered an inhibitory region that
when deleted increases Jub recruitment to the AJ, increases Yki target gene expression and leads to bigger adult wings. These results support the hypothesis that a conformational change in α-Catenin regulates Jub binding, and contribute to defining α-Catenin as an important mechano-regulator by its recruitment of proteins under tension. In parallel, we investigated the role of different populations of Myosin II (medial vs junctional) on Jub subcellular localization. Laser ablation experiments have been done to decrease tension and examine how Jub level changes under reduced tension and the dynamics of those changes. Overall, this work could give us a better understanding of how mechanical tension regulates growth.

222 β-Integrin is required for wound-induced polyploidization. R.S. Besen-McNally, K. Gjelsvik, V.P. Losick Mount Desert Island Biological Laboratory, Bar Harbor, ME.

A key step in tissue repair is to replace cells that have been lost or damaged by injury. One strategy occurs by restoring cell number through cell proliferation and another occurs by increasing cell size through polyploidization. Studies in several Drosophila tissues, mouse liver, and most recently the zebrafish epicardium have demonstrated that polyploid cells arise in adult tissues, at least in part, to promote tissue repair and restore tissue mass. However, the signals that cause polyploid cells to form in response to injury remain poorly understood. In the adult Drosophila epithelium polyploid cells are generated by both cell fusion and endoreplication resulting in a giant polyploid syncytium that is essential for wound repair. The Hippo signal transduction pathway regulates wound-induced polyploidization (WIP) by influencing both endoreplication and cell fusion, suggesting that altered biomechanical cues in the tissue could be a driver for polyploid cell growth. In a preliminary screen, we identified the β-Integrin, Myospheroid (Mys), as a candidate WIP activator. We have found that Mys is upregulated 2-3 fold in the wound-induced polyploid cells. Epithelial specific knock down of mys, using the Gal4/UAS system, caused a significant defect in wound healing by altering syncytium formation. We are currently investigating whether Mys signals directly to the Hippo pathway to regulate WIP. In conclusion, our findings point to β-integrin as one of the early signals required for generation of polyploid cells in response to injury.

223 The role of Yorkie in different stages of eye development through the utilization of different binding partners of Drosophila melanogaster. M. Anderson1, B. Nasser1, T. Cook2, J. Kagey1 1) Department of Biology, University of Detroit Mercy, Detroit, MI; 2) Center For Molecular Medicine and Genetics, Wayne State University, Detroit, MI.

The transcription factor Yorkie (Yki) is being studied. Yki regulates organ size and other key developmental aspects, and has a human homolog, Yes-Associated Protein (YAP). We utilized the UAS/Gal4 system and RNAi to study the knockdown of Yki and its binding partners to identify their role temporal and spatial role in eye development. Yki is known to have a roll in growth, survival, and differentiation throughout different stages of eye development and will serve as an excellent system for this study. When overexpressed, Yki leads to overgrowth of the tissue; when Yki is under-expressed, it leads to a reduction in tissue size. Using the eye has allowed Yki to be studied during both the adult stage to look for phenotypic changes, and the larval stage to use microscopy to stain for molecular changes. To conduct this experiment we are using the Gal4 drivers, eyeless, GMR, and, Mirror, to identify the role Yki and potential binding partners, scalloped and smad, in the development of the eye. Preliminary data suggests that a decrease in the size of the eye when using the driver eyeless in YkiΔ corresponds with a similar phenotype in tkΔ and dppΔ. Imaginal eye discs have been studied by fluorescent microscopy, to identify and molecular changes accompanying the adult phenotypes observed. Determining the effects on cell growth, regulation and signaling in Yki and its binding partners is important to understanding the effects of YAP in many cancers.

224 Dorsal/NF-κB in the Drosophila Embryo Exhibits a Ventral-to-Dorsal Gradient in Mobility. H. Al Asafen, N. Clark, R. Sozzani, G. Reeves chemical and bimolecular engineering, NC state university, raleigh, NC.

Morphogen-mediated patterning of developing tissues is a highly dynamic process. However, there are only a handful of quantitative measurements of biophysical parameters associated with morphogen gradients. Here we report measurements of the mobility of Dorsal, a Drosophila homolog of NF-κB, in the early embryo using scanning fluorescence correlation spectroscopy techniques. We find that the diffusivity of Dorsal varies along the dorsal-ventral axis, with lowest diffusivities on the ventral side. Further analysis shows that it is only Dorsal in the nucleus that has a spatially-dependent diffusivity; the cytoplasmic pool of Dorsal has a constant diffusivity in the embryo. Furthermore, nuclear export rates appear to also be lower on the ventral side of the embryo. Analysis of mutants in which Dorsal nuclear levels are uniform has confirmed this DV asymmetry in diffusivity and nuclear export rates. These observations could be explained by a significant pool of DNA-bound Dorsal on the ventral side of the embryo. We propose that either Toll-mediated phosphorylation of Dorsal or Cactus binding to Dorsal explains the DV asymmetry in these two biophysical processes.

A large class of human developmental abnormalities, known as the RASopathies, is caused by deregulated RAS signaling. Most of the structural and functional phenotypes observed in humans can be successfully mimicked in model organisms, but the underlying mechanisms remain largely unclear. We are using the first wave of RAS signaling in the *Drosophila* embryo to dissect these mechanisms, focusing on the gain-of-function mutations in MEK1, a critical component of the RAS pathway. We found that mutant MEK1 variants cause ectopic signaling in the middle of the embryo, consistent with their constitutive activity in vitro. Surprisingly, endogenous signaling at the embryonic poles is diminished, pointing towards a negative feedback mechanism. To decipher the nature of this feedback, we used RNA-seq to identify the genes upregulated in response to the uniform expression of the activating MEK1 variants. One of the top identified genes was *sprouty* (*sty*), which encodes a negative regulator of RAS signaling in multiple developmental systems. Moreover, loss of *sty* leads to further increase of ectopic signaling caused by the activating mutations. These results demonstrate how the effects of activating mutations are modulated by feedback and illustrate the power of using *Drosophila* for the mechanistic studies of a large class of developmental abnormalities in humans.

### 226 A functional screen identifying novel *Drosophila* Egf receptor targets with roles in eggshell morphology

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Signaling by the *Drosophila* epidermal growth factor receptor (Egfr) plays an important role in many aspects of development, including oogenesis, embryogenesis and proper development of both the eye and the wing. For example, in the wing Egfr signaling plays an important role in vein tissue specification, and in the ovary the pathway is known to play a key role in the establishment of the body axes during oogenesis. Microarray screens by our lab and others have been used to identify potential downstream transcriptional targets of the Egfr receptor using the *Drosophila* ovary as a model system. Our initial work compared gene expression in fly ovaries in which the activity of the Egfr-pathway was reduced (*gurken* mutant), normal (OreR), or constitutively active (CY2/λTop). We have employed a number of approaches to further investigate the expression, biological function, and mechanism of action of a subset of putative genes of interest, focusing primarily on genes of previously unknown function. A small-scale functional screen using available libraries of UAS-RNAi transgenic flies and P-element insertion lines was used to investigate the possible functions of a group of novel EGFR-responsive genes. A number of these genes have been found to play roles in normal eggshell structure and morphogenesis. Gene knockdown phenotypes include decreased chorionic integrity, shortened eggs, and various dorsal appendage malformations. We are further investigating these genes in several ways, including examination of expression patterns in *gurken* mutant, OreR, and CY2/λTop ovaries via in situ hybridization and determination of the fertility of the knockdown flies. In addition, we are creating mutant fly lines using the CRISPR-Cas9 system to induce null mutations in our genes of interest and will be using these lines for further study.

### 227 Investigation of eRpL22-like function in *Drosophila melanogaster* eye development through consequences on EGF signaling

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The *Drosophila melanogaster* eRpL22 ribosomal protein family contains two paralogues: eRpL22 and eRpL22like. eRpL22like shows unique structure and tissue expression pattern from eRpL22, potentially indicating paralogs in this family have tissue-specific roles as components of specialized ribosomes, or they confer or possess extra-ribosomal function. eRpL22 is ubiquitously expressed whereas eRpL22-like expression is specific to the testes and eye. Using the core ribosomal component eRpL23a as a positive control for ribosome presence in third instar eye discs, immunohistochemistry analysis revealed eRpL22 co-localizes more with eRpL23a, than eRpL22like does with eRpL23a. Less co-localization with eRpL23a indicates an extra-ribosomal role for eRpL22like and this differential expression pattern reveals a potential function of eRpL22-like in early eye tissue.

Mosaic knockout of eRpL22-like showed several eye phenotypes in midpupal stages, most significantly 5-8 ectopic cone cells instead of the typical 4. This phenotype in particular is indicative of epidermal growth factor receptor (EGFR) pathway over-activation. EGF/EGFR functions to induce growth, differentiation, migration, adhesion and cell survival pathways, all of which are critical for proper eye development to occur. Ras is a downstream component in the EGFR signaling pathway – Ras overexpression results in eye ommatidia fusions. Overexpression of Ras alone does not result in the ectopic cone cell phenotype, indicating that eRpL22-like must interact upstream of Ras within the EGFR pathway. Ambiguous results from this EGFR pathway effector screen suggests pathway cross-talk, potentially implicating notch-delta signaling.

Given these preliminary findings, EGFR, its ligands, and its downstream effectors (a narrow candidate pool of accessory proteins upstream of Ras), emerge as potential molecular communication targets for eRpL22like. Initial characterization of eRpL22 family paralog expression patterns provide evidence for the functional divergence of eRpL22like from eRpL22, supporting the idea that ribosomal proteins have extra-ribosomal roles, or alternatively, indicating ribosomes themselves are more complex than previously thought.
228  **Probing PLC-γ function in Drosophila by in vitro mutagenesis.** C. Naidu, J. Thackeray  Biology Department, Clark University, Worcester, MA.

Drosophila has a single PLC-γ homolog encoded by small wing (sf). Previous studies have shown that SL is a negative regulator of the highly conserved EGFR signaling pathway, but also participates as a positive regulator of insulin signaling during growth. To investigate the role of the various domains in the fly PLC-γ homolog we generated a series of sf germline transformation constructs that each knock out a critical amino acid in one of the many domains in this protein. In examining the capability of these altered forms to function during photoreceptor R7 and wing vein development, as well as wing growth, the most striking finding is that the tyrosine residue predicted to be phosphorylated during activation of the molecule at the activated receptor is required for EGFR signaling, but is dispensable for SL's role during insulin signaling in the wing. This indicates that there is a fundamentally different mechanism of activation for PLC-γ when activated by the InR than when activated by EGFR.

229  **A Deficiency Screen for Genetic Interactors of Jagunal in Drosophila.** Sydney Alvarado, Khayla Shabazz  San Francisco State University, San Francisco, CA.

The Endoplasmic Reticulum (ER) is an essential organelle involved in protein secretion, calcium homeostasis, and lipid synthesis. However, not much is known about how it partitions and is inherited during cell division. A recent study has shown that the ER divides asymmetrically during mitosis in the Drosophila embryo early during gastrulation. This asymmetric partitioning of the ER relies on the highly conserved ER transmembrane protein Jagunal (Jagn), however the molecular pathway that drives jagn-dependent ER partitioning is currently unknown. Preliminary data demonstrates that ectopic inhibition of jagn in the Drosophila compound eye results in a rough eye phenotype. In order to identify Jagn interactors, we performed a dominant modifier screen involving deficiency lines covering the entire 3rd chromosome. Here, we crossed JagnRNAi line with deficiency lines covering the entire 3rd chromosome. We have identified 29 suppressors and one enhancer of the Jagn-induced rough eye phenotype, which included genes involved in asymmetric cell division, regulation of cell shape, and response to ER stress. Future directions include secondary screening of identified targets and examination of their role in ER asymmetric partitioning. Further validation of the identified targets will provide important insight into the molecular pathway involved in ER partitioning.

230  **Characterization of Antibodies Developed against Two Basement Membrane Degraders in Drosophila melanogaster.** A.A. Aromiwura, A. Srivastava  Biology, Western Kentucky University, Bowling Green, KY.

Basement Membranes (BMs) are an evolutionarily conserved specialized form of extracellular matrix that surround most organs and tissues. Among several other functions, BMS provide structural support to the tissue, and act as barriers that prevent tumor metastasis. Previously in our lab several putative BM degraders were identified in a genetic screen. To further understand the role of these genes in development we generated antibodies against two BM degraders. The specificity of these antibodies was confirmed by utilizing western blots and immunohistochemistry on tissues overexpressing and downregulating these genes. Additionally, the antibodies were used to assess the localization of the respective proteins in various tissues. Data from this analysis will be presented.

231  **Genetic dissection of interommatidial cell calcium signaling.** H.C. Chang, D.F. Ready  Department of Biological Sciences, Purdue University, West Lafayette, IN.

Ca²⁺ waves, characterized by cell-to-cell spreading of cytosolic Ca²⁺ spikes, have been implicated in numerous processes, including electrical synapse formation, glia-glia and glia-neuron communications, and wound healing. To further understand the physiology and functional significance of Ca²⁺ waves, we have developed a powerful model that allows non-invasive imaging of Ca²⁺ spikes at single-cell resolution. In the process of exploring whether Ca²⁺ waves play a role in Drosophila visual system, we expressed GCaMP6, a genetically encoded Ca²⁺ sensor, with IGR-GAL4 (long form GMR; active in all retinal cells except the bristle cells), and observed robust Ca²⁺ waves in adult eyes. These Ca²⁺ waves, propagating specifically through honeycomb-like lattice of interommatidial cells (IOCs), appear to be capable of initiating from any region of the retina, advance equally in all directions, and move across the eye with a speed of 4-5 um/sec. In young IGR>GCaMP6 adult eyes, the dynamics of calcium waves are regular, with each cytosolic Ca²⁺ spike rising sharply to a maximum in approximately 2 sec, followed by a slower return to baseline. Each spike lasts ~30 sec, followed by a baseline level of ~30 sec, with cells thus spending approximately equal times active and resting. The dynamics of IOC Ca²⁺ waves deteriorate significantly in older IGR>GCaMP6 flies, implying this phenomenon has an age-dependent functional relevance. Using both RNAi-mediated knockdown and FRT/FLP-induced mutant clones, we provide evidence that IOC wave propagation requires inositol 1,4,5-triphosphate receptor (IP3R), indicating that these spikes are facilitated by Ca²⁺ release from the ER stores. Removal of IP3R function in the eye has been shown to cause photoreceptor degeneration (Acharya et al. 1997; Raghu et al. 2000). This, along with our observation that IP3R is required for IOC Ca²⁺ waves, raises an intriguing possibility that these accessory cells are
communicating via Ca\(^{2+}\) waves to ensure photoreceptor maintenance. Taken together, our preliminary work demonstrates the ease of this \(\text{IGMR}>\text{GCaMP6}\) platform can be used to systematically identify additional factors required for Ca\(^{2+}\) wave initiation and propagation, which should pave ways to further dissect the functional relevance of this cellular communication.

232 **Ecdysone regulates epithelial barrier maturation in wing imaginal discs.** D.F. DaCrema, A. Halme Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

The epithelial barrier, formed by septate junctions in invertebrates, compartmentalizes the body and provides a barrier to pathogens. To create and sustain distinct compartment environments, the formation and permeability of the epithelial barrier is highly regulated. This regulation is especially evident during development. We modified a barrier-permeability assay to measure changes in the function of the epithelial barrier in maturing wing imaginal discs. *Drosophila* imaginal discs are epithelial, pouch-like tissues derived from the embryonic epidermis and invaginate from the larval epidermis. They contain a primary, pseudostratified epithelium, which becomes the adult tissue, and a squamous epithelium, which undergoes histolysis during metamorphosis. Using our assay, we observed that the epithelial barrier of wing imaginal discs is permeable early in the third larval instar, but becomes impermeable prior to pupariation. This maturation of epithelial barrier function correlates with an increase in circulating levels of the steroid hormone ecdysone. To determine if ecdysone signaling regulates this change in the epithelial barrier, we blocked ecdysone signaling by overexpressing ecdysone receptor dominant-negative constructs. The expression of dominant-negative Ecdysone Receptor A prevented the maturation of the epithelial barrier in the wing imaginal discs. To determine whether ecdysone is sufficient to induce barrier maturation earlier, we fed larvae ecdysone and found that this accelerated barrier maturation. To determine how the septate junctions change during epithelial barrier maturation, we examined the localization of several septate junction components over the timecourse of barrier maturation. Coracle, a *Drosophila* homolog of protein 4.1 and a core component of the septate junctions, is diffusely localized along the lateral cell membrane prior to epithelial barrier maturation. Following barrier maturation, Coracle becomes tightly localized to the septate junctions. We are currently examining whether ecdysone regulates the maturation of the epithelial barrier by regulating the localization of Coracle to the septate junctions.

233 **4e Binding Protein is essential for adaptation to hypoxia in *Drosophila.*** M.J. Katz\(^1\), J Perez Perri\(^1,3\), J Acevedo\(^1,4\), A Valko\(^1\), M Melani\(^1,2\), E Sorianello\(^1,2,5\), P Wanner\(^1,2\)

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mRNA translation plays a key role during the cellular response to different stress conditions. The cap-dependent translation inhibitor 4eBP is highly regulated by diverse signal transduction pathways that respond to energy availability. On the other hand, the transcription factor HIF mediates adaptation to hypoxia through mechanisms that inhibit mRNA translation, reduce metabolism and oxygen consumption. Since 4eBP is involved in energy homeostasis balance determination, in this work we sought to evaluate its requirement in adaptation to hypoxia in an in vivo model. Our results show that 4eBP transcription is strongly induced in hypoxia in embryos, larvae and adult flies. Moreover, mutant flies experiments have revealed that 4eBP transcription induction during hypoxia depends on the transcription factors dFOXO y dHIF. In silico analysis of 4eBP promoter has predicted the presence of four putative hypoxia response elements (HRE). Using a luciferase reporter we have confirmed that one of the predicted HRE sequences is necessary for 4eBP transcriptional induction in hypoxic conditions. *Drosophila* 4eBP mutants are viable and fertile in normoxia but fail to survive in hypoxia. In adult stage, 4eBP mutant flies accumulate reactive oxygen species in comparison to wild type flies. In addition, superoxide dismutase over expression partially revert lethality in 4eBP mutant flies exposed to hypoxia. Finally, 4eBP mutant flies increase their mitochondrial content suggesting an aberrant mitochondrial metabolism in this background. We conclude that 4eBP is a central regulator of energy balance essential for adaptation to hypoxia in vivo.

234 **Tissue homeostasis in the context of DNA damage, cell death and cellular signalling: Non-apoptotic role of Dronc in DDR and cell protective function of JNK via dp53.** C. Khan\(^1,3\), S Muliyil\(^2\), C Ayyub\(^1\), B.J Rao \(^1\)

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DNA damages provides with the stressful situation to the cells, where it need to decide amongst various choices available to a cell which consists of cell cycle arrest, DNA repair and cell death. Moreover, genomic stress need to be communicated within the tissue for efficient homeostatic response. In my talk, I will be talking about the role of non-apoptotic cell death machinery in DNA damage signalling as well as how DNA damages are compensated within the tissue via crossstalk of cellular signalling pathways with DNA damage signalling, using *Drosophila* wing imaginal discs as model system. The phosphorylation of the histone variant H2Ax (denoted yH2Avx; yH2Av in flies) constitutes an important signalling event in DNA damage sensing, ensuring effective repair by recruiting DNA repair machinery. In contrast, the yH2Av response has also been reported in dying cells, where it requires activation of caspase-activated DNases (CADs). Moreover, caspases are known to be required downstream of DNA damage for cell death execution. We show here, for the first time, that the *Drosophila* initiator caspase
Dronc acts as an upstream regulator of the DNA damage response (DDR) independently of executioner caspases by facilitating γH2Av signalling, possibly through a function that is not related to apoptosis. Such a γH2Av response is mediated by ATM rather than ATR, suggesting that Dronc function is required upstream of ATM. In contrast, the role of γH2Av in cell death requires effector caspases and is associated with fragmented nuclei. Our study uncovers a novel function of Dronc in response to DNA damage aimed at promoting DDR via γH2Av signalling in intact nuclei. Where, we propose that Dronc plays a dual role that can either initiate DDR or apoptosis depending upon its level and the required threshold of its activation in damaged cells. In addition, we will also be talking about the role of JNK and dp53 signalling following DNA damage assaults, in cell protection and cell death, by positively feedback regulation. Where, I will show the role of JNK and dp53 in regulating the expression pro-apoptotic genes rpr and hid for executing cell protection and cell death. These opposite roles of JNK and p53 in regulating cell death, protects cells from excessive death, which in turn be decided by the levels of DNA damages or the persistence of DNA damages. Further, I will also allude to the crosstalk of DDR signalling in regulating activation of JNK and dp53 which in turn bring about overall tissue homeostasis.


Adenosine (Ado) is a crucial metabolite and signaling molecule, which affects energy homeostasis and cell proliferation. It is released from cells under stress conditions, so that its extracellular concentration may increase to micromolar range. The level of extracellular Ado can be controlled by Adenosine deaminases (encoded by Adenosine deaminase-related growth factor genes in Drosophila). Most of the physiological functions of Ado are mediated by G-protein coupled Adenosine receptor or by transport into cells by Exquisitivertebrate and Concentrativemembrane transporters. The Ado uptake is followed by phosphorylation with Adenosine kinases, which can interfere with AMP/ADP/ATP energy homeostasis in the cells. Key proteins regulating Ado signaling, transport and metabolism are conserved among vertebrates and invertebrates.

Here we examined levels of transcriptional response of four Drosophila cell lines, which exhibit various sensitivity to high extracellular adenosine. We compared imaginal disc cell line CI.8+, hematopoietic cell line Mbn2, embryonic cell line S2 and neuroblast cell line Bg2-c6 and focused on the differences in basic transcription of genes involved in Ado signaling, transport and metabolism and also possible transcriptional adaptations after exposure to medium with high extracellular Ado.

Our results show that different types of Drosophila cell lines use different pathways for Ado conversion and suggest that such differences may be an important part of complex mechanisms maintaining energy homeostasis in the body. We also revealed importance of Adenosine signaling in the regulation of cell energy metabolism.

236 Functional characterization of concentrative nucleoside transporter 2 (CNT2) in Drosophila melanogaster.  H.O. Maaroufi1,2, L. Cota Vieira2, Y. Lin1,2, M. Zurovec1,2 1) University of South Bohemia, Faculty of Sciences; 2) Biological Centre of the Czech Academy of Sciences.

Adenosine (Ado) is a ubiquitous metabolite that plays a prominent role as a paracrine homeostatic signal of metabolic imbalance within tissues. It quickly responds to various stress stimuli by adjusting energy metabolism and influencing cell growth and survival. Ado homeostasis in tissues is maintained by Adenosine deaminase (ADA) which converts Ado into Inosine, by Adenosine Receptor (AdoR) through which Ado activates a specific signaling pathway regulating the cell growth and by nucleoside transporters. There are two types of nucleoside transporters: the Concentrativtransporters (CNTs) and the Exquisitivertate transporters (ENTs) which are membrane transport proteins delivering purine and pyrimidine nucleosides across the cytoplasmic membrane. In Drosophila, there are two types of CNTs which mediate nucleoside uptake from the extracellular space to the cytoplasm: CNT1 and CNT2. In this project, our focus is directed to describe the physiological function of CNT2 in Drosophila melanogaster. CNT2 is mostly expressed in the Drosophila digestive system. Our results show that cnt2 mutation causes high lethality in the larval stage, indicating that CNT2 is important for Drosophila development. This lethality is linked to the appearance of a strong melanization (due to cell death) which is restricted to the hindgut. These “melanotic tumors” are highly similar to those found in the adenosine deaminase (enzymes of Ado metabolism) mutant and over-expression of adoR. Both adgf-A mutants and adoR over-expressing flies also showed high mortality due to over-activation of AdoR signaling. Moreover, the RNA expression pattern provided by Flybase showed that cnt2 is highly expressed in the midgut, while adoR is in the hindgut. Hence, we hypothesize that cnt2 mutation causes the accumulation of Ado in Drosophila gut lumen. This accumulation leads to the over-activation of AdoR in the hindgut which gives rise to melanization and the death of the cnt2 mutant larvae.

237 Role of STIM and Orai Ca2+ signaling proteins in developmental cardiomyocyte growth.  C.E. Petersen, J.S. Smyth. Anatomy, Physiology, and Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD.

The store-operated Ca2+ entry (SOCE) mechanism of Ca2+ signaling has been strongly implicated in rodent models of pathological cardiac hypertension, suggesting that SOCE is an essential regulator of heart physiology and disease. An important hallmark of cardiac hypertrophy etiology is the re-activation of signaling mechanisms that regulate cardiomyocyte growth during heart development. Consistent with this, expression and function of STIM and Orai proteins that mediate SOCE are high in fetal cardiomyocytes and drop precipitously in adult cardiomyocytes, with restoration of high expression levels in
Identification of Novel Proteins Required for Ras Membrane Localization Using the Drosophila Eye.  
Juliet King, Sarah Lombel, Kyle Shtern, Julie Gates  Biology, Bucknell Univ, Lewisburg, PA.

Mutations in the small GTPase Ras are associated with 30% of all human cancers. The most common oncogenic Ras mutation disrupts its GTPase activity, leaving Ras in its constitutively active, GTP bound form. Both wild type and mutant Ras must undergo a post-translational prenylation in order to associate with the cell membrane. Once anchored to the membrane, GTP bound Ras is able to stimulate downstream signaling cascades to initiate processes like cell division and differentiation. To gain insight into the post-translational modification of Ras, James Mahaffey and Mark Philips at the NYU School of Medicine used a mammalian cell culture screen to identify ten novel proteins that are required during this process. These candidate proteins include receptor tyrosine kinases (RTK), non-receptor tyrosine kinases and phosphatases. To determine whether these candidates perform a similar role in an intact organism, we are using the Drosophila eye as a model. Ras is a component of the Boss-Sevenless RTK pathway that, when activated, results in one of five Sevenless RTK-expressing cells taking on the R7 photoreceptor cell fate. When GTP bound Ras is expressed in all five of these cells, the Boss-Sevenless RTK pathway remains active and they all become R7 photoreceptors. This results in the ommatidia losing their tight organization and becoming enlarged, giving the eye a rough appearance. We have used the sevenless-Ga4 driver to express GTP bound Ras (UAS-Ras85D.v12) and UAS-RNAi for each candidate in the developing eye. We would expect that lowering levels of the candidate proteins that are involved in Ras prenylation would prevent GTP bound Ras from associating with the membrane and activating downstream signaling cascades, leading to wild type eyes. If the candidate protein is not necessary for membrane localization, then when protein levels are lowered, Ras would still be able to localize to the membrane, leading to rough eyes. Thus far we have examined four of the ten candidates and found that lowering the levels of two of these candidates in the presence of GTP bound Ras resulted in a shift from rough to wild type eyes. We are currently using RT-qPCR to confirm that this change in phenotype correlates with a decrease in the candidate mRNA levels. This work will allow us to determine whether the candidate proteins are required for Ras membrane localization in an intact organism and enhance our understanding of how Ras membrane localization is regulated.

Identification of a conserved Rabex-5 ubiquitination signal in Ras.  
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Ras proteins are conserved GTPases that act downstream of several signal transduction cascades and play crucial roles in cell proliferation, development and differentiation. Misregulation of Ras signaling by activating mutations in the Ras proteins or upstream regulators and downstream effectors is observed in a number of human cancers and developmental disorders (collectively called Rasopathies). Analogous activating mutations in the Drosophila Ras, Ras85D (also called Ras1; hereafter called “Ras”) result in developmental abnormalities and tissue hyperplasia recapitulating human tumor phenotypes. Thus, in a cellular context, limiting the activity of endogenous Ras proteins is crucial to maintain tissue homeostasis. We previously identified a role for Rabex-5, an E3 ubiquitin ligase, to inhibit Ras activity by ubiquitination in vivo. Crucially, Rabex-5 can inhibit both the oncogenic and wild-type (WT) forms of Ras. Here, we identify a tyrosine-based ubiquitination signal necessary for Rabex-5-mediated inhibition of Ras. This conserved signal is also present in mammalian Ras and represents a transposable signal able to autonomously confer Rabex-5-mediated ubiquitination on target proteins. Characterizing the effect of expressing Rabex-5 ubiquitination insensitive Ras results in Ras activation phenotypes in epithelial tissues such as the wing and the eye. In contrast, expressing a phosphomimic Ras to increase ubiquitination is able to suppress the phenotypes of oncogenic Ras G12V. Together, these data indicate that inhibitory ubiquitination of Ras is important in physiological contexts and that evading Rabex-5-mediated ubiquitination could initiate a malignant transformation.
The small GTPase Rala is required for lymph gland homeostasis in *Drosophila*. H. Knaevelsrud1,2, G. Gavory1, C. Baril1, J. Enserink2,3, M. Therrien1,4 1) Institute for Research in Immunology and Cancer, Laboratory of Intracellular Signaling, Université de Montréal, Montréal, Québec, Canada; 2) Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; 3) Section for Biochemistry and Molecular Biology, The Department of Biosciences, Faculty of Mathematics and Natural Sciences, University of Oslo, Oslo, Norway; 4) Département de pathologie et de biologie cellulaire, Université de Montréal.

The small GTPase Ral regulates important membrane trafficking events, including exocytosis, endocytosis and autophagy. In mammalian cells RalA and RalB are activated downstream of active Ras, which directly interacts with RalGEFs. Over the recent years it has become clear that RalA/RB plays important roles in signal transduction leading to cancer formation and metastasis, both in Ras-dependent and independent manners. *Drosophila melanogaster* has one single Ral protein, Rala, which has been implicated in eye, notum and wing development independently of Ras. In this study we have investigated the involvement of Rala in the hematopoietic system of *D. melanogaster* larvae. We found that perturbation of Rala function by RNAi or dominant negative Rala increased the the number of circulating hemocytes and the size of the cortical zone of the lymph gland, which contains differentiated hemocytes. Moreover, we determined that Ras and Rala affect hemocytes independently of each other. Instead, Rala activity in S2 cells and in the lymph gland is regulated by RalGPS. Finally, we establish that the exocyst and Rab11 is involved in maintaining lymph gland homeostasis downstream of Rala. In conclusion, we present a new RalGPS-Rala-exocyst-Rab11 axis active in the maintenance of lymph gland homeostasis.

Calcium-dependent regulation of actomyosin contractility in epithelia. M.K. Levis1, J. Jangula1, A. Nematbakhsh2, M. Alber2, J. Zartman1 1) Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN; 2) Department of Mathematics, University of California Riverside, Surge, Riverside, CA.

Wound healing in epithelial cells is crucial to prevent infection in many tissues including the skin, lungs, and stomach. However, there is a poor understanding of how biochemical signals and mechanical forces are coupled to coordinate cellular processes during epithelial regeneration. Among their many signaling roles, calcium ions (Ca\(^{2+}\)) act to regulate mechanical forces generated by actomyosin contractility after wounding. Here, we quantitatively investigate the impact of Ca\(^{2+}\) channel activity on wound healing dynamics in the *Drosophila* wing imaginal disc, a powerful model system to study wound healing and regeneration. Multiple Ca\(^{2+}\) channels including IP\(_3\),R and SERCA were pharmacologically inhibited in the wing disc and the dynamic response to wounding was measured. Precise laser incisions were made along the edge of the cell membranes of two adjoining cells. The recoil of tricellular junctions connecting the cut cell provides a gauge of relative tissue tension. Additionally, the dynamics of the gap size created by the cut were quantified. On short time scales, inhibiting IP\(_3\),R pharmacologically, did not significantly impact the recoil of tricellular junctions or growth of the wound margin. Inhibition of SERCA increased the net recoil of tricellular junctions, consistent with increased actomyosin contractility, but dynamics of the wound margin were unaffected. We are quantifying the dynamic relationships between Ca\(^{2+}\) ion concentrations, actomyosin accumulation, and cell bond tension to explain these surprising and non-intuitive results. These data also feed into a multi-scale subcellular element model of wound healing that incorporates Ca\(^{2+}\) dependent regulation of cell mechanical properties to examine different hypotheses in this regard. Many FDA approved drugs are available that manipulate Ca\(^{2+}\) homeostasis. Consequently, mechanistic insights into how Ca\(^{2+}\) signaling impacts actomyosin dynamics and cell contractility can lead to promising therapeutic targeting strategies for improving chronic wound healing outcomes.

Investigating precise regulation of the RhoA GTPase in tissue folding. M. Denk-Lobnig, A.C. Martin 1) Institute for Research in Immunology and Cancer, Laboratory of Intracellular Signaling, Université de Montréal, Montréal, Québec, Canada.

Rho-family GTpases and actomyosin-driven contraction are precisely regulated, which is crucial for tissue morphogenesis and development. *Drosophila melanogaster* mesoderm invagination depends on both positive and negative regulation of the Rho-family GTPase RhoA. In the mesoderm, RhoA is activated by the guanine nucleotide exchange factor RhoGEF2. A GTPase activating protein, Cumberland GAP (C-GAP; RhoGAP71E), inhibits RhoA and is required for proper organization of actomyosin contractility. How RhoGEF2 and Cumberland GAP are coordinated to precisely control RhoA and actomyosin is unclear. I have used CRISPR-mediated genome editing to generate endogenously tagged Cumberland GAP to determine its localization in the *Drosophila* embryo. We find that C-GAP localizes to the cytoplasm where it could result in global RhoA inhibition away from a polarized RhoGEF2 activation. In addition, we observe local enrichment of C-GAP at the lateral cell membrane, which could further polarize RhoA activity. We aim to understand how RhoA’s regulators are coordinated spatially and temporally.

Protein Kinase C δ regulates the structure and dynamics of cellular protrusions of migrating border cells. Felix Gunawan, Syed Saad Husainie, Adam Kramer, Jing Lu, Amad Bhatti, Dorothea Godt Dept Cell & Systems Biology, University of Toronto, Toronto, ON, CA.

Actin-based membrane protrusions are essential for many cell migration processes, including the migration of the border cell cluster (BCC) during *Drosophila* oogenesis. In a transcriptome-wide screen, we identified Protein Kinase C delta (PKCδ) as a putative downstream target of the transcription factor Traffic Jam, which we had previously shown to regulate BCC migration. Here, we show that the serine/threonine kinase PKCδ regulates the formation and organization of actin filament
bundles in migrating border cells. We find that PKCδ is enriched along the plasma membrane and in the leading protrusion of the BCC. Examination of PKCδ mutants, generated through CRISPR/Cas9 technology, revealed its requirement in maintaining the normal morphology and dynamics of cellular protrusions, and in limiting tumbling movements of the BCC. Increased PKCδ expression caused delays in BCC migration by disrupting the formation of actin filament bundles and reducing cellular protrusions. Expression of catalytically inactive PKCδ affirmed that the kinase activity of PKCδ is necessary for the effect of PKCδ on the F-actin network. In summary, our analysis identifies PKCδ as an important regulator of F-actin organization and cellular protrusion dynamics during collective cell migration.

244 Role of α-Catenin actin-binding domain in regulating cadherin complex interaction with the F-actin cytoskeleton. **Ritu Sarpal**, Noboru Ishiyama, Mistuhiko Ikura, Ulrich Tepass 1) Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada; 2) Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; 3) Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada.

α-Catenin (α-Cat) is a mechanosensory protein that operates at the interface of the E-cadherin-β-Catenin complex and the F-actin cytoskeleton. However, the mechanism by which the actin-binding domain (ABD) of α-Cat interacts with F-actin and the function of the direct α-Cat-actin interaction remain largely unknown. Using a structure-guided mutagenesis approach, we identified residues in the α-Cat ABD that are important for binding to F-actin to ask whether direct F-actin binding is crucial for α-Cat function in vivo. A mutant form of α-Cat, α-Cat3A, that carries point mutations in these residues is effectively recruited to adherens junctions (AJs) but is unable to rescue the α-Cat mutant zygotic phenotype. Next, we generated an α-Cat variant, α-CatABD*, that showed a significantly higher binding affinity to F-actin in vitro compared to α-Cat. In flies, α-CatABD* is recruited effectively to AJs but shows only a minor rescue of the α-Cat zygotic mutant phenotype. Further, over-expression of α-CatABD* (as well as α-Cat3A) resulted in larval or pupal lethality in contrast to over-expression of α-Cat which is completely viable. To assess the impact of α-CatABD* on specific cell movements, we expressed α-CatABD* in embryos maternally depleted of endogenous α-Cat. Maternal siRNA knockdown of α-Cat compromises mesoderm invagination, a defect rescued by the co-expression of a siRNA-resistant α-Cat transgene. In contrast to α-Cat, expression of α-CatABD* is unable to rescue mesoderm defects. Together, these findings indicate that either loss or enhancement of direct F-actin binding of α-Cat severely interferes with its function, and suggest that the dynamic regulation of the direct interactions between α-Cat and actin is essential for the function of AJs during Drosophila morphogenesis.

245 Using pathway-specific downstream genes to quickly evaluate changes in signaling status in RNA-seq data. **Wei Song**, Yanhui Hu, Aram Comjean, Stephanie E. Mohr, Norbert Perrimon 1) Department of Genetics, Harvard Medical School, Boston, MA 02115, USA; 2) Drosophila RNAi Screening Center, Harvard Medical School, Boston, MA 02115, USA; 3) Howard Hughes Medical Institute, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.

Gene expression profiling has been widely used for revealing the regulatory mechanisms of diseases, cellular stress responses and other biological processes. Signaling pathways that control growth, differentiation, aging, as well as metabolism, are at the core of many regulatory systems. To uncover mechanisms underlying cellular phenotypes, it is essential to analyze gene expression signatures in the context of signaling pathways. However, when a pathway is activated, the expression levels of pathway core components including transcription factors (TFs) and cofactors are not necessarily affected, as they are constantly regulated in a post-translational manner, such as by phosphorylation. By contrast, the expression levels of the downstream targets of TFs are more reliable indicators of pathway activities. For example, expression levels of FoxO target genes such as 4EBP and INR (insulin receptor), but not IRS or AKT, have been used as an indicator of insulin pathway activity. Currently, curated resources of TF target genes for signaling pathways are not well established for Drosophila melanogaster, which hampers accurate prediction of pathway activities. We evaluated all relevant publications for the 11 major signaling pathways and assembled TF target gene lists based on the literature. The resource is supported by a new online tool, PathPT, that lets users upload RNA-Seq data and quickly evaluate down or up-regulation of the major signaling pathways in response to a perturbation.

246 Coordinated regulation of microRNAs by ATM/E2F1/p53 in Drosophila at physiological condition and at DNA damage response. **Rui Zhu**, Ying Ge, Ze Zhao, Qinghua Wang, Xiaolin Bi 1) Department of Biological Sciences, Dalian Medical University, Dalian 116044, CN; 2) Institute of Cancer Stem Cells, Cancer Center, Dalian Medical University, Dalian 116044, CN.

To preserve the genome integrity, highly coordinated signaling pathways and repair mechanisms named DNA damage response (DDR) have been evolved in cells. ATM kinase is a central player to facilitate cellular response to the DNA double strand breaks (DSBs) and can phosphorylate a variety of targets such as p53 and E2F1. The tumor suppressor p53 is one of the major players for protection of genome. Another family of transcription factors that affect cell fate is the E2F family. There is extensive crosstalk between the E2F and p53 pathways to influence vital cellular decisions. The microRNAs (miRNAs) regulate virtually every biological process through regulation of gene expression at the post-transcription level, large quantity of studies have shown miRNAs are regulated by DDR and play a role at the DDR. A few systemic studies have demonstrated that miRNAs are functional components of the p53 pathway. Recent studies suggest that
ATM regulates miRNAs biogenesis at the primary miRNA level in response to DNA damage through phosphorylation of KH-type splicing regulatory protein (KSRP), a key component of Drosha and Dicer miRNA processing complex, ATM can also regulate nuclear export of precursor miRNAs at DNA damage response. However in vivo analysis of coordinated regulation to miRNA expression by ATM, E2F1, and p53 is still lacking.

In this study, we systemically investigated differentially expressed (DE) miRNAs at embryonic stage between p53 mutant and wild-type (wt) flies, and at third instar larval stage among atm/e2f1/p53 mutants and wild-type flies, at both normal physiological condition and after ionizing radiation (IR). We identified 28 DE miRNAs between p53 mutant and wt in embryos and 20 DE miRNAs in L3 under physiological condition, and 34 DE miRNAs between p53 and wt in embryos and 8 DE miRNAs in L3 after irradiation, respectively. Between e2f1 mutant and wt flies, we identified 27 DE miRNAs under physiological condition and 40 DE miRNAs after irradiation. And between atm mutant and wt flies, we identified 34 DE miRNAs under physiological condition and 33 DE miRNAs after irradiation. Among all DE miRNAs, some were identified in atm, e2f1, and p53 mutants. We found that Drosophila p53 regulates miRNAs in a different mode during development, and ATM and E2F1 play more important roles in regulating miRNAs after irradiation. We also detected sensitivity to irradiation of most DE miRNAs mutated flies and explored interrelations between p53 and E2F1 in regulating miRNAs. Further more, we found some miRNA mutated flies were highly sensitive to irradiation, showed more irradiation induced apoptosis, or S phase checkpoint defect. The study of intrinsic mechanisms underlying non-coding RNAs at DNA damage response are still ongoing.

247 Defining the mechanisms by which the Crk family of adaptor proteins regulate cell adhesion and actin dynamics during neural development and morphogenesis.  A.J. Spracklen1, A.N. Bonner2, E.M. Thornton-Kolbe2, M. Peifer1,2 1) Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

Crk family proteins, including Crk and Crk-like (Crk-L), are a well conserved family of small adaptors that help assemble multi-protein signaling complexes to modulate diverse biological processes, including cell adhesion, migration, and immune synapse function. They also play important roles in many cancers, including leukemia where Crk-L is a key mediator of oncogenic forms of Abelson tyrosine kinase (Abl). Knockout mouse models demonstrated that Crk and Crk-L have essential, but non-overlapping roles during embryogenesis; however, double mutants have not been examined, and thus key questions about their in vivo function remain. Our lab and others have defined key roles for fly Abl in both embryonic morphogenesis and central nervous system (CNS) patterning. We were surprised to find a short, conserved motif (PXXP) within the linker region was more important for CNS patterning and epithelial morphogenesis, than both kinase activity and F-actin binding. This led us to hypothesize that PXXP-interacting partners, like Crk, are critical for mediating Abl's activities during embryogenesis. To test this hypothesis, we are using genetic and cell biological approaches to define the morphogenetic roles of Crk, the sole Crk family member in the fly, during normal development. Using both RNAi and a powerful gene replacement platform we generated using CRISPR, we find Crk is essential for embryonic viability and several aspects of embryonic morphogenesis. Given Abl's well-defined roles in CNS patterning, we first explored Crk's roles there. We found loss of Crk disrupts proper axon guidance, resulting in both aberrant midline crossing defects and gross perturbation of CNS morphology, including loss of commissural axon bundles. Interestingly, these defects are reminiscent of mutants either completely lacking Abl or where Abl-Crk interactions are disrupted, suggesting Abl and Crk work together during CNS patterning. However, loss of Crk also results in earlier morphogenic defects, including a failure to properly stabilize contractile actomyosin rings during cellularization, resulting in entrapment of nuclei during furrow ingression, as well as defects in early syncytial divisions. These defects are not observed in Abl mutants, suggesting Crk has Abl-independent roles during early embryogenesis. We are continuing to define the mechanisms by which Crk works with interacting partners, including Abl, to shape dynamic cell behaviors throughout development.

248 Identifying kin17 as a potential novel regulator of autophagy, using GWAS technologies in D. melanogaster.  A. Weeger 1) Genetics and Developmental Cell Biology, Iowa State University, Ames, IA.

Using the autophagy pathway, a cell under stress can recycle its own damaged proteins and organelles to continue supplying energy to the organism. To better understand this pathway, and uncover novel regulation factors, we have used the DGRP fly panel to assess autophagy response before and after starvation stress in genetically diverse backgrounds of Drosophila Melanogaster. We applied GWAS technology after gathering phenotypic data both in a well fed fly, and following a starvation challenge. We were able to isolate 298 unique SNP associated with a constitutive, non stress induced, autophagy response and 222 unique SNPs associated with a response to stress. These SNPs are linked with 112 unique genes in the case of stress independent autophagy and 97 genes of interest in the case of stress dependent autophagy. Following RNAi testing of selected candidate genes, we further identified kin17, a zinc-finger protein, as a potential negative regulator of autophagy function along with other promising candidates.

249 Establishing a model of BM damage and analyzing its repair.  A. Howard1,2, G. Bhave1,3, A. Page-McCaw1,2 1) 1Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN; 2) Program of Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN; 3) Department of Medicine, Vanderbilt
University Medical Center, Nashville, TN.

The basement membrane is a sheet-like extracellular matrix that wraps around muscle fibers and underlies epithelia. Although the basement membrane is often considered to be static, there are indications that the BM is a dynamic structure in vivo, as it can grow, shrink, and repair. We have developed a system to analyze basement membrane repair in adult animals, using an adult gut injury model in Drosophila. The gut has a well-defined architecture of epithelial cells (enterocytes) residing on top of a basement membrane sheet, and the gut tube is wrapped in visceral muscles also surrounded by basement membrane.

To injure the basement membrane of the gut, flies are fed Dextran Sodium Sulfate (DSS); DSS administration has been previously used as a model for ulcerative colitis in mice. In Drosophila, DSS induces morphological changes consistent with basement membrane damage. Using fluorescently tagged DSS, we determined that DSS becomes lodged in the gut BM. Both electron and structured-illumination microscopy indicate that the BM thickens after DSS feeding. The stiffness of the BM is decreased upon DSS damage, as assessed by a stress/strain analysis. Moreover, there are clear morphological changes to the muscles that indicate the weakening of the basement membrane. Importantly, the basement membrane is repaired within 48 hours after removal of the DSS irritant. Interestingly, inhibiting or knocking down a collagen-IV crosslinking enzyme, peroxidasin, mimics the tissue changes seen in response to DSS. In addition, peroxidasin and laminin are required for the repair of basement membrane upon damage with DSS. Peroxidasin transcription levels are increased as a result of damage. We are investigating whether the requirement for peroxidasin indicates a structural change in BM during repair.

250 Calcium signaling dynamics in the early response to epithelial wounds. J. O’Connor1, E. Shannon1, A. Stevens2, M. S. Hutson2, A. Page-McCaw1 1) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2) Department of Physics and Astronomy, Vanderbilt University, Nashville, TN.

Epithelial tissue is an important structure in all complex organisms, lining all surfaces that come in contact with the environment and protecting the inside of the organism from the outside world. Normally, these epithelial cells are stationary, non-invasive, non-proliferative, and polarized along the apical-basal axis. However, upon sensing a wound, these cells can become migratory, invasive, proliferative, and polarized along the front-rear axis. This state change serves as a mechanism by which these cells can rearrange in order to heal wounds and restore epithelial structure and function. One fundamental question is how these cells can detect and interpret signals from a wound in order to properly respond to the epithelial breach.

The earliest detectable signal in wounds is a dramatic increase in cytosolic calcium that spreads from the wound margin, which is conserved among model organisms and humans. This signal has been shown to trigger a healing response around the wound margin, as well as in cells distant from the wound. Through a collaborative effort, we have used complex genetic tools coupled with quantitative analysis to create a wound model in the Drosophila pupal notum, an epithelial monolayer on the dorsal side of Drosophila pupae. We created mosaic-like flies that express genetic changes in one part of the pupal notum, while maintaining an internal control in the neighboring section of epithelium. By wounding on the border of gene expression in this tissue using pulsed laser ablation, we can create reproducible epithelial wounds, and monitor the dynamics of the calcium signal that is released in real time using a genetically encoded GCaMP.

We have shown that an initial influx of calcium is due to plasma membrane micro-tears at the wound site, allowing extracellular calcium to flood into the cells. This calcium then diffuses from the wound through gap junction mediated diffusion. Here we will present data showing that a second calcium expansion is mediated by a G-protein signal transduction pathway that releases calcium stored in the endoplasmic reticulum. Our goal is to develop an integrated model of how wounded cells signal their neighbors and control their epithelial state change in order to properly respond to damage.

251 A targeted RNAi screen for conserved cell junction genes involved in collective cell migration of border cells in the Drosophila ovary. N.S. Kotian1, K. Hylen1, J.D. Lathia2, J.A. McDonald1 1) Division of Biology, Kansas State University, Manhattan, KS; 2) Department of Cellular and Molecular Medicine, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

Collective cell migration is a complex and fascinating process, fundamental not only to wound healing, immune response and embryogenesis but also to tumor invasiveness. A critical question in this process is how groups of cells break away from the epithelium and continue migrating as a single unit, establishing dynamic cell-cell junctions to stay together and communicate amongst cells of the collective. The relatively simple border cells from the Drosophila ovary are an excellent genetic model system to study in vivo collective cell migration and invasion. The 6-10 border cells migrate collectively to the large oocyte at the posterior end of the developing egg chamber, the functional subunit of the ovary. Recently, in collaboration with the Lathia lab (Cleveland Clinic), we demonstrated that patient-derived glioblastoma cancer cells can undergo collective cell invasion. An RNAi screen in border cells was designed to target conserved cell junction genes whose elevated expression was associated with glioblastoma patient survival. This approach was used with the Lathia lab earlier and two genes were identified in both systems to play an important role in collective cell invasion. With this current screen, the top four candidate genes- alpha-Catenin, Dachsous, Lachesin and Symplekin- were identified with consistent migration
defects. From here, we focused on alpha-catenin which had the strongest migration defect. Further, live imaging in border cells showed splitting of the border cell cluster along the path of migration. Knocking alpha-catenin down specifically in border cells or in polar cells also caused the cluster to split. Our current work involves looking at the mutant alleles of top four candidate genes to confirm the phenotypes observed in the RNAi screen. This will allow us to assay how cell-cell junctions are regulated to keep cells together as they migrate collectively.

252 Study the roles of mir-274 on cell invasion in the Drosophila wing epithelia model.  C. Chang¹, F. Chang¹, J. Li², C. Chen², Y. Tsai¹ 1) Life Science, Tunghai University, Taichung, TW; 2) National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, TW.

Most human solid tumors are derived from epithelial tissues. The epithelial cells are polarized and well organized structure. The molecular mechanism of epithelial cell migration is extensive studied in culture cells. However, it is difficult to monitor tumorigenesis in vivo. MiRNAs are small noncoding RNAs containing 21-22 nucleotides. Drosophila is a good genetic system and has powerful genetic tools. We used Drosophila wing epithelia as a model to study whether miRNAs promote the epithelial cells into invasive cells. We used the GAL4/UAS system to express miRNAs and screened for microRNAs that can promote epithelial cell migration. We isolated a novel microRNA, mir-274. In this study, we explored the molecular mechanism of mir-274 in epithelial cell migration in vivo. We found that expression of mir-274 induced epithelial-mesenchymal-transition like cell migration through the JNK pathway. However, activated the JNK signaling induces extensive apoptosis. Our results showed that expression of mir-274 alleviated hid-induced apoptosis in wing discs. It has been shown that moderate apoptosis does not lead to cell death but induce cell migration. When mir-274 and p35, an inhibitor of cell death, were coexpressed in wing discs, the mir-274-induced cell migration was repressed. These suggest mir-274 may regulate the apoptotic related genes. In the future, we will further study whether the endogenous function of mir-274 is involved in cell migration during early development.

253 Defining the Role of the Novel Protein CG1674 in Adult Muscle Development.  E.R. Czajkowski, M. Cisneros, R.M. Cripps  Department of Biology, University of New Mexico, Albuquerque, NM.

Drosophila provides us with an excellent model for studying muscle related diseases in humans. By identifying and classifying genes involved in muscle development, we can better understand the mechanisms responsible for muscle growth and deterioration, and help us discover treatments and therapies for muscle related diseases. Proteome sequencing of flight muscle confirmed CG1674 protein is present in the sarcomere, suggesting it plays a role in sarcomere assembly, and may be a functional component of the flight muscle. To further characterize CG1674, we identified its role in normal muscle formation by creating an RNAi that targeted CG1674 transcript. When crossed with muscle drivers Mef2-Gal4 and 1151-Gal4, the flies became flightless due to defects in myofibril formation confirmed by immunofluorescent staining, identifying CG1674 as a major component in normal flight muscle formation. In addition, expression of a UAS-CG1674-FLAG construct confirmed that CG1674 protein localized to the Z-disc, a structural component between adjacent sarcomeres. This localization suggests Z-disc formation to be the major function of CG1674 within the sarcomere.

254 Maintenance of retinal integrity by the Abelson kinase during Drosophila eye morphogenesis.  X. Sun¹, N. Sanchez-Lujege², I. Rebay¹ 1) Committee on Development, Regeneration & Stem Cell Biology, University of Chicago, Chicago, IL; 2) Medical Scientist Training Program, University of Chicago, Chicago, IL.

Formation of multicellular tissues requires proper arrangement of terminally differentiated cells. In the Drosophila eye, each ommatidium has a cluster of 8 photoreceptors that elongate along the apical-basal axis as the retina develops. The photoreceptors are surrounded and supported by a ring of glial-like pigment cells. The pigment cell feet form an actin-enriched fenestrated membrane at the basal side of the retinal epithelium that allows the photoreceptor axons to project to the brain while providing a barrier that keeps retinal cells from entering brain. We are interested in understanding how photoreceptors coordinate signaling inputs to maintain their morphology during terminal differentiation. The non-receptor tyrosine kinase Abelson (Abl) is well known to regulate cortical actin dynamics in response to cell-cell signals during axon outgrowth and epithelial morphogenesis. In the developing fly retina, Abl localizes to both the photoreceptor apical cortex and the pigment cell feet where F-actin is enriched. We found that Abl loss causes axon mistargeting and apical domain disruption in newly specified photoreceptor neurons. At later developmental stages, the photoreceptors fall through the fenestrated membrane of the retinal epithelium and end up in the lamina layer of the brain. abl loss also increases the number of pigment cells per ommatidium and leads to disorganized actin bundles on the fenestrated membrane. These phenotypes raise the possibility that both photoreceptor cell-autonomous and non-autonomous interactions between pigment cells and photoreceptors contribute to the photoreceptor falling phenotype. We are performing conditional knockout experiments to both specify the temporal requirement of Abl and assess the cell-type specific contributions that influence photoreceptor morphogenesis.

The male accessory gland (AG) is the major source and store of seminal fluid in Drosophila melanogaster. Main cells, the principle cell type in the AG's epithelial monolayer, secrete into the lumen a wide range of important seminal proteins, including Sex Peptide (SP). SP is a primary reproductive signal controlling female long-term post-mating responses, including increased ovulation and suppression of receptivity to subsequent matings. In females, SP binds to sperm after mating and is then released gradually from sperm storage organs to mediate profound long-term effects. Using a range of staining techniques and fluorescently tagged proteins, we demonstrate that several seminal proteins, including SP, are loaded on to an abundant population of 'microcarriers' in the AG lumen. These spindle-shaped structures stain with lipophilic dyes and therefore appear to contain lipid as well as proteins, some of which form fibril-like structures. On transfer of the microcarriers into the female reproductive tract during mating, SP is rapidly released. Some microcarriers go on to contribute to the anterior mating plug, a structure which prevents sperm escape from the uterus. Remarkably, in SP mutant and knockdown males, microcarriers in the accessory gland lumen lose their shape and seem to fuse into large coagulates, particularly after mating. This prevents normal seminal protein loading on to these structures and therefore affects seminal protein transfer to females during mating. We show that this AG-localised function of SP in regulating microcarrier morphology makes a contribution to SP's post-mating effects, which have previously been assigned solely to SP signalling in mated females. In summary, our work highlights a novel function for SP in reproduction. We also uncover an important new strategy for packaging and storage of poorly soluble seminal proteins in male reproductive glands, tailored such that subsequent release in females after mating rapidly delivers signals that promote fertility.

Coordination and crosstalk between muscle development and innate immunity in Drosophila melanogaster. N.M. Green1, J. Walker1, A. Bontrager2, M. Zych1, E.R. Geisbrecht1 1) Biochemistry & Molecular Biophysics, Kansas State University, Manhattan, KS; 2) Biology, Kansas State University, Manhattan, KS.

Tissue communication is required for maintaining organismal homeostasis during development. The coordination of metabolism, immune activation, and circadian rhythms typify the complex tissue networks necessary for organismal health. Muscle tissue is uniquely synchronized with other tissues due to its high metabolic demand, release of myokines, and attachments to nervous and connective tissues. Our lab is using the Drosophila muscle attachment site (MAS) as a model to understand the connection between innate immune activation and muscle maintenance. A pupal lethal screen for abnormal pupal morphology revealed a previously unknown role for the extracellular matrix (ECM) protein, Fondue (Fon), in muscle development. Previously characterized for its role in clot integrity, loss of fon caused a reduction in larval locomotion due to the detachment of body wall muscles. Transmission electron microscopy (TEM) analysis of fon mutant MASs revealed a depletion of electron-dense matrix accumulation and disruption of cellular support structures in both tendon and cuticle. More interestingly, a sensitized background screen revealed a subset of coagulation proteins, Fon, Tiggrin (Tig), and Larval serum protein 1 γ (Lsp1γ), that are secreted from the fat body and incorporated into MASs for stabilization. Further investigation into gene expression profiles of MhcC1 mutants with hypercontraction-induced muscle stress indicated a clear trend of innate immune activation, suggesting a broader connection between muscle and immunity. In fon mutants with muscle detachment, we also observed abnormal melanin accumulation along the MAS, pathogen-independent translocation of Dorsal (Dl) in the fat body, constitutive expression of the antimicrobial peptide (AMP) drosomycin, and recruitment of hemocytes to damaged muscle. In a fon-sensitized background assay, we identified genetic interactions between fon and Toll pathway genes, including loss of the NFXB inhibitor/ixB, cactus, and overactivation of SPE which enhance muscle detachment. At the local level, fon-mediated muscle detachment and muscle hypercontraction mutants, MhcC1 and Brkd29, cause JAK/STAT activation within muscle tissue. Activation of JAK/STAT using hopmut mutants was sufficient to induce Toll signaling in the fat body, but not reciprocally. Understanding the mechanisms by which these two biological processes are intertwined will advance our knowledge of how tissue stresses can be sensed and the molecular mechanisms eliciting multi-tissue responses.

Elucidating the Role of Eip63E in Drosophila Axonal Transport. S. Klinedinst Schreiner University, Kerrville, TX.

A critical element of the neuronal cytoskeleton is the network of microtubules that provides structural support, allows motility and serves as a transportation network for the organized movement of molecules within the neuron. The microtubule cytoskeleton is critical for the normal functioning of the nervous system and dysfunction of the microtubule cytoskeleton appears to make significant contributions to neurological diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's Disease and Huntington's Disease. The central focus of this project is to characterize the potential role that Eip63E plays in Drosophila axonal transport. Eip63E is a kinase that has homology to a family of mammalian cyclin dependent kinases (Cdk's) called PFTAIRES, whose function is currently poorly understood. Cdk's are traditionally involved in regulating cell cycle progression, however the functions of some Cdk's include neuronal apoptosis, neuronal migration, axon guidance, synaptic transmission, and membrane transport. We have begun to characterize the role of Eip63E, which has been shown to
genetically interact with both molecular motors, Dynein and Kinesin, in *Drosophila*. We hypothesize that the Eip63E PFTAIRE functions as a regulator of one of these neuronal functions of Cdk5. Our data thus far indicates that Eip63E functions in axonal transport and is required for the proper delivery of presynaptic vesicles and active zones to the presynaptic axon terminal.

258 Neuroblast populations are dependent on ER conserved protein responsible for asymmetric division

Jagunal. Alonso Castro, Jose Ortega, Blake Riggs  Department of Biology, San Francisco State University, San Francisco, CA.

Adequate neuroblast populations are key in the proper development of the central nervous system in *Drosophila*. Neuroblasts (Nbs) undergo type I or type II modes of asymmetric division identifiable by transcription factors Deadpan (Dpn), Worniu (Wor), PointedP1 (PntP1) and Asense (Ase). These modes of asymmetric division give rise to neural cell diversity; however, the molecular mechanisms that establish neuroblast populations are unknown. Here we hypothesize that Jagunal, a conserved endoplasmic reticulum (ER) protein responsible for ER asymmetric division during mitosis, is a key protein in establishing Neuroblast populations in the *Drosophila* brain. Expression of Jagunal RNAi via an Elav-Gal4 driver in the *Drosophila* brain leads to death at the third-instar stage of development. We suspect that upon comparison of WT neuroblast populations with Elav-Gal4 RNAi fly strains, we will observe discrepancies in neuroblasts populations. These results suggest a role for Jagunal in the molecular pathways that establish neuroblast populations and therefore cell fate determination.

259 Sensing mechanical force during cell division. I. Cristo, D. Pinheiro, J. de las Heras, I. Gaugué, Y. Bellaiche Institut Curie, Paris, FR.

Cell division is an important process in the maintenance and maturation of epithelial tissues. Cytokinesis, the physical division of the cell components, relies on highly coordinated processes between the cellular membrane and cytoskeleton components. During this process, adherens junctions remodeling and the formation of a contractile ring are essential for the division and *de novo* membrane formation between the two daughter cells. This occurs in a tightly organized manner in order to maintain epithelial integrity and tissue cohesiveness. In the Drosophila notum epithelium, this process is a collective effort: i) as the dividing cell promotes the ingestion of the membrane due to the activity of the contractile ring, ii) neighboring cells promote the juxtaposition of the deforming membrane in order to create a long new adhesion contact between the future daughter cells. This event is achieved through the proper regulation of non-muscle MyosinII (MyoII) activity via a mechanosensing mechanism that relies on E-Cadherin dilution. We will describe the mechanisms that ensure the timely localization of Myosin to promote proper sensing of mechanical forces.

260 Crumbs and Xpd regulate mitotic motor kinesin-5 for chromosome segregation in Drosophila. J. Hwang1, L. Vuong1, K. Choi1 1) Korea Advanced Institute of Science and Technology, Daejeon, South Korea; 2) Icahn School of Medicine at Mount Sinai, New York, U.S.A.

Mitosis is an essential process in all eukaryotes. Crumbs(Crb) is a transmembrane protein required for the regulation of apical basal cell polarity and Hippo growth signaling. Our previous study suggests that Crb plays a role in chromosome segregation. In this function, Crb forms a complex with Xpd, Galla-1 and Galla-2 (named the CGX complex) to regulate proper chromosome segregation in early embryogenesis. Human Xpd (Xeroderma pigmentosum D) is a DNA helicase involved in DNA repair, transcription and cell cycle regulation. Galla proteins are homologs of human MiP18 that is a subunit of the MIP18-MMS19-XPD complex known to regulate chromosome segregation independently of transcription. However, it is unknown how the CGX complex controls mitosis. Here we show that CGX complex proteins interact with a microtubule motor protein Klk61F (*Drosophila* kinesin-5) that is important for assembling bipolar spindles during mitosis. CGX complex proteins show physical interaction with Klk61F and localize along spindle microtubules during mitosis. Depletion of CGX results in abnormal mitotic spindle phenotypes in embryos. These phenotypes are rescued by overexpression of Klk61F. Depleting any one of CGX proteins leads to strong reduction in the Klk61F level. Taken together, we suggest that CGX complex plays a critical role for mitosis by regulating the level of Klk61F protein.

261 IRBIT promotes differentiation during tissue regeneration in the Drosophila midgut. A. Arnaoutov1, K. Plevock Haase1, H. Lee1, M. Serpe1, B. Oliver2, M. Jarnik1, M. Dasso1 1) National Institute of Child Health and Human Development, NIH, Bethesda, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda MD.

Enteroctyes (ECs) of the intestinal epithelium serve both as an absorptive conduit for conveying ingested nutrients to the organism and as a barrier for protecting the core of the body from the microbes in the intestinal lumen. The maintenance of gut epithelium in adult Drosophila, like in mammals, is achieved by the controlled proliferation and differentiation of intestinal stem cells (ISCs) that can replenish all cell types in the intestinal epithelium. As ISCs divide, they produce enteroblasts (EBs), committed non-dividing cells that ultimately mature into adult ECs. Ribonucleotide reductase (RNR) is the enzyme that is essential for the de novo formation of deoxynucleotides (dNTPs), and it plays an indispensable function during cell cycle. IRBIT (AhcyL1) is a conserved metazoan protein that regulates RNR. Here, we show that the suppression of RNR activity by IRBIT in EBs is critical for their differentiation. The intestine of flies lacking IRBIT demonstrates symptoms of
hyperplasia, with accumulation of EBs. Un-replenished and frail ECs in ∆IRBIT flies fail to protect against microbes; such damaged intestines were susceptible to attack of enteric bacteria and invoked a continuous anti-inflammatory response. RNAseq analysis revealed that the midguts of ∆IRBIT flies displayed gene expression patterns consistent with a failure of EB maturation and increased antibacterial response. Our results point out to the importance of RNR regulation during tissue renewal and uncover a concerted response, orchestrated by IRBIT, that is essential for the shaping of a healthy intestinal epithelium.

262 Collective Dynamics of Cell Cycles in the Drosophila Germline. C. Doherty1,2, L. Gavis3, S. Shvartsman1,2,3 1) Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 2) Department of Molecular Biology, Princeton University, Princeton, NJ; 3) Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ.

How cells within a developing tissue coordinate growth is not well understood. Different properties of growth, such as the length of a cell cycle, must be regulated in order to prevent aberrant growth. We use the Drosophila ovary as a model to discern parameters whose regulation leads to coordinate growth. In early Drosophila oogenesis, a germline precursor cell undergoes four rounds of synchronous division with incomplete cytokinesis to produce an interconnected cluster of 16 cells. One of these cells differentiates as the oocyte, and the other 15 enter the endocycle, becoming polyploid nurse cells that grow to over 100 times their original size. However, this growth is not evenly distributed amongst the nurse cells. A recent study revealed that the nurse cells grow in groups. Specifically, the more directly connected nurse cells are to the oocyte, the larger they are. For example, the four nurse cells that are connected to the oocyte by one cytoplasmic bridge are larger than the six nurse cells that are connected to the oocyte through one other cell. Thus, the nurse cells appear to fall into four groups according to their growth properties and these groups reflect the topology of the 16 cell cluster. We are investigating the mechanisms that underlie these groupwise dynamics within the nurse cell cluster. Through quantitative image analysis, we found that cells within a group have the same total DNA content and that DNA content differs 2-fold among groups at later stages of oogenesis. Our results further indicate that these differences arise from the groupwise spatial regulation of endocycles within the nurse cell cluster. We are currently studying how different growth inputs and feedback mechanisms are integrated to control the periodicity of cell cycle regulators.

263 Noncanonical functions of Phenylalanyl tRNA synthetase. Tin Manh Ho1, Dominique Brunssen1, Jiongmung Lu1,2, Beat Suter1 1) Institute of Cell Biology, University of Bern, Bern, Switzerland; 2) present address: Max Planck Institute for Biology of Ageing, Köln, Germany.

Aminoacyl tRNA synthetases (aaRSs) are essential enzymes for loading the appropriate amino acid onto their cognate tRNA. Additionally, recent publications revealed non-canonical functions for an increasing fraction of these proteins. Several diseases and tumorigeneses are shown to be associated with malfunctioning aaRSs. Expression of Phenylalanyl tRNA synthetase (PheRS) is elevated in stem cells and in multiple cancers. We also found that overexpression of PheRS increased cell growth and cell proliferation in different fly tissues while downregulation of PheRS by RNAi had the opposite effect. To test if the function of PheRS in regulating cell growth and proliferation is carried out by a noncanonical function of PheRS, we made a mutant α-PheRS gene, which codes for a protein that is not able to carry out aminoacylation. We found that overexpression of this mutated PheRS protein also increased cell proliferation in follicle cell twin-spot experiments in the fly ovary, indicating that the effect is caused by a noncanonical function of PheRS.

Cytoplasmic PheRS consists of two α-PheRS and two β-PheRS subunits. These subunits usually need each other to be stable. However, we found that some cell types in the larval midgut can accumulate higher levels of β-PheRS even if only this subunit is overexpressed. Additionally, upon overexpression of the catalytic subunit α-PheRS we observed unexpected accumulation of smaller isoforms. Based on their size, these are predicted to lack most of the catalytic domains. The resulting larvae isoform patterns are tissue specific. We are presently investigating whether these observations can be linked to the non-canonical growth regulating function of PheRS.

264 Genetic control of tissue-specific growth in the larval trachea of Drosophila. K. Wilson, L. Hill, R. Ward 1) University of Kansas, Lawrence, KS.

In humans and many animals, post-embryonic development is achieved through allometric growth, which is characterized by organs and tissues that grow at different rates relative to each other. While the growth of each organ or tissue is likely dependent on its function in development and homeostasis, the mechanisms that control this tissue-specific growth are poorly understood. In order to elucidate tissue-specific growth mechanisms, we are using the larval trachea, the gas exchange organ in Drosophila melanogaster, as a model tissue. Larval tracheal growth is an excellent model to study tissue-specific growth because the trachea can be easily imaged and measured in live animals, it is composed of a tubular epithelial network that exhibits allometric growth, and various genetic approaches can be employed to manipulate gene expression specifically in the trachea. The genes uninflatable (uif) and Matrix metalloproteinase 1 (Mmp1) have been identified as tissue-specific growth regulators of the larval trachea. Larva with homozygous mutations in these genes have trachea that are about half the relative size of trachea in wild type animals. To identify genes involved in larval trachea growth, we screened through a collection of EMS-induced larval lethal mutations, and identified 7 mutants that have an abnormal ratio of trachea to body.
length in 3rd larval instars. Three of the mutants show reduced tracheal growth similar to mutations in \textit{uif} and \textit{Mmp1}, whereas the remaining 4 mutant lines show increased tracheal growth. Using deficiency mapping, complementation analysis, and RNAi we have determined that the reduced tracheal growth mutant, \textit{I(3)LL15149}, has a mutation in the gene \textit{Tenectin (tnc)}. One of the overgrown tracheal mutants (\textit{I(3)L12265}) fails to complement a pair of overlapping deficiencies that spans 99B5 to 99B7 and includes 13 genes. We are continuing to conduct complementation analysis using overlapping deficiencies and RNAs to identify candidate genes in order to identify the specific gene responsible for this mutant phenotype. In addition, we are examining the cellular phenotypes associated with all of these mutations in order to uncover molecular mechanisms associated with tracheal-specific growth that can be used as a paradigm to better understand allometric growth in all organisms.

265 Understanding coiled-coil function during synaptonemal complex assembly. Katherine Kretovich Billmyre, Cori K. Cahoon, R. Scott Hawley Stowers Institute of Medical Research, Kansas City, MO.

Coiled-coil motifs are a common secondary structure that is present in about 10% of all eukaryotic proteins. These motifs have been implicated in many cellular processes including oncogenesis and are being investigated as therapeutic targets. The synaptonemal complex (SC) is an ideal model for studying coiled-coil domains as the genes that encode SC coiled-coil proteins evolve rapidly, but the secondary protein structure is conserved. Recent findings in \textit{Saccharomyces cerevisiae} have identified specific coiled-coil regions as necessary for SC function. We are investigating the importance of coiled-coil domains in the Drosophila SC protein, C(3)G. C(3)G is the main transverse filament protein in the SC and removal of C(3)G results in a complete loss of SC structure and recombination. A partial deletion of 211 amino acids (ccΔ1) in a coiled-coil region of C(3)G displays a shortening of the SC, a premature disassembly defect and altered recombination. To determine which amino acids were responsible for the various observed mutant phenotypes, smaller deletions were made by CRISPR/Cas9 specifically in regions where amino acids may not be in a coiled-coil confirmation. A small deletion in one of these regions (ccΔ3) has severe defects in SC assembly, which was not observed in the original X204 mutant. We are currently investigating the effects of this deletion on recombination, crossover distribution, and nondisjunction. Furthermore, we are characterizing the dynamics of SC assembly and centromere clustering in this mutant by immunofluorescence. This information will allow us to investigate individual functions of C(3)G during meiosis. Because of the structural conservation between coiled-coil proteins, information regarding the functional importance of coiled-coil interactions will be of a broad relevance to many species.

266 Regulation of the meiotic spindle and sister centromere cohesion in oocytes by antagonism between PP2A and Aurora B kinase. A. Gladstein, J. Jang, K. McKim Waksman Institute Rutgers University, Piscataway, NJ.

Meiosis in females is especially vulnerable to errors due to a lack of the major microtubule-organizing center, the centrosome, in oocytes. Defects during meiosis in oocytes can have detrimental effects in the progeny, and are the leading cause of infertility, birth defects, and spontaneous abortion in women. Using \textit{Drosophila} as a model organism, we investigate how the meiotic spindle is assembled and regulated. A key player in \textit{Drosophila} meiosis is the four-protein chromosomal passenger complex (CPC), which is primarily responsible for regulating spindle assembly and making the correct microtubule attachments, to ensure proper chromosome segregation. Aurora B, a kinase, is the catalytic component of the CPC and is responsible for phosphorylating spindle proteins to establish bipolar spindle formation. Using a drug (Binuclein 2) to inhibit Aurora B activity in oocytes, we observed the loss of all spindle microtubules in meiosis I. Thus, continuous Aurora B activity is required to preserve the spindle during meiosis I. The necessity of a kinase for spindle regulation suggests that spindle dynamics may be regulated by a phosphatase. The protein complex Protein Phosphatase 2A (PP2A) has been shown to oppose CPC functions by dephosphorylating spindle proteins. When Aurora B was inhibited in PP2A RNAi oocytes, the spindle was maintained, demonstrating that PP2A antagonizes the Aurora B spindle maintenance function. PP2A comes in two distinct forms, differing in their B subunits - Twins (TWS= B55) and Widerborst (WDB = B56). Both complexes contain PP2A and MTS (Microtubule star), which is the catalytic protein of the complex. Our findings suggest that both PP2A complexes play a role in antagonizing the CPC. When one complex is knocked out, in conjunction with the inhibition of Aurora B, spindle maintenance is partially reversed. We have also found that loss of WDB is required to maintain sister chromatid cohesion and the metaphase I arrest in oocytes.

267 Female meiotic drive of B chromosomes in \textit{D. melanogaster}. S.L. Hanlon1, R.S. Hawley1,2 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Female meiosis produces four products, but only one becomes the oocyte nucleus while the remaining three are discarded. A chromosome that acts selfishly can exploit this asymmetry and ensure its inclusion in the oocyte nucleus, a phenomenon referred to as chromosomal meiotic drive (CMD). Here we follow newly-arisen B chromosomes discovered in \textit{Drosophila melanogaster} and observe that their segregation is subject to strong CMD. These B chromosomes originated in a laboratory stock carrying a null allele of the female meiosis-specific polo kinase inhibitor \textit{mtrm} held over a balancer chromosome and are present in unexpectedly high numbers (an average of 8-10 per fly). We purified and sequenced the B chromosomes and find they are comprised primarily of heterochromatic repeats derived from chromosome 4 and contain no unique sequence...
elements. We then tested the transmission frequency of B chromosomes from individual females from the original stock and observed strong CMD in these females, but not in males from the same stock. When the B chromosomes were in a wild-type background, the meiotic drive was reduced. To our knowledge, this is the first evidence of true female chromosomal meiotic drive in an animal system, providing a glimpse into how newly-arisen chromosomes may be able to propagate within a population by exploiting the complexity of female meiosis.

268 Redox state alteration at the onset of development in D. melanogaster. Boryana Petrova1, Caiping Tian2, Elizabetha Freinkman1, Maiko Kitaoka1,3, Jing Yang2, Terry Orr-Weaver1,3 1) Whitehead Institute, Cambridge, MA; 2) National Center for Protein Sciences - Beijing, Beijing, China; 3) Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA.

The transition of an arrested oocyte to a totipotent embryo, enabling the fast cell divisions at the onset of development, demands rapid multidimensional remodeling. Although fundamental for this process, changes in metabolic output and its byproduct ROS are only beginning to be characterized. The significance of these changes is evidenced in Drosophila by the essential role of the ovary-specific thioredoxin, deadhead (dhd). Thioredoxins are general ROS scavengers. The absence of Dhd in the oocyte causes a block in embryonic development and a failure of protamine to histone exchange in the male pronucleus of fertilized eggs (1,2). We find that in addition there are independent meiotic defects in the oocyte. To define changes in redox state in the oocyte as it transitions to an embryo we used redox-sensitive GFP (roGFP) imaging as well as metabolomic profiling by mass spectrometry. Both approaches demonstrated that mature oocytes are in a reduced state compared to early embryos. Using mass spectrometry we cataloged the reactive cysteine proteome at both developmental stages. In the absence of Dhd, oocytes become oxidized and by thiol-redox profiling, we identified the proteins whose cysteines became oxidized in dhd mutants. Our current efforts are directed towards understanding the roles of Dhd interaction partners we identified and likely downstream targets during meiosis and early embryogenesis. Finally, reduction in the oocyte of further ROS scavengers leads to defects in early embryogenesis, and preliminary epistatic analyses indicate Dhd functions as part of a broader redox network. As changes in redox state have been observed in mammalian oocytes, our work will define likely conserved links between developmental control of cell cycle progression and regulation by metabolic and environmental cues. This may have important implications for female fertility.


269 The roles of Dalmatian in meiotic cohesin regulation in Drosophila. Z. Sisco Waksman Institute of Microbiology, Rutgers University, New Brunswick, NJ.

The cell cycle is filled with opportunities for mistakes. Whether it be the loss of cell cycle checkpoints or errors in DNA synthesis, an alteration in any component of the cycle can cause a multitude of problems to arise. One vital aspect of the cell cycle is the correct grouping and separation of chromosomes. The cohesin complex is responsible for sister chromatid cohesion, which is essential for chromosome bi-orientation and proper chromosome segregation. There are at least three proposed cohesin complexes within Drosophila, all serving distinct functions. The mitotic cohesin complex is comprised of four core subunits: SMC1, SMC3, Rad21, and SA. There are two different meiotic cohesin complexes, both containing the SMC1/SMC3 subunits, but one containing SA and C(2)M and the other containing SUNN and SOLO. The complex containing SA/C(2)M is required for synaptonemal complex assembly, and that containing SUNN/SOLO is required for sister chromatid cohesion. How SA/C(2)M promotes SC assembly is not known. For cohesion, these subunits come together to form a ring that is believed to entrap sister chromatids from S phase until anaphase onset. Cohesins are loaded onto the chromosomes with the help of Soronin and establish cohesion through acetylation of its SMC3 subunit. Cohesin is dissociated from the chromosomes by the prophase pathway and the actions of Wapl, or Separase cleavage of the kleisin subunit. Cohesin dissociation is antagonized through the protective actions of MEI-S332, which recruits PP2A and prevents phosphorylation of Soronin and cohesin. However, while the protection by MEI-S332 is essential during meiosis II, it may not operate during mitosis and meiosis I. In Drosophila, Dalmatian (dmt) encodes a Soronin ortholog and has been shown to be essential in mitotic cells for both cohesion establishment and protection. We first used the germ line clone method to determine if dmt is required in Drosophila meiosis for sister chromatid cohesion and oocyte development. Preliminary results suggest that homozygosity for dmt results in a lack of oocytes, suggesting that dmt is essential for oocyte development. We are currently developing RNAi tools to study dmt in meiosis. This research will determine if Dalmatian is required for SC assembly, and whether it is required for protection of cohesion during meiosis I.

270 PP1-87B antagonizes Polo and BubR1 in controlling microtubule dynamics to achieve sister chromatid co-orientation in metaphase I in Drosophila oocytes. L. Wang1, A. Das2, K. McKim1 1) Waksman Institute of Microbiology, Piscataway, NJ; 2) Department of Biology, University of Pennsylvania, Philadelphia.

In meiosis I, a unique mechanism called co-orientation ensures that sister chromatids co-orient and segregate to the same
pole at metaphase I. In budding yeast, co-orientation is generated by a complex called Monopolin, while in fission yeast and mice, co-orientation has been shown to be mediated by specialized kinetochore proteins, Moa1 and MEIKIN, functioning with meiosis specific cohesin, Rec-8, to regulate sister chromatid co-orientation. Even though meiosis specific co-orientation is needed for chromosome segregation, knowledge of the mechanism remains limited. Here we show that Drosophila protein phosphatase PPI-17B is required for sister chromatid co-orientation. In PPI-17B depleted oocytes, sister centromeres separate, implying defective co-orientation. In fact, this sister centromere separation in PPI-17B depleted oocytes, is depends on several proteins, including Aurora B, Polo, BubR1 and the synaptonemal complex (SC) protein C(3)G. This indicates antagonistic mechanisms involved in co-orientation. In addition, we have confirmed that maintenance of co-orientation in PPI-17B depleted oocytes is microtubule-dependent, unlike the co-orientation defect in the absence of kinetochore protein SPC105R, which is due to Separase-dependent loss of cohesion. Taken together we propose a mechanism where PPI-17B establishes proper sister chromatid co-orientation in meiosis I, by inhibiting Aurora B kinase and possibly through structural chromatin components like the SC. We further conclude that maintenance of this co-orientation requires careful regulation of microtubule dynamics by the antagonism between PPI-17B and Polo/BubR1. This balanced microtubule dynamic in metaphase I is the other crucial pathway in oocytes to achieve sister chromatid co-orientation other than specialized co-orientation protein.

271 Structural-Functional characterization of Pericentrin like protein (Plp). R. Varadarajan1, K. Plevock2, C.J. Fagerstrom3, N.M. Rusani1 1) Cell biology and Physiology, National Heart, Lung, and Blood Institute, NIH, Bethesda, Maryland, MD; 2) National Institute of Child Health and Human Development, NIH, Bethesda, Maryland, MD.

Centrosomes are the major microtubule organizing centers in most eukaryotes. A typical centrosome consists of a pair of centrioles encircled by a proteinaceous pericentriolar material (PCM). Centrosome maturation is a key cell cycle processes that facilitates recruitment of PCM proteins to the centrioles thereby enable centrosome-driven functions including microtubule nucleation. Mutations in PCM proteins (Pericentrin and Centrosomin) are associated with many human genetic disorders, including cancer and ciliopathies. Drosophila is an excellent model system to study the centrosome and diseases that are related to their dysfunction. Here, we focus on understanding the molecular mechanisms underlying PCM recruitment and microtubules nucleation. Pericentrin like protein (Plp) is a centrosome protein shown to regulate PCM recruitment, however, its interdependent interaction with other essential PCM components such as Asl, Sas4, Spd2 and CNN remains unclear. To investigate the precise role of Plp during centrosome maturation, we performed a structure-function analysis by generating Plp protein truncation and characterized its function in vivo. We found that the C terminal region of Plp contains a promoting signal for PCM recruitment, while the N terminal region contains an inhibitory signal encoded within amino acids 649-933 of Plp. Interestingly, we found direct binding between the inhibitory and promoting regions (aa 2538-2747) of Plp, suggesting a sophisticated autoinhibition mechanism at play. Our working model is that Plp resides in a closed/inhibited conformation in interphase to prevent PCM recruitment. In mitosis, this autoinhibition is relieved and the C-terminus is then allowed to recruit other PCM components. We are now focusing on identifying the molecular interactions between Plp and other PCM protein, which may further explain the role of Plp in PCM recruitment and microtubules nucleation.


During the early stages of Drosophila embryogenesis, maternally loaded Blm DNA helicase is essential for proper DNA replication; Blm mutant females, who fail to provision Blm to their eggs, exhibit a maternal effect lethality. Embryos that lack maternally loaded Blm suffer DNA damage and reduced survival to adulthood due to replication challenges that arise during the rapid early syncytial cell cycles of Drosophila embryo development. Repetitive DNA sequences are especially vulnerable; the Drosophila Y chromosome, which harbors vast amounts of repetitive DNA, poses a distinct challenge to DNA replication during these early cell cycles. Indeed, sons survive to adulthood at lower frequencies than daughters when their mothers lack Blm. Consequently, surviving progeny are female-biased. We hypothesized that specific Y chromosome DNA sequence motifs require Blm DNA helicase for proper replication in early syncytial cycles, and thus are more deleterious in the absence of Blm. To test this hypothesis, we screened naturally derived global populations of Drosophila melanogaster and identified genetic variation that impacts Blm-dependent progeny sex ratio skew. Consistent with our model, this variation maps to the Y chromosome. We are currently exploring both the Y-linked locus responsible for this variation and the molecular mechanism underlying Y chromosome-sensitive replication challenges that arise during syncytial embryonic cycling.

273 Error-prone DNA repair in Drosophila: the missing link between polymerase theta structure and function. J. Blanch, K. Beagan, M. McVey Tufts University, Medford, MA.
DNA damage leads to toxic interstrand crosslinks (ICLs) and double-strand breaks (DSBs) in the genome that must be repaired to maintain cell viability. ICLs are resolved by protein signaling networks that cleave DNA during removal and produce DSBs as intermediates. To resolve DSB gaps, cells use homology-directed repair and canonical end-joining. When these pathways are not available, a series of alternative end-joining (alt-EJ) pathways can align DNA ends. Alt-EJ generates numerous mutations in the genome that drive tumorigenesis if they occur in critical tumor suppressor genes. Polymerase theta (Pol theta) is an error-prone translesion polymerase that repairs ICLs and is important for alt-EJ in Drosophila (Chan et al., 2010). Pol theta structure is made up of helicase, linker, and polymerase domains. Our lab has shown that the polymerase and helicase domains are required for ICL repair in Drosophila (Beagan et al., 2017). In contrast, only the polymerase domain is required for facilitating alt-EJ events. Even though the helicase domain is not required, it can facilitate complex insertions into the Drosophila genome.

The function of the pol theta linker domain has not been elucidated for ICL or alt-EJ repair. We hypothesize that the linker domain might act as a flexible tether between the helicase and polymerase domains during alt-EJ and could mediate the proper removal of ICLs from DNA strands. To test this, we constructed transgenic Drosophila in which the Pol theta linker domain has been deleted. As a result, the Pol theta protein product has helicase and polymerase domains that are directly adjacent to each other. We are measuring survival of these transgenic flies after treatment with ionizing radiation (to induce DSBs) and with nitrogen mustard (to induce ICLs). We are also measuring alt-EJ repair in these mutants using a site-specific gap repair assay. Alt-EJ repair phenotypes in “linkerless” mutants are being compared to trans-heterozygotes that have intact Pol theta linker domains, but dysfunctional polymerase or helicase domains in opposite copies. In this way, we are assessing if functional copies of Pol theta helicase and polymerase domains must be tethered by the linker for proper alt-EJ repair. Overall, these experiments will clarify how the linker domain impacts the function of Pol theta in ICL and error-prone DSB repair processes.

274 **Elucidating the multiple functions of POLDIP2 in Drosophila melanogaster.** J.C. Castaneda, T. Hanscom, M. McVey Tufts University, Medford, MA.

DNA polymerases are essential components of DNA repair processes. They depend on a variety of proteins to be recruited to the site of synthesis and to increase their processivity. Polymerase delta interacting protein 2 (POLDIP2) was identified as a novel protein that interacts with both polymerase δ and PCNA in the yeast two-hybrid system. POLDIP2 has also been shown in human cells and other yeast-two hybrid systems to interact with polymerase η, polymerase λ, and PrimPol, which are recruited to bypass lesions induced by external damage. In the case of pol η and λ, POLDIP2 increases their processivity and catalytic activity. POLDIP2 appears to be pleiotropic in nature as it has been implicated in a variety of cellular processes, including tau aggregation in human cells, mitotic spindle rearrangement in rat brain endothelial cells, and cooperating with NADPH oxidases in yeast. Homozygous POLDIP2 knockout mice incur perinatal lethality and significantly lag in development in comparison to wildtype mice. For this reason, studying POLDIP2 function in other model systems could provide additional information about its multiple roles.

We are interested in the potential role POLDIP2 might play regarding polymerase recruitment and switching during DNA repair in Drosophila melanogaster. Complete deletion of the gene was induced via the CRISPR Cas-9 editing system and a DsRed marker was inserted into the POLDIP2 locus via homology-directed repair. Intriguingly, flies homozygous for the deletion incur lethality at the second-instar larval stage. We are currently utilizing several approaches to determine the genetic basis for this lethality. In addition, we are conducting a P-element imprecise excision screen to obtain deletion mutants that could give insight into the functional domains of POLDIP2. Mutagen sensitivity assays will be conducted with these mutants to better characterize the interplay between POLDIP2 and the DNA repair and replication machineries.

275 **Uncovering new regulation of acentric DNA segregation.** D. Clay¹, D. Fox¹ ² ¹) The Department of Cell Biology, Duke University, Durham, NC; ²) The Department of Pharmacology and Cancer Biology, Duke University, Durham, NC.

Faithful segregation of genetic material during mitosis is essential for maintaining genomic integrity. DNA damage compromises this process, requiring cells to respond through conserved DNA damage responses. While the mechanisms underlying DNA damage responses during interphase of the cell cycle have been described in detail, much less is known about how a cell responds to damage during mitosis. The presence of acentric DNA, broken DNA lacking a centromere, during mitosis is particularly problematic. The ability of cells to align and segregate acentric DNA during mitosis has been recently appreciated, but the mechanisms driving this process remain incompletely understood. Our laboratory has pioneered the use of Drosophila rectal papillar cells as a powerful and genetically tractable in vivo model to uncover the mechanisms that mediate segregation of acentric fragments during mitosis. Previous findings from our laboratory identified the Fanconi Anemia DNA repair proteins FANCD2 and FANCI, as well as BLM helicase, to be involved in this mitotic DNA damage response¹. Further, our data suggest acentric segregation appears to occur independently of the canonical Fanconi Anemia DNA repair pathway. To further understand mechanisms of acentric segregation, we performed a candidate screen. Our screen analyzed the roles of all Drosophila genes identified to be required for G2/M arrest after double-strand DNA breaks ². Results of the screen will be presented at the meeting.
276 Conservation of RecQ helicases between Drosophila and humans. R.L. Cox, C.M. Hofley, J.R. LaRocque Human Science, Georgetown University, Washington.

RecQ helicases are a family of proteins involved in maintaining genome integrity as they function in DNA repair, recombination, and replication. Mutations in the RecQ helicases can result in multiple genetic diseases that disrupt chromosomal stability, thus increasing the possibility of developing cancer. This study focuses on the conservation of RecQ helicases between humans and Drosophila melanogaster. The human RecQ helicase family consists of five helicases: RECQL, RECQL4, RECQL5, BLM, and WRN while the Drosophila RecQ helicase family consists of four: DmRecQL4, DmRecQL5, DmBlm, and DmWRNexo. Human cells deficient in RECQL or BLM have increased chromosomal instability and defects in DNA repair. While Drosophila have no ortholog to RECQL, DmBlm has functional similarities to human BLM, as DmBlm mutants have an increase in sensitivity to ionizing radiation (IR) and a decrease in repair of DNA double-strand breaks (DSBs). To determine if RECQ helicase functions are conserved across species, human RECQL was expressed in Drosophila using the GAL4-UAS system, and the ability for human RECQL to rescue DmBlm mutant sensitivity to IR was tested. Preliminary data indicates that the DmBlm mutants expressing human RECQL may be more sensitive to IR than DmBlm mutants not expressing the transgene. This may suggest that expression of human RECQL in DmBlm mutants is interfering in the IR-induced DSB repair pathway. Alternatively, a failure to rescue DmBlm mutant IR sensitivity suggests that human RECQL function has diverged from other RecQ helicases. This hypothesis will be tested by expressing human BLM in DmBlm mutants using the GAL4-UAS system and determining if this expression can rescue the hypersensitivity to IR. Because BLM is expressed in both humans and Drosophila, there may be functional conservation between human BLM and DmBlm that was not observed between human RECQL and DmBlm.

277 Drosophila chromosome fragile sites. Hunter Hill, Kent Golic Biology, University of Utah, Salt Lake City, UT.

The earliest evidence for chromosome fragility arose about a century ago when Calvin Bridges observed constrictions in polytenic chromosomes that were also weak regions where chromosomes tended to break during squashing. Since then, chromosome breakage has been repeatedly linked with gross dysfunction of the genome including aneuploidy, polyplody, deletions, inversions, and translocations. Human chromosomes are also known to exhibit constrictions and breaks at select locations when grown under conditions of replication stress. This finding spurred the idea that perhaps replication wasn't complete in those regions prior to division, and indicated that replication timing may play a critical role in chromosome fragility.

Dicentric chromosomes may be produced through various means, including natural meiotic recombination between inverted chromosomal segments, or experimentally induced site-specific recombination. When attached centromeres segregate to opposite poles, the chromosomes may break, and the broken ends can be healed by de novo telomere addition. Recovery of such chromosomes, and hence further examination, is generally limited by the resulting aneuploidy. To overcome this limitation we devised a genetic screen that would allow recovery of chromosomes with breaks anywhere along their length. Sister chromatid exchange was induced on a ring chromosome, which generated double dicentric bridges in anaphase. We found that breakpoints clustered into a limited number of hotspots, and concluded that dicentric bridges do not break randomly, but tend to break in a few “fragile” regions. Interestingly, the hotspots often coincide with regions identified as late replicating, implicating DNA replication timing as a factor that determines chromosome strength under tension. We also find that different chromosomes have different breakage hotspots. In particular, a semi-lethal ring chromosome (“filicidal”) exhibits a concentration of breakpoints in the region that is linked to its lethality, suggesting that replication timing may also underlie the mechanism of lethality.

To test the role of replication timing in chromosome weakness we are examining the effect of replication stress on breakage sites using aphidicolin (inhibitor of DNA polymerase α). If dicentric bridge breakage is altered after feeding flies aphidicolin, it will substantiate replication timing as a major factor for chromosome integrity in Drosophila.

278 Altering substrate specificity of the Holliday junction resolvase GEN. C. Moffatt, D. Rognstad, J. Sekelsky Biology, UNC-Chapel Hill, Chapel Hill, NC.

Branched structures are important intermediates in DNA damage repair, and if left unresolved, often result in cancerous cell growth. Many nucleases are responsible for cutting these intermediates; GEN1 in humans and Yen1 in yeast are two that have specificity for Holliday junctions (HJs), but cleave other substrates during DNA damage repair, such as 5’ flaps and forked DNA. This begs the question: what is the most critical DNA intermediate structure for these proteins to cleave in vivo? This is difficult to answer because GEN1/Yen1 are backups to Mus81. However, in Drosophila melanogaster, these roles are reversed—GEN assumes a primary role with Mus81 operating as a backup nuclease. This makes Drosophila an especially

qualified model in which to study GEN. It has been shown that *H. sapiens* GEN1 is structurally similar to its ortholog from *D. melanogaster*. Despite this, GEN cleaves 5' flaps more efficiently than HJs. We hypothesize that 5' flaps are the more biologically important intermediate for GEN and its orthologs to resolve in vivo. To test this, I made two chimeric proteins predicted to have altered substrate specificity. I substituted 60 residues from GEN with that of the same region from *C. thermophilum* GEN1 and *H. Sapiens* FEN1. The region swapped out of GEN was its helical arch, which is thought to help thread flapped structures through for better cleavage. In the protein GEN[GEN1], the helical arch is replaced with the corresponding region from CIGEN, which lacks the arch, and thus is limited to HJ specificity. The second protein, GEN[FEN1], has the arch from GEN substituted with a slightly different arch from HsFEN1, which limits activity to 5' flaps. I have expressed and purified both chimeric proteins and will be testing their cleaving abilities on 5' flaps and HJs in nuclease assays. This experiment will also be carried out in vivo to confirm any biological benefit of cleaving flaps over HJs. I plan to make the same changes to endogenous GEN in *D. melanogaster* to test the functions affected, including the response to DNA damaging agents. We hope to gain novel insights into the role of this nuclease, the substrates it preferentially cleaves, and how this can be understood in the greater context of DNA damage repair pathways in *Drosophila* and humans alike.

279 The Role of DmBlm in DNA Double-strand Break Repair and Gene Conversion.  
N. Srivastava, J.R. LaRocque  
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Maintenance of genome integrity is essential for cell viability and the transfer of genetic information from one generation to the next. Compromising this integrity can lead to several genetic diseases, including cancer. DNA double-strand breaks (DSBs) are a type of DNA damage that threaten genome stability. Fortunately, the cell has reparation mechanisms in place to ensure that DSBs do not result in mutations within the genome or death of the cell. Two major DSB repair pathways include non-homologous end joining (NHEJ) and homologous recombination (HR). One feature of HR repair products is the presence of gene conversion tracts (GCTs); the length of GCTs may be determined by various steps of HR, such as end resection and Holliday junction resolution. *Drosophila melanogaster* Blm (DmBlm), the ortholog of human BLM, has been shown to participate in DNA gap repair. To test DmBlm's involvement in DSB repair of a simple break, the DR-white repair assay was used. This assay measures the contribution of HR, NHEJ, single-strand annealing, and crossover repair of I-SceI-induced DSBs. A constitutively-active-I-SceI enzyme created DSBs within the DR-white reporter assay, located in the genomes of DmBlm mutants and heterozygote controls. A statistically significant decrease in HR was observed in DmBlm mutants compared to heterozygote controls. These data suggest that DmBlm plays a role in repairing simple DSBs through intrachromosomal HR. However, it is not completely understood where in the HR pathway DmBlm plays a role. Gene conversion tract (GCT) lengths may reveal DmBlm's potential role in several steps of the HR pathway. To test DmBlm's role in determining GCT length, the DR-white.mu reporter assay was used. This assay contains silent polymorphisms along the donor sequence to measure GCT length. After constitutively-active-I-SceI enzyme created DSBs within the DR-white.mu reporter, the DNA of HR flies from DmBlm mutants and heterozygote controls was collected. GCTs lengths of these HR events were measured and compared for statistically significant differences.

280 The role of systemic factors in regulating mitotic and hypertrophic injury responses.  
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Two widely conserved forms of injury responses at the cellular level include mitosis (cell division) and hypertrophy (cell enlargement without division). While much work has focused on the cell-intrinsic mechanisms that drive a cell towards one response or the other, there has been relatively little exploration of the systemic factors influencing cellular injury responses. We recently found that the *Drosophila* pylorus is a new model tissue for studying a developmentally regulated switch from a mitotic to hypertrophic injury response (see abstract by Cohen and Fox, this meeting). The *Drosophila* pylorus is a sphincter that regulates the passage of food into the hindgut, and while the larval pylorus undergoes mitosis in response to injury, the adult pylorus undergoes hypertrophy. The developmental timing of this mitotic-to-hypertrophic injury response switch is tightly regulated and appears to correlate with a large systemic pulse of ecdysone steroid hormone signaling. Ecdysone has previously been shown to modulate cellular gene expression as well as to activate wound-responsive migration in hemocytes, the *Drosophila* innate immune cells. Adult hemocytes actively migrate towards sites of injury, whereas larval hemocytes do not, and this ability is acquired during early pupation in an ecdysone dependent manner. Therefore, it is possible that ecdysone signaling could regulate the injury response switch either by directly modulating pyloric gene expression, or by activating a pro-inflammatory immune response in adults that itself regulates the injury response switch. Transcriptome analysis of adult pylorus following injury reveals an upregulation of numerous immune response genes including antimicrobial response genes and the cytokine Unpaired-3. We are currently investigating the potential involvement of ecdysone signaling and the innate immune response in regulating the hindgut injury response switch. The results of these studies will be presented at this poster.

281 An RNAi screen to discover deubiquitinases (DUBs) important for cell division.  
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Proteolysis and ubiquitination play important roles in regulation of the cell cycle. Although there are several E3 ubiquitin ligases known to function in cell cycle control, relatively few deubiquitinases (DUBs) with opposing activities have been identified. The *Drosophila* genome contains 41 DUBs belonging to all DUB subclasses and most have a mammalian ortholog. Although other published studies have characterized the RNAi phenotypes of all *Drosophila* DUBs, none have focused specifically on cell cycle phenotypes. I will express RNAi against each of the *Drosophila* DUBs in multiple tissues and examine those tissues for resulting cell cycle defects. This screen will be performed in ovaries, as this will allow the identification of DUBs required for canonical mitotic cycles, endocycles and chorion amplification, along with two cell cycle transitions. In addition, screening will be performed in pupal eyes and wings to assay for a cell cycle exit role. With this screen, I expect to identify DUBs that antagonize the activity of the E3 ubiquitin ligases that drive cell cycle progression by targeting cell cycle proteins for proteolysis, namely the APC/C and the SCF and SCF-like complexes. In addition, I may discover DUBs that function in developmental signaling pathways that interface with the cell cycle machinery.

282 Stem cell proliferation is regulated by Myt1 in the *Drosophila* intestine. R.J. Wilms, S.D. Campbell Biological Sciences, University of Alberta, Edmonton, Alberta, CA.

Cdk1 is the master mitotic kinase responsible for propelling cells into mitosis. To prevent mitotic activity during interphase, the Wee-like kinases Wee1 and Myt1 phosphorylate Cdk1. Though Wee1 and Myt1 are partially redundant, previous work from our laboratory has demonstrated that *Drosophila* Myt1 is the major Cdk1 inhibitory kinase in sensory organ development and male meiosis. In these contexts, as well as during oogenesis, we have observed a common theme wherein loss of Myt1 activity results in either failure to exit the cell cycle, or misregulation of progenitor cell homeostasis. Consistent with these observations, we recently identified Myt1 as a regulator of adult intestinal stem cell (ISC) equilibrium. This finding not only supports a conserved role for Myt1 in regulating progenitor cell populations, but also provides us an effective system for characterizing the mechanism by which Myt1 accomplishes this task.

The *Drosophila* intestine is an excellent model for studying stem cell renewal and cell differentiation and has well-defined similarities to the mammalian intestine with respect to cell types, morphology, and signaling pathways. An initial characterization of the midgut in 7-day old myt1 null mutants showed an approximate 40-fold increase in the mitotic index relative to controls. Edu labeling used to analyze the rate of turnover in the gut indicated that this increase corresponded to cell hyperproliferation. RNAi depletion of Myt1 in adult ISCs via a temperature sensitive UAS-Gal4 system phenocopied the mutant phenotype, indicating that the increased mitotic index of myt1 mutants was not due to disruption of the developmental program or to loss of Myt1 in other cell types. We are currently analyzing how each epithelial cell type in the gut of myt1 mutants is affected in order to define the molecular mechanism Myt1 employs to regulate stem cell division in *Drosophila*. Furthermore, recent work identifying Myt1 as essential to glioblastoma stem-like cell proliferation highlights how understanding Myt1 regulatory mechanisms could have implications for practical biomedical application.

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283 *Jim Lovell (lov)* acts downstream of *dmyc* to regulate endopolyploid growth in the larval stages. K.M. Beckingham, F. Zhou, R. Dibbs, S. Hsu, S. Green, M. Karki, I. Chu, O. Casimir Dept BioSciences, Rice Univ, Houston, TX.

*Jim Lovell (lov)* encodes a putative transcription factor of the BTB/POZ domain family. Tissue specific knockdown of *lov* using the Gal4-UAS system has established that *lov* is required during the larval stages for endopolyploid growth: knockdown of *lov* in the tracheae, epidermis, salivary glands and fat body produces smaller cells with smaller nuclei. BrDU DNA labelling in the salivary glands has established that these smaller nuclei undergo less DNA replication than control nuclei. Lov protein is primarily located in the nucleolus, indicating that its role in growth involves regulation of ribosome synthesis. dMyc is a major regulator of endopolyploid growth and a known regulator of ribosome biosynthesis. We are therefore investigating interactions between these two transcriptional regulators. During larval life, altering the levels of dmyc expression does not alter the levels of *lov* transcripts and similarly *lov* knockdown or overexpression has no effect on dmyc mRNA. However, overexpression of dmyc increases Lov protein levels significantly. A role for dmyc in regulating *lov* by a post-translational mechanism is therefore indicated. Our initial epistasis experiments also indicate that dmyc is upstream of *lov* in the larval endopolyploid growth pathway. Thus in the tracheae, the *lov* knockdown phenotype is epistatic to the dmyc overexpression phenotype.

Given that BTB/POZ domain transcription factors can act as heterodimers we are testing the hypothesis that *lov*'s role in endopolyploid growth involves interaction with a BTB/POZ domain partner. So far none of candidate proteins assayed as possible *lov* partners during larval life (tramtrack, lola, lolal and ribbon) produces the same phenotype as *lov* when knocked down in the tracheae. However, there are similarities between the ribbon null phenotype and the *lov* overexpression
phenotype during embryogenesis. Ongoing studies are addressing a possible ribbon-lov interaction during embryonic
development.

284 A switch from compensatory proliferation to compensatory hypertrophy in the injured Drosophila hindgut. E. Cohen\textsuperscript{1}, S. Allen\textsuperscript{1}, D. Fox\textsuperscript{1,2} 1) Department of Cell Biology, Duke University Medical Center, Durham, NC; 2) Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.

As development progresses, many organs lose their ability to respond to injury by compensatory cell proliferation. In such organs, like the human heart and kidney, tissue injury activates a non-proliferative cellular response known as hypertrophy. After injury, tissues that use hypertrophy for repair increase the size and often the DNA copy number of remaining cells to restore lost organ mass. Although many cues have been identified that are required for either compensatory proliferation or hypertrophy after tissue injury, little is known about molecular cues that could switch an injured organ between these injury responses.

We have identified the Drosophila hindgut pylorus as an accessible model to understand regulation of the switch between compensatory proliferation and hypertrophic repair. Using a highly tunable, site specific genetic ablation system, we found the pylorus undergoes compensatory proliferation when injured during larval stages, but then switches to a hypertrophic repair program by adulthood. The difference in these injury responses reflects a difference in injury-induced cell cycles. While the adult pyloric injury response is driven by the endocycle, the injured larval pyloric cells instead undergo mitotic cell cycles. We narrowed the time of the switch between injury-induced cell cycles to a 12-hour window during mid-pupation, coincident with a mid-pupal spike in Ec dysone hormone.

We then conducted a candidate screen for regulators of the injury response switch. To do this, we developed a new method for temporal control of injury separate from the control of transgenic construct expression. The method, which we term DEMISE (Dual Expression Method for Induced Site-specific Eradication), uses the Gal4/Gal80 and FLP/FRT systems to simultaneously induce mosaic tissue injury while independently expressing transgenes of interest. Using DEMISE, we identified the conserved cell cycle regulator (fizzy-related-\textit{fzr}), which normally acts to degrade mitotic cyclins, as a regulator of the pyloric injury response switch. \textit{fzr} RNAi completely restores compensatory proliferative capability to the normally hypertrophic adult pylorus. Our work has provided new insight into cell cycle control after organ injury, has developed novel tools for studying tissue injury repair, and is likely to inform efforts to regenerate tissues.

285 Cell cycle re-entry in the adult Drosophila melanogaster brain. Shyama Nandakumar, Emily Lerner, Chelsea Yu, Olga Grushko, Laura Butitta Molecular Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI.

Discoveries in recent years suggest that neuronal aneuyploidy and hyperploidy are far more common than previously thought, and prevalent in many organisms. Increased ploidy in postmitotic neurons has also been associated with age-related cognitive decline and neurodegeneration. We recently discovered that under normal physiological conditions cells in the aging adult fly brain exhibit age-associated cell cycle re-entry and hyperploidy, making it an excellent model system to study this process. How and where is hyperploidy generated, and what role does it play in age-related neurodegeneration? We developed a sensitive flow cytometry based assay to quantify changes in ploidy in individual fly brains. Our work thus far indicates that cell cycle re-entry occurs in multiple cell types, increases with age, and that high levels of hyperploidy can cause neurodegenerative phenotypes. In addition, we have found that increased oxidative damage leads to an earlier onset of hyperploidy in the brain and that the levels of hyperploidy can be manipulated through the genetic modulation of DNA damage and DNA replication licensing factors. We also observe a constant rate of cell death in the ageing brain, suggesting that the brain suffers a potential loss of cells over the lifespan of the organism. Studies in other postmitotic tissues have demonstrated cases where postmitotic cells re-enter the cell cycle and become hyperploid in response to wounding and damage. We thus hypothesize that the hyperploidy that we observe may compensate for the loss of cells that cannot be replaced by stem cells initially, but with time the accumulation of hyperploid cells could result in increased neurodegeneration. We have characterised where cell cycle re-entry occurs in the brain by examining specific cell types systematically. We have also developed new tools and assays to specifically label hyperploid cells in the adult brain to address whether specific cell types are more susceptible to or have a propensity for cell cycle re-entry. We have found that the optic lobes contribute to the majority of hyperploid cells in aged Drosophila brains. This is significant as recent work uncovered a small population of slowly dividing stem cells in the adult optic lobes suggesting that this region of the Drosophila brain requires higher cell turnover. Thus, the population of stem cells may become insufficient as the animals age to adequately compensate for normal aging-related cell loss and consequently, hyperploidy could result. If true, this would suggest that initially, cell cycle re-entry in neurons may play a protective, and prolonged accumulation of the same results in neurodegeneration.

Introduction

The formation and development of an organ is a complex process which involves several programmed events, including changes in cell shape and adhesion, proliferation, and differentiation. These events are defined by the expression and combination of specific Transcription Factors (TF), and aberrant processes lead to malformations and lethality. The Malpighian Tubules (MTs; equivalent to human kidney and liver) of Drosophila melanogaster is an important model for tubulogenesis, and for omics and integrative physiology. Therefore, we have used this system to study different functions of the tubule-enriched TF dGATAe during the development and physiology of this organ.

Methods

In this study, we have downregulated spatially and temporally the expression of dGATAe in the MTs using the Gal4/UAS and Gal80TS systems, and examined the defects in the function and morphology of this organ. Larval, pupal and adult tubules were dissected, and were immunostained with different markers to confirm the morphological phenotypes. We quantified the expression of possible downstream candidate genes of dGATAe by qPCR. In addition, we also challenged the flies with different stress assays (starvation, desiccation, etc.), to address the functional defects.

Results

We found that silencing dGATAe in the Principal Cells of the MTs induced strong defects in the morphology of the tubules in pupal and adult stages. They are shorter and have tumorous growth. We therefore stained these tubules with different markers and could observe over proliferation of small nuclei cells in the main segment. These cells were positively stained with Stem Cell and proliferation markers, such as Delta, Armadillo, or Phospho-Histone 3, suggesting that these cells could be Renal Stem Cells (RNSCs). Knockdown of dGATAe also induced strong upregulation genes involved in control of apoptosis and growth control. Finally, we observed that these flies were significantly less tolerant to starvation and desiccation stress, and their lifespan is heavily compromised. Further investigation is in progress to characterize the mechanism behind the role of dGATAe in the tubule.

Conclusion

Altogether, our results show that dGATAe is required during pupal stage in the Malpighian Tubules to maintain their integrity. We purpose that dGATAe is required for the control of tubule cell population, regulating the expression of downstream genes, and therefore, the morphology and function of this organ.

287  Scaling up the myoblast precursor pool through motor neuron signaling.  J.J. Fernandes1,2, Kumar Vishal1, Katie Lincoln1, Erin Enright1, Kole Sedlack1, Justin Crookes1, Arya Chalke1 1) Biology Dept, Miami Univ, Oxford, OH; 2) Center for Neuroscience.

About 80 muscle fibers are present in the hemi-thorax of the fruit-fly, Drosophila, and a majority of them, found in the dorsal thorax, are derived from precursors in the wing imaginal disc. Among these are the large indirect flight muscles (IFMs), comprising of 6 DLM fibers and 7 DVM fibers. Each of the six DLM fibers contains about 3000 nuclei. A carefully regulated build-up of the IFM myoblast pool needs to occur during its 24 hour myogenic period, to account for the almost 100-fold increase in nuclei as compared to the pool present in the wing-imaginal disc. Denervation of the developing fibers compromises fiber formation mainly through an effect on myoblast proliferation. These results led us to propose that innervating motor neurons regulate myoblast proliferation. A role for motor neurons as signaling cells was examined by blocking motor neuronal secretion using a dominant negative form of Shibe. As a result of the manipulation, a significant reduction in the number of nuclei [33%, n=20] was observed, which is a function of myoblast proliferation. To investigate the underlying mechanism of motor neuron communication the role of EGF ligands was tested by manipulating the production of Vein, Keren and Spitz. Blocking Vein [RNAi] resulted in the most severe effects: a 22% decrease in myoblast proliferation led to a 27% reduction in number of DLM nuclei. Overexpressing Vein in motor neurons led to almost doubling of the number of DLM nuclei, confirming that motor neurons use EGF signaling to scale up the DLM myoblast pool.

288  A new paradigm for regulation of apoptosis by intracellular pH dynamics.  J. Peralta, B. DuPriest, B. Grillo-Hill 1,2 Biological Sciences, San Jose State University, San Jose, CA.

Growth control at the tissue level requires communication between cells and coordination of both proliferation and apoptosis. Apoptosis is a conserved pathway that eliminates unnecessary cells to precisely pattern tissues and avoid developmental errors. Regulated pH dynamics are seen during programmed cell death in cultured mammalian cells, with alkalinization of mitochondrial matrix pH and subsequent cytosolic acidification resulting in decreased intracellular pH (pHi). We recently developed Drosophila models wherein over-expression of the ubiquitous sodium-proton exchanger DNhe2 (homolog of mammalian NHE1) showed increased pHi, increased cell proliferation and aberrant patterning of imaginal discs. We predicted that increased pHi in our system would inhibit apoptosis, however external examination of adult fly eyes showed decreased overall size. We next performed cell counts in pupal retinae, and found a significant decrease in the number of interommatidial lattice cells at the end of pattern formation, from an average of 15 cells in control to 11.4 cells in GMR>DNhe2.
This is despite increased proliferation that we reported in GMR>DNhe2 larval eye discs. The lattice cells are pruned through developmentally regulated apoptosis during pupal eye development, suggesting that the existing model where increased pH inhibits cell death may not apply in this system. Therefore, we hypothesize two models to describe our data: 1) increased pH promotes both proliferation and cell death; or 2) increased proliferation induces compensatory cell death, which exceeds the normal number of cells targeted by apoptosis to result in fewer cells at the end of patterning. We will distinguish between our models using temperature-shift experiments and phenotypic analysis. These findings will elucidate mechanisms for pH-regulation of conserved, critical developmental processes and provide evidence for new paradigms in growth control.

289 Modulation of CRL4<sup>cad</sup> activity in the syncytial embryo.  

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CRL4<sup>cad</sup>-mediated protein destruction is a developmentally critical phenomenon required for proper progression of both canonical and non-canonical cell cycles. CRL4<sup>cad</sup> induces replication-coupled destruction of its substrates, which include the Drosophila proteins Double-parked (Dup), E2f1, and Dacapo (Dap). Despite its critical role in overseeing S phase progression and maintaining genome stability, we have observed modulation of CRL4<sup>cad</sup> function in several developmental contexts. For example, our preliminary data suggest that CRL4<sup>cad</sup> activity may be modified in the rapid, non-canonical cell cycles of the syncytial embryo. More specifically, we have observed differential stability of substrates in this context: Dup appears to be targeted for destruction during S phase as normal, while E2f1 and Dap are stable. These data suggest that CRL4<sup>cad</sup> is active, but that at least two of its substrates are protected during these early cell cycles. We have identified sequences within the E2f1 protein that are required for protection in the early embryo. Continuing experiments seek to elucidate the mechanism, and ultimately the developmental function, of differential CRL4<sup>cad</sup> substrate stabilization during early embryonic development. Our findings suggest that CRL4<sup>cad</sup> activity may be modulated to accommodate unique cell cycle programs such as the rapid cycles of the syncytial embryo.

290 Regulation of Dap protein stability in the female germline.  

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Highly conserved proteins called CDK inhibitors (CKIs) play important roles in regulating cell proliferation both during development and during adult homeostasis. CKIs inhibit cell cycle progress, thereby limiting cell proliferation. Drosophila melanogaster possesses just one major CKI, a gene called Dacapo (Dap). Dap expression is carefully regulated throughout the lifespan of the fly; while insufficient Dap expression can lead to runaway proliferation, excess Dap expression can prematurely halt tissue growth and severely disrupt organ function. Dap is important developmentally and is also thought to contribute to adult tissue homeostasis in several tissues, including germline stem cells in the female reproductive system. Within germline stem cells, for example, Dap expression is tightly regulated by miRNAs to control cell division. Here we explore the potential role that regulated proteolysis of the Dap protein might play in development of the female germline. The Dap protein has previously been shown to be targeted by CRL4<sup>cad</sup> for replication-coupled destruction. Our preliminary data suggest that this mechanism may also regulate Dap expression in the female germline. Disruption of Dap degradation in the female germline, either by RNAi-mediated depletion of Cdt2 or by expression of a version of Dap that cannot bind Cdt2, leads to defects in both the cell cycle and oocyte specification. Our findings suggest that Dap degradation is required for normal oogenesis and for female fertility.

291 CRL4<sup>cad</sup> function during chorion gene amplification.  

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The CRL4<sup>cad</sup> ubiquitin ligase targets a number of proteins for replication-coupled destruction, including Double-parked (Dup), E2f1, and Dacapo (Dap). This function of CRL4<sup>cad</sup> has been shown to be critical for normal DNA replication, cell cycle progression, cell survival, and maintenance of genome stability in a number of cell types and for both canonical and non-canonical cell cycles. However, its role has not yet been investigated in the unique DNA replication cycles that occur during chorion gene amplification in follicle cells. Here, we explore a potential role for CRL4<sup>cad</sup> in chorion gene amplification using RNAi to deplete Cdt2 and by expressing stabilized versions of CRL4<sup>cad</sup> target genes. We find that disruption of CRL4<sup>cad</sup> function in follicle cells leads to aberrant DNA replication in Stage 10 egg chambers. These data suggest that CRL4<sup>cad</sup> may indeed play a role in overseeing this unique cell cycle program.

292 Modification of tumorigenesis microenvironment by a microRNA.  

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Tumorigenesis depends not only on mutations in oncogenes or tumor suppressor genes, but also on the tissue microenvironment where the mutant cells are located. Using the *Drosophila* wing imaginal disc, we have shown that the folded hinge area is a “tumor hotspot” for neoplastic tumor suppressor gene (nTSG) mutations, whereas the pouch area is a tumor coldspot. In the hinge area, growth-promoting JAK-STAT signaling activity is high, and the organization of the
epithelium forces the pro-tumor cells to undergo apical extrusion into the lumen, thus allows tumor formation and progression. In contrast, the pro-tumor cells undergo basal extrusion and apoptosis in the coldspot pouch area. In an effort to identify molecules that can change tissue microenvironment for tumorigenesis, we found that microRNA miR-133 misexpression converts the nTSG tumor coldspot to a hotspot in the wing disc. In the pouch area where tumorigenesis is normally suppressed, misexpression of miR-133 induces ectopic epithelial folds in the wing pouch, a feature resembling the hinge area. However, miR-133 misexpression alone resulted in downregulation of Jak-STAT signaling, which was opposite to what we had expected. Through surveying a number of growth regulatory signaling pathways and genetic epistasis analysis, we found that miR-133 instead promotes JNK signaling to facilitate tumor growth in the pouch coldspot area. miR-133 target analysis revealed that Rim and Rau are likely responsible for this conversion of the tumor coldspot to a hotspot. Together, this study reveals that tissue microenvironment can be changed from hostile to favorable for tumorigenesis based on regulatory systems that involve microRNAs and their regulatory targets.

293 Expression pattern observation of CG6191 (Mary Shelley) in Drosophila melanogaster and the link between its homolog Cables1 and epithelial based cancers in humans.  
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The gene CG6191 in the Drosophila melanogaster genome has little known about its function. However, it has a mammalian homologue, Cables1 is known to play a role in cell cycle regulation. Cables1 has been shown to be down regulated and frequently mutated in various forms of cancers including ovarian cancer. Due to observed eye and head phenotypes, CG6191 has been referred to as Mary Shelly (MS). Here we are investigating the expression pattern of the MS gene in different Drosophila melanogaster tissue. These results are then utilized to disrupt MS through targeted RNAi expression. Expression pattern of MS has been observed using the Green Fluorescent Protein (GFP) introduced to the fly's genome through the MiMlC fly lines. Two different types of fly lines were used to visualize the MS expression pattern. The first was a direct tagging of MS through a MiMlC-MS-GFP fruit fly. The second line was a cross between MS-Gal4 and UAS-GFP flies. This cross created a gene trap enabling GFP to only activate where MS was directly expressed. The two different lines were used as confirmation for MS expression patterns in various tissue. Preliminary data has been observed in three different tissues (imaginal wing discs, imaginal eye discs, and adult ovaries). Each studied tissue type demonstrates a unique pattern of gene expression. RNAi disruptions have produced phenotypic changes in the wing discs but have minimal to no growth alterations in the eyes of the fruit flies. When expressed in the posterior compartment of the wing, RNAi has been shown to disrupt Wg signaling and has also induced apoptosis. In mammalian cell culture, the Cables1 protein has been observed in several mammalian cell types, both epithelial tumor cell lines and normal epithelial cell lines, and screened on Western Blots. Implementing SiRNA transfection, the link between Cables1 loss of expression and epithelial tumor growth can be observed and implemented into human cancer therapies.

294 Regulation of dronc in development and cancer.  
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The Hippo pathway is an evolutionarily conserved pathway that regulates organ size and tissue homeostasis in Drosophila and mammals. The pathway functions by regulating the nuclear availability of transcriptional cofactor Yorkie (Yki), mammalian YAP, which is regulated by the activity of a core kinase cascade comprising the serine threonine kinases Hippo (Hpo) and Warts (Wts) and their accessory proteins. Yki binds with transcription factors like Scalloped (Sd) or Homothorax (Hth) to regulate target genes involved in cell proliferation and survival. Downregulation of the Hpo pathway causes increased cell proliferation and overgrowth, whereas hyperactivation of this pathway leads to cell death due to activation of these caspases. Previous work in our lab identified the initiator caspase Dronc (mammalian Caspase 9) as a transcriptional target of Yki. Caspase proteins are cysteine aspartic proteases which play essential roles in cellular signaling, development and cell death via apoptosis or Programmed Cell Death (PCD). We found that loss of Hippo signaling leads to downregulation of dronc expression suggesting that Yki could act in co-repressor complexes to provide growth and survival cues to cells where Hippo pathway is downregulated. We hypothesize that Yki functions both as an activator and a repressor simultaneously in association with the TEAD family transcriptional factor Sd to control dronc expression. Here, we present our work on the regulation of dronc by the Hippo pathway, and its implications in organ size control, and in disease conditions like cancer.

295 Yorkie drives tumor progression by antagonizing Pointed/ETS-mediated cellular senescence.  
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Cooperative action of key oncogenic drivers Ras and Yorkie/YAP is critical for human cancer progression, yet the underlying mechanism remains unknown. Here, through a genetic screen in Drosophila, we show that the Hippo effector Yorkie abrogates oncogenic Ras-induced cellular senescence by antagonizing the ETS transcription activator Pointed, thereby driving tumor progression. We show that Pointed acts as a novel tumor suppressor that is sufficient to induce cellular senescence downstream of Ras signaling. Interestingly, malignant tumors with Ras activation and cell polarity defects significantly
downregulate Pointed expression, and forced expression of Pointed in such malignant tumors dramatically suppressed their growth and metastatic behavior. Mechanistically, elevated Yorkie activity in malignant tumors represses Pointed expression by regulating FoxO signaling, thereby inhibiting cellular senescence. Our data provide a mechanistic understanding of a novel oncogenic cooperation between Ras and Yorkie/YAP via Pointed/ETS-mediated regulation of cellular senescence.

296 Tumor suppressive roles of Nucleoporins 98 and 96 in Drosophila epithelium. Ajai Joseph Pulianmackal,1 Kiriaki Kanakousaki,1 Saranyaarajan Varadarajan,1 Kerry Flegel,1 Norman Zielke,2 Laura Buttitta1 1) MCDB, University of Michigan, Ann Arbor, MI; 2) University of Helsinki, Institute for Biotechnology, Helsinki, Finland.

The Nucleoporin 98KD (Nup98) is one of the most promiscuous translocation partners in hematological malignancies, contributing to at least 31 different truncation-fusion proteins. To date, nearly all disease models of Nup98 translocations involve ectopic expression of transgenes recapitulating the fusion protein under study, leaving the endogenous Nup98 loci unperturbed. Hence, they cause either the upregulation or the downregulation of Nup98 activity. The contribution of Nup98/96 to the leukemic disease phenotype remains unknown. Nup98 and 96 are also mutated in many other cancer types and located near a tumor suppressor region known to be epigenetically silenced, suggesting that their disruption may not be limited to blood cancers.

We found that reducing Nup98/96 function via an RNAi approach in Drosophila melanogaster (where the Nup98/96 shared mRNA and reading frame gene structure is conserved) de-regulates the cell cycle. We find evidence of overproliferation in Nup98/96-deficient tissues, counteracted by elevated apoptosis and aberrant Wingless, TNFα and JNK signaling associated with wound healing. When the knockdown of Nup98/96 is combined with inhibition of apoptosis, we see synergism leading to overgrowth consistent with a tumor-suppressor function for endogenous Nup98 and/or 96. Nup98/96 being nuclear porins, we expected that reducing its function may affect the transport of proteins to nucleus. Interestingly, we found that the nuclear export of a ribosomal protein RPL10 is misregulated. We find evidence for decreased protein synthesis in Nup98/96 knockdown cells. Knockdown of proteins required for export of ribosome from the nucleus to cytoplasm recapitulated phenotypes similar to Nup98/96 knockdown. This suggests that the phenotypes by Nup98/96 knockdown maybe caused by aberrant ribosome assembly. The overexpression of Nup98/96 also phenotypes similar to its knockdown. Based upon our data, we suggest that the overexpression or partial loss of Nup98 and Nup96 function de-regulates the cell cycle to cooperate with other mutations in cancer.

297 Cell-type-specific role of Snr1 in the developing optic lobe in Drosophila. S. Keegan, S. C. Hughes Department of Cell Biology, University of Alberta, Edmonton, AB, CA.

The loss of the human gene, SMARCB1, has been associated with nerve sheath tumors in Schwannomatosis, a rare cancer. There are a number of questions remaining regarding the genetic cause and progression of Schwannomatosis. It has been suggested that SMARCB1 has a distinct function in different cell types during nervous system development. Some reports indicate that the cellular localization of SMARCB1 may contribute to these changes in function. Although SMARCB1 is part of the SWI/SNF chromatin remodeling complex, it can also be found in the cytoplasm. To gain better understanding of the role of SMARCB1 in the developing nervous system, we study the orthologous gene in Drosophila melanogaster, Snr1. We hypothesize that cytoplasmic Snr1 decreases proliferation, and that knockdown of Snr1 in the cytoplasm will lead to over-proliferation. We used immunofluorescence and confocal microscopy to look at the localization of Snr1, and to characterize the effects of the loss of Snr1 in the developing optic lobes in the brains of Drosophila larvae. Snr1 appears to be localized to the nucleus in symmetrically dividing neuroepithelial cells, and post-mitotic neurons and glia. In asymmetrically dividing neuroblasts however, Snr1 is localized to the cytoplasm. When Snr1 is knocked down specifically in neuroblasts with RNA interference, the optic lobe is very overgrown. When expression of Snr1 is lost in neuroepithelial cells, there is a significant decrease in neuroepithelial tissue and the optic lobe is disorganized. Finally, when Snr1 is knocked down in glial cells, there is a significant reduction in the number of glial cells, however the glial cells are larger and disorganized. The differing role of Snr1 in certain locations in the cell will also be confirmed by expressing Snr1 with a deletion in the nuclear export sequence. Our results suggest that cytoplasmic Snr1 does play a role in controlling proliferation in neuroblasts, and that Snr1 may also have a role in promoting proliferation of symmetrically dividing cells in the developing optic lobe in Drosophila.

298 Cell Type-Specific Response to Spindle Misorientation and Effects on Tissue Growth. A.S Parra, C.A

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Coordination of cell polarity and spindle orientation with cell growth and proliferation ensures mitotic fidelity and thus proper animal development. Mitotic errors have been associated with aberrant tissue growth in both epithelial cells and stem cells. Mutations in cell cycle-promoting genes in neural stem cells cause a mild increase in the Drosophila central nervous system (CNS). Conversely, identical mutations in Drosophila imaginal wing discs (IWD), terminally differentiated cells, lead to massive tissue overgrowth. The mechanisms underlying the differential responses of these cells to errors in cell division, however, are unknown. Here we seek to build a stem cell model and a differentiated cell model to elucidate the varied tissue-specific responses. We found that mutated growth-promoting genes cause substantial overgrowth in epithelial cells, while no significant change in the CNS was observed when expressed in neural stem cells. Additionally, loss of Mud, a
gene essential for proper mitotic spindle orientation, results in apoptosis-mediated inhibition of IWD growth in response to mutated growth-promoting genes. Our results further highlight the differential response of epithelial cells and stem cells to mutated growth-promoting genes. Taken together, these results point to an overgrowth-inhibitory mechanism in epithelial cells stemming from errors in spindle orientation caused by these mutations. Further analysis will provide a better understanding of the cell signaling pathways that govern tissue level responses to defective cell division, which will be important to improving our understanding of the underlying molecular bases for numerous human diseases.

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**Cell Competition as a Model for Pre-neoplastic Development.**  
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The Cancer Genome Atlas makes it clear that cancers frequently descend from a single proliferative cell exhibiting altered expression of one or more oncogenes/tumor suppressors. Although the mechanisms allowing a rogue cell to expand into a centimeters wide field - probably at the expense of healthy tissue - remain unclear, the early stages of tumor growth bear striking similarities to cell competition. This dynamic developmental process describes the ability of cells in a tissue field to sense each other's relative fitness and actively eliminate less fit, but otherwise viable cells. When these competitive interactions occur between healthy wildtype (WT) cells and cells overexpressing the proto-oncogene Myc, it is the WT cells that are eliminated. This not only results in the erosion of healthy tissue, but stimulates proliferation of Myc overexpressing cells, a process called super-competition. Myc induced super-competition is an evolutionary conserved process and was recently shown to occur naturally in mouse embryos. The list of mutations known to induce super-competition has expanded to include Hippo, Wnt/Wg, and JAK/STAT. Data from our lab indicates that Myc induced super-competition has co-opted a branch of the innate immune response to create a novel pathway resulting in the NF-κB mediated apoptosis of WT cells. We are attempting to unravel the genetics underlying this mechanism, examining the full extent of NF-κB activity during other contexts of cell competition, cataloging the transcriptional program underlying Myc induced competition through RNA-seq, and determine the prevalence of endogenous Myc induced competition with a transgenic reporter based on the role of Relish in Myc induced competition.

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**Cell competition eliminates aneuploid cells after irradiation.**  
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Cell competition refers to a special type of cell-cell interaction between two group of cells within multicellular organs. Those cells generally contain distinct genotypes and are homogeneous viable, however, one group will be eliminated from the niche if they encounter the other group of cells, which will later take their places. For example, cells heterozygous for most ribosomal protein (Rp) genes will be out-competed if their neighbor wild-type cells. Since Rp genes are randomly distributed in chromosomes, heterozygosity of Rp genes could serve as a marker for cells with chromosomal deficiencies. Therefore, cell competition has been proposed to act as a surveillance mechanism to actively remove genomically-defective cells by p53-independent cell death, such as has been observed following ionizing irradiation. In order to test whether this p53-independent cell death reflects cell competition, we have generated RpS12<sup>G97D</sup> and p53 double mutant flies. RpS12<sup>G97D</sup> is a recessive mutation that prevents cell competition. We found that following irradiation, p53-independent cell death is RpS12-dependent, suggesting that it is cell competition. In addition, aneuploid cells, which can be identified in adult bristles, are more numerous after irradiation when cell competition is blocked. In summary, our evidence supports a role for cell competition as a surveillance mechanism for aneuploid cells. We are investigating the significance of this process under physiological conditions.

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**Growth regulation by the bZip-domain protein Xrp1 in Minute (Rp<sup>+</sup>) cells.**  
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Tissue homeostasis is essential for precise development, regeneration and physiology of an organism. Cell competition which involves elimination of slow growing (sub-optimal) cells when surrounded by fast growing cells is one such phenomenon by which tissue homeostasis may be maintained. In *Drosophila*, cells lacking a single copy of many ribosomal proteins genes (*Rp<sup>+</sup>*, also known as *Minute*) display slow growth behavior and are eliminated when present next to wild type cells. We are working on bZip-domain protein Xrp1 which not only regulates elimination of *Rp<sup>+</sup>* cells during cell competition but high expression of same is also responsible for slow growth behavior of *Rp<sup>+</sup>* cells. To understand the key signals through which Xrp1 regulates *Rp<sup>+</sup>* growth, we have performed whole-genome mRNAseq of *Drosophila* wing imaginal discs. Our data suggests that changes in gene expression of *Rp<sup>+</sup>* cells is because of high expression of Xrp1 as it loss revert those changes to wild type levels. Preliminary data related to various pathways identified through this method will be presented. Moreover, apart from transcriptional targets, we have also identified a translational target gene of Xrp1 in the regulation of *Rp<sup>+</sup>* growth and result for same will be presented as well.
Mechanisms of clonal extrusion maintain epithelial homeostasis. J.L. Lahvic, I.K. Hariharan Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Epithelia have diverse mechanisms for eliminating unfit cell types, including tissue-inappropriate cells and over- or under-proliferating cells. These endogenous homeostatic processes may also eliminate pre-cancerous cells. While certain cases of these so-called cell competition processes, such as the slow-growing Minute mutants or fast-growing Myc overexpressing cells, have been well studied, the elimination of RasV12 cells from normal tissue has received less attention. RasV12 cell competition appears to have a mechanism unique from other cases- RasV12 cells do not undergo apoptosis like Minute mutants, and they do not intermingle with wildtype cells as Myc overexpressing cells do. We have observed entire RasV12 clones to extrude apically or basally from the Drosophila wing epithelium, which matches observations seen in cell culture. Specific molecular mechanisms likely regulate this extrusion process. Clones more frequently extrude from the pouch of the wing disc, a tissue known to experience high rates of cell competition, than from the notum. Additionally, JNK signaling is activated surrounding some RasV12 clones, as indicated by an AP-1 reporter line and staining for the JNK target, MMP1. We have developed a new genetic system for manipulating gene expression outside of RasV12 clones in order to screen for non cell-autonomous regulators of extrusion. We are additionally adapting a synthetic Notch receptor system to specifically label RasV12 clone neighbor cells and identify genes differentially expressed in these neighbors compared to other wild-type cells. These experiments will define the molecular mechanisms of a little studied form of cell competition- RasV12 clonal extrusion. Importantly, many dangerous cancers begin as small clones of Ras overactive cells, and these experiments have the potential to identify novel methods of targeting these cancers, by enabling wildtype cells to directly eliminate tumor cells.

Hyperinsulinemia abrogates tumor-suppressive cell competition. Y. Sanaki, D. Kizawa, T. Igaki Graduate School of Biostudies, Lab of Genetics, Kyoto Univ., Kyoto, Kyoto, JP.

Epithelial tumor development is restrained by an intrinsic tumor-suppressive mechanism called cell competition. In particular, pre-malignant cells mutant for the apico-basal polarity gene scribble (scrib) are actively eliminated from Drosophila imaginal epithelia when surrounded by wild type cells. While cell competition is thus a critical mechanism for removing potentially dangerous cells, pre-malignant cells could somehow escape cell competition and become cancers. Importantly, cancer incidence varies between individuals according to predispositions such as heredity, past medical history, and lifestyle. We therefore hypothesized that physiological differences could affect tumor-suppressive cell competition. To understand the regulatory mechanisms of tumor-suppressive cell competition, we conducted a dominant modifier screen for suppressors of scrib-induced cell competition in Drosophila. Interestingly, we found that heterozygous insulin receptor substrate mutants (chico+/) exhibited massive scrib tumors resulting from dysfunctional cell competition. Unexpectedly, chico remotely modulated cell competition from Insulin-Producing Cells (IPCs); chico knockdown in IPCs facilitated scrib cells to evade elimination and phenocopied chico/+ heterozygotes. Mechanistically, IPC-knockdown of chico upregulated Drosophila insulin-like peptides (DILP) 2 and 5, and overexpressing DILP2 in IPCs abrogated scrib cell competition and triggered tumors. Thus, hyperinsulinemia enables pre-malignant cells to become tumors by attenuating tumor-suppressive cell competition. This may partially explain the higher tumor incidence seen in type II diabetics, who frequently exhibit hyperinsulinemia.

Loss of foxo rescues stem cell aging in Drosophila germ line. H. Ruohola-Baker1,2, F. Artoni1,2, O. Palmeira3 1) Dept Biochemistry, Univ Washington, Seattle, WA; 2) Institute for Stem Cell and Regenerative Medicine, University of Washington, School of Medicine, Seattle, WA; 3) Nucleus of Multidisciplinary Research, Federal University of Rio de Janeiro, Duque de Caxias, Brazil.

Aging is a complex biological process, comprised of cellular and organismal changes that, together, lead to the progressive decline in tissue and organ function. In addition to diminished function in differentiated cells, aging tissues are marked by a decrease in the proliferative capacity of pluripotent cells, hampering their regenerative capacity and ability to respond properly to injury and insult. However, the mechanisms that govern the regenerative competence of stem cells early in the aging process remain unclear. We utilized the ability of Drosophila melanogaster germ line stem cells (GSCs) to survive exposure to low doses of ionizing radiation (IR) as a model of adult stem cell injury. Previous work in the young fly has shown a remarkable ability of Drosophila germ line stem cells (GSCs) to survive IR, even when their progeny undergo rapid apoptosis (Xing, Su et al. 2015): when flies are exposed to low doses of ionizing radiation GSCs survive, while their progeny, the transiently amplifying cells, do not. Dying GSC daughter cells secrete the ligand Pvf1, which signals via the Tie receptor and microRNA bantam to inhibit the apoptotic machinery in GSCs (Bilak, Uyetake et al. 2014, Xing, Su et al. 2015). After a period of quiescence, the GSCs re-enter the cell cycle and, ultimately, regenerate the germline. Using this model we identified a regeneration defect in aging GSCs: while aging GSCs survive exposure to IR, they fail to reenter the cell cycle and regenerate the germline in a timely manner (Artoni et al., 2017). Mechanistically, we identify loki (Ma et al., 2016), foxo, mTOR homologue, Tor and Tsc1 as important regulators of GSC quiescence following exposure to ionizing radiation. foxo and Tsc1, downstream of loki, are required for entry in quiescence, while Tor is essential for cell cycle reentry. Importantly, we further show that the lack of regeneration in aging germ line stem cells after IR can be rescued by loss of foxo. These data suggest that misregulation of foxo may underlie the decline in regenerative capacity post-injury, observed in aging.
Apoptosis shapes Drosophila neural development from embryonic to adult stages. For example, a subset of neural stem cells undergo apoptosis in each abdominal hemisegment at the end of embryogenesis, leaving only three out of thirty per abdominal hemisegment survive until larval life. When this apoptosis is inhibited, these cells which are known to be larger than wild type. These findings suggest that the Tor pathway regulates RA transcripts to properly coordinate cell growth and proliferation during development.

The Tsc/Tor pathway regulates specific de2f1 transcript isoforms to control cell size and proper development. M. Bradley-Gill, M. Kim, N. Moon Department of Biology, McGill University, Montreal, Quebec, CA.

The coordination between growth and cell division in multicellular organisms is complex and still poorly understood. The Tsc/Tor pathway is an important growth regulator known to respond to nutrient levels. Tor promotes growth by increasing translation whereas Tsc1 negatively regulates this pathway. In a previous study, our lab found that loss of tsc1 upregulates an E2F cell cycle transcription factor in Drosophila, de2F1, suggesting a critical link between growth and cell division pathways. The upregulation occurred post-transcriptionally, consistent with the idea Tor activity promotes translation. We asked whether this regulation was important to couple cell size and division. Complicating the analysis is that de2F1 is transcribed from 6 different transcript isoforms that differ in their 5'UTR and expression pattern. We concentrated on the "RA" region, coding for three transcript isoforms, which contains conserved translational control elements and increased the most in response to tsc1 mutation. Here, we show that the RA region transcripts are regulated by the Tsc/Tor pathway to control cell size and development. Removal of these specific transcripts resulted in inability of de2F1 to increase in response to tsc1 mutation. Furthermore, RA tsc1 double mutant cells that should be differentiating began ectopically entering S-phase and dying. Interestingly, these double mutant cells were larger than tsc1 mutant cells which are known to be larger than wild type. These findings suggest that the Tor pathway regulates RA transcripts to properly coordinate cell growth and proliferation during development.

An RNAi based screen to identify upstream regulators and downstream effectors of the TORC1 inhibitor GATOR1. Yingbiao Zhang, Kuikwon Kim, Elena Ghaniam, Nicolas Johnson, Mary Lilly NICHD, NIH, Bethesda, MD.

The nutrient sensitive Target of Rapamycin Complex 1 (TORC1) is a master controller of metabolism in eukaryotes that regulates physiology, metabolism, and aging. However, the upstream inputs that influence TORC1 signaling are extremely complex and not fully understood. In Drosophila, oocyte growth and development is strongly influenced by metabolic pathways. The Gap Activity Towards Rags (GATOR) complex is a highly conserved upstream regulator of TORC1 that is comprised of two subcomplexes, GATOR1 which inhibits TORC1 in response to amino acid starvation and GATOR2 which opposes the activity of GATOR1. We are using Drosophila oogenesis as a model to define the function of the GATOR1 and GATOR2 complexes in metazoans. The GATOR2 complex is comprised of five proteins, Mio, Wdr24, Wdr59, Sec13 and Seh1. Mutations in seh1 and mio cause the deregulation of the GATOR1 complex and the constitutive inhibition of TORC1 activity in the female germline. Reduced TORC1 activity in mio and seh1 mutants results in a block to oocyte growth and development. RNAi based depletions of the seh1 transcript in the female germline phenocopied the seh1 mutant. To identify upstream regulators and downstream effectors of the GATOR complex, we undertook a large-scale RNAi based screen using lines from the Transgenic RNAi Resource Project (TRiP). Specifically, we screened for genes that when co-depleted with seh1 in the female germline rescued the seh1 null ovarian phenotypes. Two of the genes identified in the RNAi screen include Charybdis, a homolog of (Regulated in development and DNA damage responses 1 (Redd1) and Sima (Hif1-α), a known upstream regulator of Redd1. Notably, Hif1-α is a master transcriptional regulator of the cellular and developmental response to hypoxia. We are working to understand the role of Charybdis/Redd1 and Sima/Hif1-α in the regulation of oocyte growth and development.

Regulation of different developmental apoptosis through fs(1)h. A. Boronsztejn, K White CBRC, MGH / Harvard Medical School, Charlestown, MA.

The control of cell number is essential for the proper formation of organs during development. In the developing Drosophila central nervous system, subsets of neural stem cells die at the end of embryogenesis. In each of the central abdominal hemisegments of the ventral nerve cord, 27 out of 30 neural stem cells (neuroblasts) undergo programmed cell death. Thus, only 3 neuroblasts per abdominal hemisegment survive until larval life. When this apoptosis is inhibited, these stem cells continue to divide and give rise to a hyperplastic central nervous system and adult lethality. Our lab previously showed that Notch and AbdominalA (AbdA) control the transcriptional activation of the central cell death regulators grim and reaper in the doomed neuroblasts, ensuring their elimination through the canonical apoptotic pathway. An RNAi screen in the lab identified the transcription factor female sterile homeotic (fs(1)h) as a new regulator of this apoptosis. Surprisingly fs(1)h appears to induces neuroblast apoptosis independently of its known function on AbdA transcription regulation. In situ analysis revealed that instead fs(1)h regulates rpr transcription through the regulation of an intergenic regulatory region already known to be involved in neuroblast apoptosis, and the transcription of other downstream component of the canonical apoptotic pathway. Finally our results show that this pro-apoptosis function of fs(1)h is not restricted to the neuroblast but is also at play during the midline glia development.

Cut alters the chromatin landscape of the reaper locus to facilitate neuroblast death. R. Arya1,2, S. Gyonjyan1,2, K. Harding1,2, T. Sarkissian1,2, L. Zhou1, K White1,2 1) CBRC, Massachusetts General Hospital, Charlestown, MA; 2) Harvard Medical School, Boston, MA; 3) University of Florida, Gainesville, FL.

Apoptosis shapes Drosophila neural development from embryonic to adult stages. For example, a subset of neural stem cells undergo apoptosis in each abdominal hemisegment at the end of embryogenesis, leaving only three out of thirty
phagoptosis is a two-step process where the nurse cells are first acidified, and secondly degraded by cathepsins. Previous work on the spatial regulation of apoptosis in the Drosophila VNC, and further our understanding of the links between cell identity and cell fate.

309 Restriction of apoptosis in Drosophila neural stem cells. K. Harding, K. White Harvard University - MGH, Charlestown, MA.

Programmed cell death is a critical aspect of normal development for all multicellular organisms. Embryonic development of the Drosophila melangaster ventral nerve cord offers a convenient model system to study the regulation of programmed cell death in vivo. Embryonic neural stem cells (neuroblasts) in the abdominal region of the VNC self-renew until late stages of embryogenesis, when the vast majority undergo apoptosis prior to larval development. However, three defined neuroblasts survive and contribute to larval neurogenesis. To learn more about the role of cell identity in regulating cell fate, we are investigating factors that determine the “doomed” and “survivor” fates in Drosophila abdominal neuroblasts. We have characterized a number of neuroblast-specific GAL4 lines that allow us to accurately identify and visualize doomed or surviving neuroblasts prior to their death. Using these reporter lines, we have shown that, prior to undergoing apoptosis, doomed neuroblasts express a pulse of the Hox gene AbdA, as reported previously by Bello et al (2003) and Arya et al (2015). However, the pulse of AbdA is not observed in surviving neuroblasts, suggesting that upstream Hox regulators are responsible for dictating the distinct fates of abdominal neuroblasts. We are currently assessing whether these upstream factors are differentially expressed between doomed and surviving neuroblasts. These findings build on a large existing body of work on the spatial regulation of apoptosis in the Drosophila VNC, and further our understanding of the links between cell identity and cell fate.

310 Modifiers of Bar eye facet cell death using DGRP sequenced genomes. T. Holy, J. Thompson Biology, University of Oklahoma, Norman, OK.

To map polygenic background modifiers of cell death, we used sequenced lines from the Drosophila Genetic Reference Panel (DGRP). We combined genomic technology and electron microscopy to measure precisely the genetic backgrounds affecting cell death in eye-facet development of the dominant mutation Bar eye. This approach focuses on heterozygous effects, since half of the genome is from the inbred Basc stock carrying the dominant mutation and the other half constitutest autosomes of individual DGRP strains. Heads were removed, bisected between the compound eyes, and mounted on SEM plugs. These were sputter-coated with gold-palladium and scanned by SEM to provide high-resolution images for facet number counts in each eye. Average facet amounts were calculated for each semi-genome from 100 DGRP lines. When available, pairs of eyes from the same individual were assayed for symmetry. Comparing both eyes from the same individual allowed the additional measurement of developmental homeostasis as reflected in fluctuating asymmetry, FA = |L – R|/((L + R)/2). We found that DGRP lines differ significantly in alleles at loci affecting the degree of cell death. For example, for strain 25185 (RAL 358), the average facet count was approximately 240, compared to strain 25745 (RAL 714), in which there were about 75 facets per eye. Relevant modifier loci are being evaluated for functional connections. These two strains were among the most different in facet phenotypes among 100 DGRP backgrounds assessed. We, therefore, found it interesting that the level of developmental symmetry in expression was quite similar between these two backgrounds. This suggests that degrees of eye facet cell death may be regulated homeostatically. Results will be discussed in the contexts of modifier loci for cell death and the effect of this disruptive process on developmental homeostasis. We thank Preston Larson, Samuel Roberts Noble Microscopy Laboratory, for help with SEM imaging.

311 Exocytosis and lysosomal gene involvement in nurse cell phagoptosis. O. Naranjo1, A. Mondragon1,2, Y. Zhang1, K. McCall1 1) Biology, Boston University, Boston, MA; 2) Molecular Biology, Cell Biology, and Biochemistry, Boston University, Boston, MA.

Cell death and clearance play an important role in the functions of organisms, and when interrupted, these processes can lead to autoimmune diseases or cancer. Previous research has been primarily focused on apoptosis, autophagy, and necrosis. Our lab studies phagoptosis, a novel form of cell death which is not clearly understood. Phagoptosis occurs when a cell uses engulfment and phagocytic machinery to kill a nearby cell. In Drosophila development, a layer of follicle cells surrounds fifteen nurse cells and an oocyte. The follicle cell layer includes a subset of cells called stretch follicle cells that directly surround the nurse cells. Nurse cells support the developing embryo by dumping their cytoplasm into the oocyte. During late oogenesis, nurse cells are encompassed and murdered by stretch follicle cells through the activation of phagocytic machinery, providing a powerful model for the characterization of phagoptosis. Our data show that nurse cell phagoptosis is a two-step process where the nurse cells are first acidified, and secondly degraded by cathepsins. Previous
work has utilized V-ATPase protein traps and antibodies to demonstrate V-ATPases are enriched and localize to the plasma membrane of the surrounding stretch follicle cells where they extracellularly acidify the nurse cells. Additionally, our lab has demonstrated that CP1 is released by the stretch follicle cells and is required for nurse cell death. We are determining the role of exocytosis machinery in nurse cell phagoptosis by knocking down exocytosis genes in the follicle cells with RNAi and examining whether CP1 is still released from the follicle cells or the acidification step occurs. Collectively, this work further characterizes the role of exocytosis and lysosomal genes in the stretch follicle cell mediated nurse cell death, and ultimately increases our understanding of phagoptosis.

312 Identifying natural genetic modifiers of apoptosis and retinal degeneration. E. Ong, R. Palu, S. Chung, D. Perez, C. Chow Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

Retinitis pigmentosa (RP) is a retinal degeneration disease associated with mutations in a number of genes in different pathways. Like many diseases, RP is characterized by phenotypic heterogeneity. This heterogeneity is thought to be caused by genetic variation between patients, yet the identity of genetic modifiers remains unknown. A previous study conducted in our lab used the Drosophila Genetic Reference Panel (DGRP) to study the effects of natural genetic variation on a model of RP. In this model, a misfolded rhodopsin protein (Rh1G69D) was overexpressed in the developing eye imaginal disc, inducing ER stress and cell death during development. Nearly half of the candidate genes identified through an association study were involved in apoptosis, suggesting that variation in apoptosis signaling may contribute to variation in retinal degeneration. Indeed, a number of RP therapies under development are aimed at inhibiting apoptosis. We hypothesized that genetic variation in apoptosis pathways might also contribute to variation in RP outcomes. Apoptosis is induced under a number of stress conditions that initiate p53 signaling. In Drosophila, p53 activates reaper (rpr), which inhibits inhibitors of Apoptosis (IAPs), allowing apoptosis to proceed. p33 and rpr are pro-apoptotic genes. To search for modifiers of apoptosis, we crossed transgenes overexpressing p33 or rpr in the eye discs onto the DGRP strains. This results in degeneration in the adult eye, similar to the RP model described above. We quantified variation in eye size and found extensive phenotypic variation among the DGRP. This was apparent for both the p53 and rpr models. We used an association analysis to identify candidate modifier genes. We identified a number of biologically interesting modifiers, including RunxB, CG3921, sm, and CG15220 which function in transcription, ER stress, mRNA processing, or DNA repair and recombination. To test the functional effects of each modifier gene, we are using RNAi to identify interactions with each apoptosis model. Identifying natural genetic modifiers of apoptosis may identify modifiers that may be applicable to a number of different retinal degeneration diseases.

313 Loss of the fatty acid elongase ELOVL6 rescues ER stress-induced apoptosis. R.A.S. Palu, C.Y. Chow Human Genetics, University of Utah, Salt Lake City, UT.

An important goal of the Precision Medicine Initiative is to address the phenotypic heterogeneity that impedes diagnosis and treatment in both Mendelian and complex genetic diseases. Individuals with the same causative mutation may display vast differences in disease penetrance and expressivity. Cryptic genetic variation is a key contributor to this heterogeneity, but the underlying genetic architecture and modifiers are largely unknown. Recently developed tools like the Drosophila Genetic Reference Panel (DGRP) allow researchers to study the effects of natural genetic variation on disease phenotypes. One process that commonly contributes to phenotypic heterogeneity is the ER stress response. Understanding how standing variation alters this response could provide therapeutic targets for a number of diverse diseases such as diabetes, obesity, and neurodegeneration. In a previous study, we utilized the DGRP to explore the impact of natural genetic variation on a model of ER stress-associated retinal degeneration. Overexpression of a mutant, misfolded rhodopsin protein (Rh1G69D) induces ER stress in the eye imaginal disc, ultimately resulting in apoptosis and a small, degenerate adult eye. Degeneration was incredibly variable among the 200 DGRP strains. Using genome-wide association methods, we identified 84 conserved candidate modifiers, many of which are associated with ER stress and apoptosis. One of these, baldspot, is the Drosophila orthologue of ELOVL6, an ER-associated fatty acid elongase. ELOVL6 activity has been linked to ER stress, insulin sensitivity, and obesity in mammals. We demonstrate that RNAi-mediated knockdown of ELOVL6 alleviates degeneration in the Rh1G69D model of retinal degeneration. This is associated with a reduction in ER stress and apoptosis. These effects are negated with elevated dietary stearate, suggesting that this function of ELOVL6 is mediated through its fatty acid elongase activity. Furthermore, loss of ELOVL6 in other tissues also reduces degenerative phenotypes induced by ER stress, suggesting that ELOVL6 is not retinal-specific, but a general modifier of ER stress. Further analysis in S2 cells suggests that ELOVL6 acts by altering the fatty acid composition of the ER and the activity of IRE1. Our findings indicate that ELOVL6 is a general modifier of ER stress that functions in a variety of contexts. ELOVL6 is a possible therapeutic target not only in retinal degeneration but in many ER stress-associated diseases.

314 Investigating the scramblase function for corpse clearance in the Drosophila ovary. J.S. Peterson, S.B. Serizier, J. Elguero, K. McCall Dept Biol, Boston Univ, Boston, MA.

Scramblases facilitate the exposure of phosphatidylinerse on the surface of apoptotic cells, which functions as an “eat me” signal to an engulfing cell. Two scramblase proteins have been identified in mammals, Xkr-8 and TMEM-16F, and we wanted to determine whether either of the Drosophila homologues of these proteins are required for corpse clearance in the ovary.
We used CRISPR Cas9 to generate a deletion in the CG homolog of Xkr-8 but found that corpse clearance was not affected and therefore we obtained mutations in subdued, a TMEM-16F homolog. Preliminary results indicated a reduction in death and clearance of nurse cells during the terminal stages of oogenesis. We will determine whether phosphatidylserine exposure, acidification, and DNA fragmentation still occur in these mutants.


The clearance of dead cells is a fundamental process in maintaining tissue homeostasis. Genetic studies in Caenorhabditis elegans, Drosophila melanogaster, and mammals identified two evolutionarily conserved signaling pathways that act redundantly to regulate this clearance process: the ced-1/6i-7 and ced-2/5i-12 pathways. Of these clearance genes, the ced-7/ABCA1 ortholog remains to be identified in D. melanogaster. Homology searches revealed a family of putative ced-7/ABCA1 homologs encoding ABC transporters in D. melanogaster. To determine which of these genes functions similarly to ced-7/ABCA1, we analyzed mutants for clearance defects in oogenesis, during which nurse cells in each egg chamber undergo programmed cell death and are removed by neighboring phagocytic follicle cells. Our genetic analyses indicate that one of the ABC transporter genes, which we named Eato, acts in the same pathway as drpr (ced-1 ortholog) and in parallel to Ced-12, and is required in the follicle cells for nurse cell clearance. We show that Eato acts in the follicle cells to promote (1) accumulation of Drpr, a phagocytic membrane receptor which facilitates recognition of dying cells, and (2) phagocytic membrane extensions around the nurse cells for their clearance. Additionally, whether ced-7/ABCA1 is required for phosphatidylserine exposure in dying cells is under debate; we show that Eato is not required for phosphatidylserine exposure. Since ABCA class transporters, such as CED-7/ABCA1 and Eato, are known to be involved in lipid trafficking, we propose that Eato acts in the follicle cells to transport membrane material to the growing phagocytic cup for cell clearance. Our work presented here identifies Eato as the ced-7 ortholog in D. melanogaster, and demonstrates a role for Eato in Drpr accumulation and phagocytic membrane extensions.

316 Characterizing stretch follicle cell dynamics during nurse cell phagoptosis. Y. Zhang, A. Mondragon, A. Ortega, K. McCall

Programmed cell death (PCD) is a genetically controlled process, playing many vital roles in development and tissue homeostasis. New research is beginning to demonstrate the importance of non-apoptotic forms of cell death such as pyroptosis, entosis, and phagoptosis in development and disease. Phagoptosis is an otherwise healthy cell being murdered by a nearby cell, but the molecular machinery used for this cell death is not well understood. We use nurse cell death in the Drosophila ovary as a model for studying phagoptosis. During oogenesis in Drosophila, there are 15 nurse cells that support the oocyte by producing supplies (RNA, proteins, etc.) throughout egg chamber development. Developmental PCD of supporting nurse cells occurs during the late stages of oogenesis and they are degraded and removed by the surrounding follicle cells; however, little is known about how stretch follicle cells degrade the nurse cells.

Vacuolar (H+)ATPases (V-ATPases) are a type of ATP-dependent proton pumps which are responsible for regulating the pH of cells and intracellular compartments. Using protein traps and confocal microscopy, we have found that V-ATPases localize to the plasma membrane in stretch follicle cells. Drosophila cysteine protease-1 (CP1) is a cathepsin L-like enzyme, which mediates degradation of proteins in Drosophila. Using antibody staining, LysoTracker staining and confocal microscopy, we have found that CP1 is highly enriched in the follicle cells and in the acidified nurse cells that they surround. These findings support a model that V-ATPases and CP1 are enriched in the stretch follicle cells and CP1 is released by the follicle cells and is responsible for nurse cell breakdown. Currently we are taking a proteomics approach to determine which proteins are secreted from the follicle cells to orchestrate nurse cell death. This work will further characterize the role of stretch follicle cells in nurse cell phagoptosis.

317 Actin cytoskeletal remodelling mediates activation of apoptosis-induced cell proliferation. L.J. Farrell

In multi-cellular organisms, stress-induced apoptotic cells frequently activate compensatory proliferation of neighbouring cells, a phenomenon termed apoptosis-induced proliferation (AiP) which is relevant to tissue regeneration and tumour development. Research in the past decade has discovered that apoptotic caspases, particularly the initiator caspase/caspase-9 orthologue Dronc, can also activate the c-Jun N-terminal kinases (JNK) pathway leading to release of mitogenic signals such as Wg, Dpp and Spi/EGF to stimulate proliferation of neighbouring cells. A recent study further revealed that extracellular reactive oxygen species (ROS) generated by the NADPH oxidase Duox in apoptotic cells attract hemocytes which in turn secret Eiger, the Drosophila homolog of TNFα, thus promoting activation of JNK non-cell autonomously. However, it is still not yet clear how Dronc activates ROS and JNK in apoptotic cells. Here, we demonstrate that accumulation of F-actin, a key component of the cytoskeleton, at the apical cortex of apoptotic cells is an early event activated by Dronc. Reduction of F-actin polymerisation via knockdown of LIM kinase 1 (LIMK1) or overexpression of Cofilin suppresses AiP. Further analyses
suggest that increase of ROS and JNK activity in apoptotic cells, essential steps leading to AiP, relies on F-actin polymerization. Therefore, actin cytoskeletal remodelling plays a key role in the process of AiP upstream of ROS production and JNK activation.

318 Use of fluorescent bacteria to probe the phagosomal environment in wild-type and mutant hemocytes. C. Harris, M. Roshandeh, C. Brennan Department of Biological Science, Cal State University Fullerton, Fullerton, CA.

Phagocytosis is an evolutionarily conserved process in which immune cells engulf microbes, first killing them, and then degrading them. Antimicrobial mechanisms in the phagosome include the production of reactive oxygen and nitrogen species, and degradation is accomplished by acidification of the phagosome and lysosomal delivery of acid-active hydrolytic enzymes including proteases and lipases. Bacteria expressing GFP have been widely used to characterize the rate of bacterial killing in wild-type and mutant hemocytes; GFP fluorescence is quenched by low pH, and persistence of GFP indicates a failure of phagosome acidification. Red fluorescent proteins (RFPs) are more acid-stable than GFP, and we have found that they are useful for characterizing phagosome maturation defects that are not related to pH, including accelerated killing by unrestrained ROS and/or RNS production. We additionally report our findings using bacteria expressing pHluorin, a GFP variant customized for pH sensing, and roGFP2, a redox-sensitive GFP variant. These fluorescent-protein-expressing bacteria are economical tools for probing the phagosomal microenvironment.

319 Determining the role of innate immunity in phagocytic defect-driven neurodegeneration. J. Elguero1, K. Tiemeyer1, J.I. Etchegaray1, M. Feany2, K. McCall1 1) Boston University, Boston, MA; 2) Harvard Medical School, Boston, MA.

In the course of normal development, neurons die and must be cleared. In Drosophila, this clearance is carried out primarily by glial cells and requires the phagocytic receptor Draper. We have previously shown that the absence of Draper results in the persistence of neuronal corpses in the brain. Additionally, Draper-deficient flies undergo age-dependent neurodegeneration. However, the mechanism underlying this neurodegeneration, as well as any causal relationship between these two phenotypes, remain to be uncovered. In other systems, persisting cell corpses have been shown to result in an immune response. In Drosophila, innate immune responses in the brain are known to induce neurodegeneration. We are thus determining whether the presence of cell corpses in Draper deficient flies induces an innate immune response, and whether this response mediates neurodegeneration.

320 Nubbin isoform antagonism governs intestinal immune homeostasis. B.G. Lindberg1, X. Tang1, W. Dantoft2, P. Gohel1, S. Seyedoleslami Esfahani1, J.M. Lindvall3, Y. Engström1 1) Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden; 2) Systems Immunity Research Institute, School of Medicine, Heath Park, Cardiff University, Cardiff, United Kingdom; 3) National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.

Gut immunity is regulated by intricate and dynamic mechanisms to ensure homeostasis despite a constantly changing microbial environment. Several regulatory factors have been described to participate in feedback responses to prevent aberrant immune activity. Little is, however, known about how transcriptional programs are directly tuned to efficiently adapt host gut tissues to the current microbiome.

The POU transcription factor Pdm1/nubbin, homologous to human Oct-1, has been studied extensively with regards to its critical functions during development. In adult flies, we have previously shown that nub is a repressor of gut immunity as mutants have a constitutively active immune response, altered gut microbiota and shortened lifespan. Its multifaceted roles are potentially further complicated by the existence of two encoded isoforms (Nub-PB and Nub-PD), of which only nub-RD has been studied previously.

In this work, we show that Nub isoforms antagonistically regulate gut immunity in Drosophila. Global transcriptional profiling revealed that Nub-PB is a strong transcriptional activator of a large set of immune genes in immunocompetent tissues including the gut. Further genetic analyses showed that Nub-PB is sufficient to drive expression both independently and in conjunction with nuclear factor kappa B (NF-κB), JNK and JAK/STAT pathways. Similar overexpression of Nub-PD did, conversely, repress expression of the same targets. Strikingly, isoform co-overexpression normalized immune gene transcription.

RNAi-mediated knockdown of individual nub transcripts in enterocytes demonstrated that both isoforms are necessary for homeostatic immune gene transcription in the midgut. Furthermore, enterocyte-specific expression levels of Nub-PB had a strong impact on gut bacterial load as well as host lifespan. Overexpression of this isoform promoted a proinflammatory signature in the midgut with activation of JNK and JAK/STAT pathways, increased apoptosis and stem cell proliferation. Moreover, these flies quickly succumbed to oral infection with Enwina carotovora carotovora 15 despite enhanced bacterial clearance and up to 1,000-fold increased expression of antimicrobial peptide genes. Our findings highlight a critical regulatory mechanism by antagonistic factor isoforms that ensures a properly tuned immune response in the midgut.
321  **Induction and inhibition of host immune responses to Kallithea Virus, a natural dsDNA virus of *D. melanogaster*.**  W.H. Palmer1, J. Joosten2, N. Medd3, G. Overheul2, R. Van Rij3, D.J. Obbard1  1) Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, GB; 2) Radboud Institute of Molecular Life Sciences, Nijmegen, Netherlands.

*Drosophila melanogaster* has played a pivotal role in the dissection of insect immune pathways, however few studies have explored fly immune responses to dsDNA viruses. Here, we take advantage of a newly isolated dsDNA nudivirus, Kallithea Virus, to characterise the *D. melanogaster* transcriptional response to dsDNA virus infection. We find differential expression of male-biased, chorion, cuticle, and immune genes, including broad downregulation of antimicrobial peptides. Antimicrobial peptide downregulation is likely due to virus-mediated inhibition of Nuclear factor kappa B (NF-kB) signalling, as a small scale screen for immune suppressors identified viral protein gp83 as a Kallithea suppressor of Toll signalling. In *Drosophila* cell culture, Kallithea Virus gp83 potently suppresses Toll signalling at the level of dorsal (dl) and Dorsal-related immunity factor (Dif), and at least partially acts through degradation of these NF-kB transcription factors. These results indirectly implicate NF-kB signalling in the antiviral immune response to DNA viruses.

322  **Defining the Transcriptional Program that Limits Enteric Viral Infections in Drosophila.**  E. Segrist, C. Sansone, J. Xu, B. Gold, S. Cherry  Department of Microbiology, University of Pennsylvania, Philadelphia, PA.

Enteric viral infections are widespread and the intestinal epithelium presents a high barrier to infection. The mechanisms that impact enteric anti-viral protection in insects are still largely unknown. We developed an oral model of enteric viral infection in the genetically tractable insect *Drosophila melanogaster*. We found that ERK signaling in intestinal enterocytes was protective, as loss of this signaling pathway led to increased susceptibility to diverse enteric viruses including Sindbis virus (SINV), a model arthropod-borne virus, suggesting that ERK signaling is broadly anti-viral. Moreover, we found that ERK signaling was activated by an antiviral cytokine, Pvf2, which was transcriptionally induced upon viral infection. Two signals were required for Pvf2 induction. First, peptidoglycan from gram negative commensals prime the transcription factor NF-kB that is necessary but not sufficient for Pvf2 expression. A second virus-induced signal is dependent on Cdk9, which allows for transcriptional pausing release, and is also required for Pvf2 induction. Since NF-kB activation was not sufficient to induce Pvf2 the identity of a virally induced, we reasoned that there may be a Cdk9 dependent transcription factor necessary for Pvf2 induction. By mining transcriptional profiling data, we identified candidate transcription factor variants involved in the activation of this innate immune response to enteric viral infection. These findings will be discussed.

323  **Drosophila crystal cells undergo pyroptosis to release pro-phenoloxidase at wound sites.**  A. Dzedziech1, R. Krautz2, Z. Wang1, M. Schmidt1, R. Markus1, U. Theopold1  1) Molecular Biosciences, Wenner-Gren Institute, Stockholm University, SE; 2) Gurdon Institute, Henry Wellcome Building of Cancer and Developmental Biology, UK.

Immune systems of all living organisms face the challenge of coping with sudden threats, including inflicted wounds, the infiltration of pathogens or internal tissue damage. Potent innate immune responses evolved as the first line of defense against sudden immune challenges in order to contain the incident on a local scale and prevent sepsis. A lack of a response would lead to autoimmune responses and jeopardize tissue homeostasis. The necessity for a localized, immediate, and intense immune response demands tight regulation. The melanization reaction in *Drosophila melanogaster* and its activation serves as a prototypical example answering these criteria. Here, we show that crystal cells, which deliver pro-phenoloxidase, the key enzyme for the melanization cascade, undergo a highly regulated mode of programmed cell death which bare striking resemblance to pyroptosis, described in the activation of immune cells in vertebrate immune systems. We identified all of the hallmark feature of pyroptosis in crystal cell rupture, including the restructuring of the cell cortex, nuclear fragmentation, swelling and rounding of the plasma membrane prior to rupture, and a dependence on caspase activity. The activation of crystal cells via caspsases allows for tight regulation of the melanization cascade as demonstrated by caspase inhibition resulting in diminished melanization of wounds. Crystal cell rupture in *Drosophila* provides a suitable model for further insights into the molecular sequence of events required for pyroptosis in immune cells.

324  **Lipid droplet dynamics during phagocytosis.**  A. Myers, E. Ogundipe, C. Brennan  Biological Science, California State University, Fullerton, Fullerton, CA.

Lipid droplets are organelles that enclose neutral lipids such as triacylglycerides and cholesterol esters, and are both nutrient storage depots and regulators of metabolic homeostasis. Within phagocytic cells including the mammalian macrophage and the *Drosophila* hemocyte, lipid droplets accumulate during diverse pathological conditions including obesity/atherogenesis and infection with intracellular pathogens such as *Mycobacteria*; such lipid-laden phagocytes are called “foam cells”. Following phagocytic engulfment, *Mycobacteria* block the normal maturation of the phagosome into a degradative organelle, and are thought to induce the accumulation of lipid droplets to serve as a nutrient source. These lipid droplets associate with the *Mycobacteria*-containing phagosome, and contribute to mycobacterial pathogenesis. However, the functions of lipid droplets during more typical phagocytosis, where the engulfed microbe is successfully killed, are not well-described. We have examined lipid droplet dynamics within larval hemocytes that phagocytose nonpathogenic bacteria such as *E. coli*, and find a transient accumulation of lipid droplets in the three hours following uptake. However, these lipid droplets were not observed in proximity of the phagosomes. Lipid droplet accumulation appears cell-autonomous: in a low
were more successful in obtaining mates. Given the unexpected results from the mating assays, we are now extracting CHCs
However, the opposite of our hypothesis has been observed; males initially infected with a higher concentration of
infected with a low, medium, or high dose of
bias toward infected or uninfected males. We hypothesized that an irregular CHC profile in infected males would decrease
These results are likely due to altered metabolic pathways in infected males, leading to variance in the production of mating
analysis suggest that the
infection on
coughing to subtle changes in chemical composition. Our project focuses on how infection alters sexual attractiveness, which
consider when investigating the human immune system in a complex environment. Invertebrates have become popular
the pathogen itself after spaceflight conditions. Being able to simulate spaceflight conditions in a controlled environment o
cultured in space, suggesting that not only do we need to consider host changes in susceptibility, but also adaptive changes
However, the common bacterial pathogen
immune systems with a high genetic similarity to humans.
models for studying human disease because they are cheap, highly amenable to experimental manip
natural variation in larval crystal cell number across the DGRP.  B. Tang1, L. Cardenas1, S. Lee1, R. Calero2, L.
The Drosophila hemolymph carries three major hemocyte cell types: plasmatocytes, lamellocytes and crystal cells. Crystal
cells (CC) express two prophenoloxidases, PPO1 and PPO2, which are activated by proteases to generate melanin in response to wounds, microbial infections and parasitization. In order to find new regulators of CC development, we measured CC counts in at least 10 wandering third instars (WL3) across 75 isogenized lines from the Drosophila Genetic Reference Panel (DGRP) collection. We activated CC melanization in the WL3 using a 10 min, 70° C heatshock and then scored the number of black CCs/WL3. The mean number of black CCs/WL3 varied across DGRP lines, ranging from 2.3 to 742.1. Compared to the OreR control line (mean of 20 CC/larvae), 30 of the 75 lines produced more CC (anova, \textit{Pseudomonas entomophila, Listeria monocytogenes, Providencia rettgeri} viral infections, except for susceptibility to \textit{D. melanogaster} sigma virus (DmelSV)). Both males and females from isolines exhibiting adult resistance to DmelSV have higher CC/W3L counts. \textit{pdom} and \textit{htl}. We are currently performing targeted misexpression studies using RNAi knockdown and cDNA overexpression to evaluate the contribution of these genes to CC abundance.

Effects of simulated microgravity on a host-pathogen system.  R. Gilbert, S. Bhattacharya  Space Biosciences, NASA Ames Research Center, Moffett Field, CA.
While it has been shown that decades of astronauts and cosmonauts can suffer from sporadic illnesses both during and after spaceflight, the underlying causes are still poorly understood, due in part to the fact that there are so many variables to consider when investigating the human immune system in a complex environment. Invertebrates have become popular models for studying human disease because they are cheap, highly amenable to experimental manipulation, and have innate immune systems with a high genetic similarity to humans. \textit{Drosophila melanogaster} has been shown to experience a dramatic shift in immune gene expression following spaceflight, but are still able to fight off infections when exposed to bacteria. However, the common bacterial pathogen \textit{Serratia marcescens} was shown to become more lethal to fruit flies after being cultured in space, suggesting that not only do we need to consider host changes in susceptibility, but also adaptive changes to the pathogen itself after spaceflight conditions. Being able to simulate spaceflight conditions in a controlled environment on the ground gives us the ability to evaluate how the microorganisms that cause immune disorders are being affected by these drastic environmental shifts. In this study, we use a ground-based simulated microgravity environment to examine the genetic changes associated with increased \textit{S. marcescens} virulence in order to understand how microgravity is affecting this pathogen, as well as how these genetic changes influence and interact with the host immune system. This study will provide us with more directed approaches to studying the effects of spaceflight on biological systems, with the ultimate goal of being able to counteract immune dysfunction in future space exploration.

Effect of Infection on Female Mating Bias and Male Cuticular Hydrocarbons in \textit{Drosophila melanogaster}.  M. McCarter, C. Filip, M. Chambers  Bucknell University, Lewisburg, PA.
Pathogenic infection results in a number of changes in living organisms, from obvious symptoms like sneezing and coughing to subtle changes in chemical composition. Our project focuses on how infection alters sexual attractiveness, which affects an organism's ability to successfully court a potential mate. We are investigating the effect of \textit{Providencia rettgeri} infection on \textit{Drosophila} sexual attractiveness behaviorally and chemically. Our initial experiments using gas chromatography analysis suggest that the cuticular hydrocarbon (CHC) profile of infected male flies is different from that of uninfected males. These results are likely due to altered metabolic pathways in infected males, leading to variance in the production of mating pheromones like CHCs (Chambers et al. 2012, Cobb and Jallon 1990). The behavioral part of our project studies female mating bias toward infected or uninfected males. We hypothesized that an irregular CHC profile in infected males would decrease their ability to mate as it would alert female flies of the infection. To test this, we performed a choice experiment with males infected with a low, medium, or high dose of \textit{P. rettgeri} competing against males injected with a sterile saline solution. However, the opposite of our hypothesis has been observed; males initially infected with a higher concentration of \textit{P. rettgeri} were more successful in obtaining mates. Given the unexpected results from the mating assays, we are now extracting CHCs
from males immediately following the mating, separating males based on infection status and mating success. This will directly allow us to connect chemical profiles with mating success. Preliminary GC data is consistent with the experiments completed independently of mating assays, with the highest infectious doses showing an increase in most detected CHCs. Future work will determine the identity of altered CHCs and determine whether it is the increase in a specific CHC or all CHCs coordinately that can explain the change in mating success.

328 Impact of Chronic Infection on Tolerance and Resistance in Drosophila melanogaster. F. P. Satriale, M. Chambers. Bucknell University, Lewisburg, PA.

Infectious microbes are often studied in the context of pathogenesis, or generation of disease and damage, but this is not their only role. Microbes live commensally in and on multicellular organisms, and these organisms play a significant role in the immune function, behavior, and metabolism of their hosts. Specifically, commensal microbes can augment immunity to future infection by pathogenic microbes, and our lab has found that this protective effect is also conferred by microbes lingering from a previous pathogenic infection.

This protection is likely conferred due to either increased tolerance or resistance to pathogens during secondary infection. Resistance to infection is a measure of how well the host inhibits bacterial growth, and tolerance is a measure of how well the host survives while carrying the pathogen. To distinguish between resistance and tolerance, we simultaneously monitored both survival and bacterial load across different combinations of microbes, as we predict that the mechanism may depend on the specific pairing of pathogens. Among the many species of bacteria that can infect Drosophila melanogaster, we selected Providencia rettgeri, Serratia marcescens, and Enterococcus faecalis because they are natural pathogens of the fly, can establish chronic infection, and each have unique cellular characteristics. Bacterial load was assessed using both selective culture methods and qPCR.

Preliminary results at 10 hours post-secondary infection suggest that the protective benefit is due to increased resistance for most combinations of chronic and secondary infection. Future experiments will better resolve the impact of chronic infection on resistance and tolerance by examining select pairs of microbes across a range of infectious doses and across a time-series. This will provide a more nuanced view of how chronic infection impacts secondary infection and will guide future mechanistic studies.

329 Enterotoxigenic E. coli heat-stable toxin disrupts trafficking to intercellular junctions. C Sera, R Schwartz, A Guichard, E Bier. Division of Biology, University of California, San Diego, La Jolla, CA.

Enterotoxigenic E. coli (ETEC) is estimated to cause over 200 million cases of diarrhea cases each year. Symptoms are caused by either of two secreted toxins: a heat-labile toxin (LT) – functionally almost identical to Cholera toxin (Ctx) – and/or a heat-stable toxin (ST). Our goal is to analyze the activity of ST using a transgenic Drosophila model and evaluate its potential synergism with LT. ST is a small peptide that activates Guanylate Cyclase C receptor (Gyc76C), causing an overproduction of cGMP. Subsequent activation of protein kinase G-II (PKGII) leads to phosphorylation and activation of chloride channel cystic fibrosis transmembrane conductance regulator (CFTR). Pathogenic upregulation of CGMP thus causes unregulated passage of chloride ions across the membrane and into the intestinal lumen, resulting in effusion of Na+ ions and water and leading to diarrhea. Whether ST also affects the intestinal epithelium is unknown.

To mimic the effect of ST, we made use of available UAS-Gyc (wt and activated*) lines. When expressed in the mid-gut, Gyc* caused physiological effects analogous to ETEC infection in mammals: mortality, epithelium thinning, and diarrhea-like symptoms as assayed by increased volume of watery feces. Further analysis revealed that GC-C* also caused loss of microvilli and abolished localization of alpha-catenin to cell-cell junctions. Additionally, levels and distribution of Rab11 and effector Sec15 were dramatically altered by Gyc*, further indicating a disruption of exocyst-mediated trafficking normally required for junctional integrity. When co-expressed, Ctx and wild-type GC-C produced synergistic thinning of the gut epithelium and also induced synergistic loss of microvilli. We are currently testing other parameters such as Rab11 distribution, fecal output, and mortality.

Overall, our experiments demonstrate a previously unappreciated mechanistic convergence between ST and Ctx on Rab11 inhibition, likely to contribute to infection symptoms.

331 Bacterial interactions drive protection against antibiotics in the fly gut. Andres Aranda-Diaz1, William Ludington2, Kerwyn Casey Huang1,3 1) Department of Bioengineering, Stanford University, Stanford, CA; 2) Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA; 3) Department of Microbiology and Immunology, Stanford University, Stanford, CA.
Symbiotic bacteria have positive and negative effects on host physiology. Efficacious antibiotic treatments should kill pathogenic bacteria, prevent the rise of resistant mutants and protect bacteria that benefit host health. Although the effect of antibiotics on bacteria has been studied for a long time, we still cannot predict their consequences on bacterial communities in complex environments like the host body. Because of its low complexity and modularity, the D. melanogaster gut microbiota provides a unique opportunity to dissect interactions between bacteria and their microenvironment and to study the effects of bacterial community dynamics and interactions with the host on antibiotic efficacy and resistance evolution.

One understudied factor that can affect antibiotics action is that of interspecies interactions. To systematically probe the effects of microbe-microbe interactions on antibiotics we performed in vitro co-culturing experiments with bacteria from the main two genera of the gut microbiota, Lactobacillus and Acetobacter. Using the RNA-polymerase inhibitor rifampin as a case study, we observed a protective effect of Acetobacter on Lactobacillus survival under rifampin treatment. We show that this protective effect can be attributed to their metabolic interactions. Understanding low-order interactions will enable predictions of growth and the effects of antibiotics in more complex communities and within the host.

Compared to in vitro cultures, the fly gut enables higher bacterial diversity to persist over time. We are using population-level growth assays and high resolution single cell microscopy at the whole gut scale to explore how this high diversity persists. Using this system, we assess the role of host microenvironments on bacterial interactions and their impact on antibiotic efficacy. Mechanisms governing resistance evolution in complex environments will help design more efficacious therapies to improve host health.

332 Microbiota-induced expression of OBP28a alters Drosophila systemic immune responses. R.C. Dziedzic, N.A. Broderick Molecular and Cell Biology, University of Connecticut, Storrs, CT.

Odorant binding proteins (OBPs) localize to the aqueous medium surrounding the olfactory neurons in adult Drosophila melanogaster and are believed to transport hydrophobic odorants to odor receptors. Unique among this class of proteins, OBP28a is also expressed in larval olfactory organs and was recently shown to not be required for olfactory responses. We recently reported that OBP28a and OBp6, its orthologue in tsetse flies (Glossina spp.), play a role in crystal cell abundance and the subsequent melanization response of tsetse and Drosophila. In both hosts, Obp expression is regulated by the presence of microbial symbionts during larval development. Here, we report on the expression pattern of OBP28a in larvae and adults. We show that flies reared in the absence of their gut microbiota (germ-free) express lower levels of OBP28a at both larval and adult stages, and this reduced expression is associated with increased susceptibility to systemic infection. The increased death is likely due to an altered melanization response that results from inefficient activation of the prophenoloxidase cascade. This can have direct effects on microbial pathogens or may affect the production of reactive oxygen species. Finally, I will describe efforts to screen members of the Drosophila microbiota and environmental bacteria for the ability to rescue OBP28a expression in germ-free larvae and their downstream impacts on crystal cell development and phenoloxidase production.

334 The uneven playing field of the gut microbiome: early colonizers dominate late arrivals. B. Obadia, V. Zhang, W. Ludington Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

The complex and highly variable gut microbiome can profoundly influence host health and fitness. Composition of the adult gut is highly correlated with early life events; however, we know little about the factors and conditions shaping the specific assembly and stability of a given microbial community. The Drosophila gut microbiome allows for the experimental reconstruction of bacterial communities within hosts guts due to its low complexity, tractability, and the ease of making germ-free flies. We sought to understand how the temporal order of species arrival during communities’ assembly could affect final microbiome species composition, an ecological phenomenon known as priority effects. Prior colonizers can impact colonization by later arrivals by either habitat creation (increase) or through competitive habitat exclusion (decrease). We fed germ-free and pre-colonized flies with precise doses of different paired bacterial species (Acetobacter and Lactobacillus spp.) and we recorded the qualitative (presence/absence) and quantitative (bacterial loads and growth rates) effects of prior and ordered colonizations on the final composition of communities. We also measured growth of the bacterial pairs in vitro to differentiate simple biochemical interactions from the complex gut environment.

Interestingly, we found that prior colonization of some endogenous bacteria promotes the colonization of some non-endogenous species. In other words, depending on the species already present, an invader can have a better colonization efficiency in pre-colonized flies than in germ-free flies. Moreover, the order in which the species were fed to the flies was key in regard to the type of interaction generated within the community (e.g., when species A then B are fed, their interaction is neutral; whereas when species B then A are fed, their interaction is competitive). Specifically, we found this relationship for certain pairs of Acetobacter sp. and Lactobacillus sp. that promote one another’s growth in vitro but can inhibit one another’s colonization in vivo. Imaging the gut at the single cell level suggests that these biochemically cooperating species which aid the host when they co-colonize nonetheless exclude one another in spatial competition, adding to the complexity of
mediated JNK activation drives epithelial cell elimination in the physiological context. To analyze JNK-mediated cell elimination mechanisms, we made use of flies heterozygously mutant for the JNK phosphatase puckered (puc), a transcriptional target of JNK signaling that directly inactivates JNK, thus forming a negative feedback loop of the pathway. puc+/+ mutant cells would thus possess sensitized JNK signaling. Indeed, the number of naturally occurring cell death was significantly increased in puc+/+ wing discs. As reported previously, blocking cell death by the effector caspase inhibitor p35 in puc+/+ wing disc resulted in strong JNK activation throughout the tissue, suggesting that JNK-activated cells are normally eliminated from the tissue in an efficient manner. Mechanistically, we found that Eiger/Grindelwald and Dronc-mediated amplification of JNK signaling occurs in such cells. Our data suggest that Eiger-Dronc-mediated JNK activation drives epithelial cell elimination in the physiological context.

335 Specificity in the early gut microbiome: species- and strain-level effects on development and their long-term fitness consequences.  A. Gould, V. Zhang, W. Ludington  Department of Molecular & Cell Biology, UC Berkeley, Berkeley, CA.

The early microbiome has critical effects on development that can result in long-term fitness consequences for a host including the activation and development of the immune system and the production of certain neurotransmitters that affect behavior and cognition. How the presence or absence of distinct species of gut bacteria affect development is not well understood and can be challenging to dissect within such highly complex communities. Taking advantage of the naturally simple gut community of Drosophila melanogaster, we used gnotobiotic flies to determine the effects of specific species and strains of bacteria on the development and fitness of the host. Results indicate that Acetobacter orientalis has an overall positive effect on its host, increasing development rate and resulting in more fit individuals. In contrast, Lactobacillus brevis has a strain-specific effect on the host; one strain isolated from wild flies (Lb.) slightly increases development rate relative to germ-free flies, whereas another strain isolated from laboratory flies (Lb) decreases development rate and produces smaller, less fit individuals. Interestingly, the negative effects on development induced by the presence of Lb can be readily overcome by the introduction of A. orientalis within the first two days of development. Lastly, we observed a fitness tradeoff induced by the early microbiome: individuals that develop quicker have higher lifetime fecundity and a shorter lifespan, while those that develop slower have lower fecundity and longer lifespans. This study highlights the importance of species and even strain specificity in the early microbiome for development and the resulting long-term fitness consequences.

336 Absorbing epithelium of the midgut as the model to study toxicity mechanisms of the heavy metal cadmium.  Olivia Brooks, Brandi Huggins, Hannah Stratton, Anton Bryantsev  Molecular & Cellular Biology Department, Kennesaw State University, Kennesaw, GA.

Cadmium (Cd) is a toxic heavy metal and environmental pollutant. Ever increasing exposure to Cd poses a health concern, because in humans Cd accumulates in kidneys and causes damage to the absorbing epithelium of proximal renal tubules. We used Drosophila as a model to study the mechanisms of Cd toxicity in the setting of absorbing cells of the midgut (enterocytes).

Chronic exposure of flies to 1mM Cd in the food significantly shortened their lifespan and affected midgut morphology. In the midgut of Cd-exposed flies, we detected an increase in the expression of the esg reporter (esg>GFP). Normally, esg>GFP is expressed in solitary intestinal stem cells, but in response to Cd exposure, its expression expanded over enterocyte clusters, each containing 4 and more cells. This change in esg>GFP signal indicates increased proliferation of midgut stem cells, which might be triggered by Cd-induced cellular damage to enterocytes. Consistently with this note, experimental overexpression of the anti-apoptotic factor Diap1 in enterocytes significantly increased fly survivorship across various Cd concentrations. We conclude that midgut enterocytes are the critical tissue target that determines overall tolerance to Cd toxicity in flies.

We used the enterocyte model to further gain insight into the mechanisms of Cd toxicity. Specifically, we addressed whether Cd induces oxidative stress in midgut enterocytes. Genetic ROS sensor, GstD1-GFP, had variable activity in the midguts of control and Cd-exposed flies, but could not reveal elevated oxidative stress in response to Cd. Overexpression of the master transcriptional regulator of anti-oxidative stress response, cnc, did not improve the survivorship of flies kept on Cd-containing food. Finally, overexpression of individual anti-oxidative enzymes Sod2 and Cat also did not improve the survivorship of Cd-exposed flies. Based on these results, we conclude that oxidative stress does not play a major role in Cd-induced toxicity of absorbing epithelial cells.

337 Dissecting JNK-mediated cell elimination in Drosophila.  M. Nakamura, T Igaki  Laboratory of Genetics, Graduate School of Biostudies, Kyoto, Kyoto, JP.

Activation of c-Jun N-terminal kinase (JNK) plays a crucial role in removing aberrant or unfit cells from epithelia, such as through cell competition. However, it still remains unclear whether and how JNK-mediated cell elimination occurs in the physiological context. To analyze JNK-mediated cell elimination mechanisms, we made use of flies heterozygously mutant for the JNK phosphatase puckered (puc), a transcriptional target of JNK signaling that directly inactivates JNK, thus forming a negative feedback loop of the pathway. puc+/+ mutant cells would thus possess sensitized JNK signaling. Indeed, the number of naturally occurring cell death was significantly increased in puc+/+ wing discs. As reported previously, blocking cell death by the effector caspase inhibitor p35 in puc+/+ wing disc resulted in strong JNK activation throughout the tissue, suggesting that JNK-activated cells are normally eliminated from the tissue in an efficient manner. Mechanistically, we found that Eiger/Grindelwald and Dronc-mediated amplification of JNK signaling occurs in such cells. Our data suggest that Eiger-Dronc-mediated JNK activation drives epithelial cell elimination in the physiological context.
Investigating neurodegeneration that arises from defective glial phagocytosis.  
K. Tiemeyer¹, J. Elguero¹, S. Jarmale², J.I. Etchegaray¹, M. Feany³, K. McCall¹  
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Cell death and subsequent phagocytic clearance are integral processes for proper development and maintenance of tissue homeostasis. In the brain, glia are responsible for the clearance and degradation of apoptotic neuronal cell bodies through phagocytosis. We have previously shown in Drosophila that the absence of Draper, a trans-membrane phagocytic receptor, results in an accumulation of developmental neuronal cell corpses that persist in an unchanging quantity throughout adulthood. Furthermore, absence of Draper ultimately leads to age-dependent neurodegeneration. However, the mechanism by which phagocytic defects induce degeneration of neural tissue has yet to be elucidated. In order to more fully explore the neurodegenerative phenotype and determine its origin, we are screening for antigens that change in accordance with the adult-onset neurodegeneration observed in the draper mutant. We aim to use these antigens to assess neurodegeneration and determine if the phagocytic defect-driven neurodegeneration is a consequence of persisting developmental corpses or of dysfunctional glial phagocytosis.

The role of FOXO during hypoxia in Drosophila.  
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Animals often live in conditions where environmental oxygen levels fluctuate. When oxygen is abundant, growth is promoted, but when oxygen is scarce, metabolic processes are altered to limit growth and promote survival. One important mechanism controlling cellular responses in hypoxia is the regulation of metabolic gene expression. In this context, the conserved hypoxia-inducible factor (HIF) has been established as a transcriptional regulator of genes important for hypoxia responses in different model organisms. However, less is known about other transcription factors important in hypoxia adaptation. We have explored this question in Drosophila.

Drosophila larvae can tolerate low oxygen conditions. At 5% oxygen, larvae slow their growth and development, but show little effect on overall viability. At 1% oxygen, larvae can survive for up to 12 hours. We find that one factor that is induced under these conditions is the Forkhead Box O (FOXO) transcription factor. FOXO has been shown to be important for regulating starvation and stress responses in Drosophila and Caenorhabditis elegans. We find that, upon switching larvae from normoxia to hypoxia, FOXO is rapidly relocalized from the cytoplasm to the nucleus in larval tissues. These tissues include the fat body and the intestine, and FOXO relocalization occurs even though animals maintain normal feeding. We also see a subsequent increase in expression of known FOXO target genes. One key regulator of FOXO is the insulin/PI3 kinase pathway, and we find that overexpression of PI3K can prevent hypoxia-induced nuclear relocalization of FOXO. Finally, we also find that foxo mutants show increased lethality in hypoxia compared to wild type animals. These data suggest that FOXO is a hypoxia-regulated transcription factor. We are currently examining how FOXO is regulated in hypoxia, whether it is required in specific cells/tissues for hypoxia tolerance, and what downstream targets may be important for its effects.

Translational control of stress responses in Drosophila.  
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During development, various environmental signals such as nutrients, lack of oxygen, temperature, infection stress affect proper growth and homeostatic balance of an organism. In order to respond to these signals, the organism alters its metabolism, the exact underlying mechanisms of which are unknown. Recent studies have highlighted the requirement for tightly regulated mRNA translation in response to various stress cues. Using Drosophila as a model system, our lab identified RNA polymerase III (Pol III)-dependent tRNA synthesis as mechanism to stimulate mRNA translation. In particular, we found that simply increasing tRNA synthesis was sufficient to enhance tissue and body growth. These results raise the possibility that translational control by regulating tRNA synthesis may play a significant role in response to environmental stress. We are investigating this using the Drosophila intestine as a model. This tissue is composed of terminally differentiated epithelial cells (ECs) that are the absorptive and barrier cells of the adult gut. Interspersed among the ECs are small intestinal stem cells (ISCs). The ISCs divide throughout adult life in order to generate new EC cells and maintain gut self-renewal in response to infection and damage. We found that oral infection increased tRNA synthesis in the gut, and we showed Pol III was required for stress-mediated stem cell proliferation. The EGFR/Ras pathway is a key regulator of ISC proliferation and we found that this pathway stimulates tRNA synthesis in the intestine and requires Pol III for ISC proliferation. We are currently investigating how tRNA synthesis is regulated in response to intestinal stress and whether changes in tRNA synthesis cooperate with other translational mechanisms to control stem cell behaviour.

Flies in a MinE: metabolomics in a particle physics lab to improve the mining workplace.  
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Underground mining is a physically demanding occupation in a physiologically stressful environment; can fundamental fly research make it safer? We are using the techniques and tools that we are developing in basic research in to the connections between genetics, environment, and metabolism in *Drosophila melanogaster* to address the applied question of how to make mining a safer occupation. In the FLIES in A MinE project, FLAME, we are quantifying the biology of working deep underground with an ultimate goal of improving worker health and Safety. FLAME is based in SNOLAB, a clean-lab facility located 2km underground in the Creighton Mine (a clean lab within a working mine) in Northern Ontario. SNOLAB is a world leader in subatomic physics research, and the location of the 2015 Nobel Prize in Physics. We use the SNOLAB environment to simulate working deep underground and quantify the impact of this working environment on fly behavior, gene expression, and metabolism. Two kilometers underground, atmospheric pressure is >20% higher than at the surface. This high pressure is one of the environmental features making underground mining physically taxing. A single day exposure to SNOLAB leads to a substantial change in metabolism in the fly, 10% of the roughly 2000 metabolites that we follow change from a single trip underground, likely a function of the elevated pressure. We are currently working to understand the metabolic pathways that are responding to the SNOLAB exposure. As we understand what the changes are, we will be able to make suggestions, e.g. simple changes in diet, that will reduce the negative effects of working deep underground and create a healthier mining workplace.

342  **A phenotypic landscape of mechanisms underlying resistance to multiple stressors.**  *Hiroshi Nishida, Mihoko Katayam, Sakan Yoo RIKEN, Kobe, JP.*

Organisms are constantly exposed to diverse stresses and respond to them to maintain a homeostatic state. Although a set of similar genes can be induced by different stressors, whether animals use the same or different mechanisms to deal with different types of stressors remains elusive. To address this question, we investigated responses of a library of mutant stocks to multiple stressors: aging, obesity, cancer, starvation and oxidative stress. For this purpose we generated 968 mutant stocks by inducing random mutations with EMS. Up to the present, we finished characterizing all stocks on responses to oncogenic stress and are in the process of completing characterization on other stressors. We are analyzing correlation among resistances to different stressors. Correlation among specific stresses was observed. We also isolated several mutants that demonstrate particularly strong resistance to single or multiple stressors. We plan to determine the genes that are responsible for the resistance by whole genome sequencing. This work provides a landscape of mechanisms by which animals resist to different stressors and also specific genes responsible for them.

343  **How does Wolbachia infection affect lifespan in *Drosophila melanogaster*?**  *J.D. Parker, M. Valentine, F. Capobianco III, S. Nandkumar, Bio 490 student group B Dept of Biological Sciences, SUNY Plattsburgh, Plattsburgh, NY.*

It is well known that the rate of aging varies among individuals according to genetic background. Another source of variation known to effect aging is infection with microorganisms and symbiotic bacterium. We previously found that genetic background can have an influence on the interaction of lifespan and a symbiotic infection (Wolbachia) in *Drosophila melanogaster*. An undergraduate group in Investigative Biology Experience (Bio 490) will be presenting their work extending these previous findings by testing for differences in oxidative damage accumulation and autophagy gene activation in the presence, and absence, of Wolbachia in these lines with lifespan differences.

344  **A transcriptional switch during oxidative stress of p38 MAP Kinases revealed through species comparisons.**  *K. Wildman1, S. Ryan23, N. Mortimer1, A. Vrailas-Mortimer1,2 1) Biological Sciences, Illinois State University, Normal, IL; 2) Biological Sciences, University of Denver, Denver, CO; 3) Chemical and Biological Engineering, Colorado School of Mines, Golden, CO.*

The highly conserved MAP Kinases, ERK, JNK, and p38K, play an important role in a variety of cellular processes from differentiation to apoptosis. In *Drosophila melanogaster*, there are three p38K genes: p38Ka, p38Kb, and p38Kc. Upon sequence analysis, we find p38Ka and p38Kb are 78% identical and have an overall similarity of 92%, while p38Kc has accumulated a number of differences that affect critical residues in the TGY motif and kinase domain that are necessary for canonical p38K function. In addition, the p38K genes have distinct roles in the fly but there is also some redundancy between p38Ka and p38Kb. In order to better understand these differences, we have analyzed the evolution of the p38K genes across the sequenced fly species. The p38Ka and p38Kb genes are found across all *Drosophila* species, however p38Kc appears to have arisen during the split between the Willistoni and Obscura groups. Interestingly, *D. psuedoobscura* has unique features with a truncated p38Kc gene and a second p38Kb gene. Looking at a ratio of non synonymous changes to synonymous changes, we find that all three p38K genes are under purifying selection, though p38Kc is under weaker purifying selection than both p38Ka and b. To further explore the differences between p38Ka and p38Kb, we compared the 1kb upstream region of each gene across multiple Drosophila species. This comparison identified three transcription factor binding sites for the p38Ka gene which were found to be highly conserved across species. Two of these binding sites are of different isofoms of Lola and the third is a homeobox binding site. For p38Kb, we identified a predicted AP-1 binding site and binding sites for two different Lola isoforms as well. Since we have previously shown that p38Kb is a regulator of the oxidative stress response in flies, we were interested in how Lola and AP-1 might be regulating p38Kb as Lola has also been previously linked to oxidative
stress. Upon qPCR analysis, we have determined that AP-1 regulates p38Kb expression under normal conditions and lola PT acts under oxidative stress conditions. Currently, we are performing survival assays to determine how well the Lola and AP-1 flies survive under oxidative conditions.

345 The control of fat storage by splicing factors in *Drosophila*. R. Bennick¹, A. Nagengast², J. DiAngelo¹ 1) Pennsylvania State University, Berks Campus, Reading, PA; 2) Widener University, Chester, PA.

In Western societies where food is abundant, these excess nutrients are stored as fats mainly in adipose tissue. Fats are stored in structures known as lipid droplets, and a genome-wide screen performed in *Drosophila* cells has identified several genes that are important for the formation of these droplets. One group of genes found during this screen included those that regulate mRNA splicing. Previous work from our lab has identified some splicing factors that play a role in regulating fat storage; however, the full complement of splicing proteins that regulate lipid metabolism is still unknown. In this study, the levels of RSF1, RBP1, RBP1-like, SF2 and SRP54 were decreased using RNAi in the adult fat body to assess their role in the control of *Drosophila* metabolism. Decreasing SF2 and RBP1 showed increased triglycerides, while inducing RNAi towards RSF1, SRP-S4 and RBP1-like had no effect on triglycerides. Interestingly, SF2-RNAi flies survived longer when starved, but RBP1-RNAi did not, suggesting that the triglyceride storage and starvation resistance were regulated differentially by SF2 and RBP1. To determine whether the triglyceride phenotype and starvation resistance were regulated differentially by SF2 and RBP1, we measured the expression of the beta-oxidation enzyme CPT1, which has been shown to be alternatively spliced into two major variants, one of which is more catalytically active than the other. In control flies, we observed higher levels of the more catalytically active CPT1 variant, while SF2-RNAi flies had no difference in the amount of either variant. This suggests that there is less CPT1 activity in flies with decreased SF2 in their fat bodies potentially promoting the increased triglycerides in these animals. Together, this study identifies novel splicing factors responsible for the regulation of lipid storage in the *Drosophila* fat body and contributes to our understanding of the mechanisms which influence the regulation of fat storage in adipose-like cells.

346 Examination of the competition between the SR proteins 9G8 and RSF1 in the alternative splicing of CPT1 in lipid metabolism. B. Borokhovsky¹, A. Aradhya², A. Nagengast¹ 1) Biochemistry, Widener University, Chester, PA; 2) Computer Science, Widener University, Chester, PA.

The method of gene regulation underlying lipid storage related to obesity is poorly understood, yet alternative splicing (AS) appears to be an important mechanism for proper lipid storage. CPT1 (carnitine palmitoyltransferase I) is a beta-oxidation enzyme involved in the breakdown of fatty acids. The gene coding for CPT1 is alternatively spliced by the SR protein 9G8 to produce two different products that vary in their activity. A linear search algorithm was developed that parsed through FASTA files of the CPT1 gene region and sought out sequences that matched known binding sequences of 9G8. We expected a result in exon 6A that would signal its inclusion but unexpectedly found a match in exon 5 of the CPT1 transcript. We theorize that 9G8 and the SR protein competitor RSF1, act in an antagonistic nature with one another for binding sites on the CPT1 gene to result in different isoforms. Alternatively, 9G8 and RSF1 could compete for access to the SR protein transporter TRN-SR shuttle which transports activated SR proteins into the nucleus for splicing activity. We are using qPCR to determine if there is a difference in the AS of CPT1 among flies with decreased expression of 9G8, the SR protein antagonist RSF1 and TRN-SR. Additionally, triglyceride (TG) assays are being conducted to determine if there is a change in TG levels among the 9G8, RSF1, and TRN knockdown flies.

347 The regulation of triglyceride storage by Ornithine decarboxylase (Odc1) in *Drosophila*. A. Fruin, K. Leon, S. Nowotarski, J. DiAngelo  Penn State Berks, Reading, PA.

Polyamines are low molecular weight, organic cations that play a critical role in many major cellular processes including cell cycle regulation and apoptosis, cellular division and tissue proliferation, and cellular differentiation; however, the functions of polyamines in regulating the storage of metabolic fuels such as triglycerides and glycogen is poorly understood. To address this question, we focused on the *Drosophila* homologs of ornithine decarboxylase (Odc1), the first step in the synthesis of polyamines, as well as Odc1’s inhibitor, ornithine decarboxylase antizyme (Oda). Mutants in both Odc1 and Oda are lethal, but heterozygotes are viable to adulthood. By eye, both Odc1 and Oda heterozygotes looked larger than their background control flies and consistent with this observation, we expected a result in the alternative splicing of CPT1 in *Drosophila* fat body. Interestingly, Odc1 heterozygous flies have augmented triglyceride storage, while Oda heterozygous flies displayed no differences in the storage of macromolecules. This lipid phenotype in the Odc1 heterozygotes is due to increased triglyceride storage per cell in addition to an increase in the number of fat cells. These results provide a link between the expression of Odc1 and triglyceride storage and may have implications in understanding the role of polyamine enzymes in regulating lipid metabolism.
348 The gene alphan shepard (shep) regulates organismal energy homeostasis in multiple metabolic organs. C. Gillette, K. Hazegh, T. Reis Division of Endocrinology, Metabolism, and Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO.

Metabolism is an integrated, multi-organ process, and is best studied within the context of the whole organism. Mounting evidence points to an important yet poorly understood role for genetic background in the control of organismal fat levels. We previously used an unbiased genetic screen to identify 66 genes that when mutated increase body fat in Drosophila larvae. One such gene, the RNA-binding protein alphan shepard (shep), has no characterized role in metabolism. We used tissue-specific RNAi to determine in which organs shep is required for regulation of organismal fat. We find that, whereas knockdown of shep in the brain phenocopies the high-fat phenotype of the mutant, knockdown in the fat body results in a lean phenotype. In both cases food consumption is unchanged, but shep knockdown in the brain decreases locomotor activity, providing a possible mechanistic explanation for the excess fat stores. We further find that Shep transcript and protein levels are regulated in a nutrient-dependent manner. Our work is now focused on determining Shep's role in different organs in regulating overall organismal energy metabolism.

349 Role of Chloride intracellular channels in the aging heart. S. Gururaja Rao1, K. Shah1, Beverly Reyes1, Piotr Bednarczyk2, Adam Szewczyk3, Elisabeth Van Bockstaele1, Soichi Tanda2, Mark Berryman2, Harpreet Singh1 1) Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA; 2) Department of Biomedical Sciences, Ohio University; 3) Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw.

Chloride intracellular channels (CLICs) are not found on the plasma membranes but on the intracellular organelle membranes. They are shown to be playing important roles in processes such as tumorigenesis and delaying apoptosis in cancer. CLIC4, the founding member of this family is known to be on the mitochondria but its physiological roles are not yet characterized. Drosophila melanogaster has one CLIC gene unlike mammalian systems that contain 6 paralogues. We set on to study CLIC in Drosophila focusing on the aging heart and cardioprotection and observed an intriguing result that the CLIC null flies show extremely shortened life span with accelerated aging phenotypes, but at the same time show cardioprotection during ischemic stress. We have also identified members of chloride intracellular channel (CLIC) proteins to be located in cardiac mitochondria in Drosophila as well as rodents. Our electrophysiological data reveal that they are functional ion channels in the mitochondria. CLIC mutant mitochondria show structural abnormalities with age, increased reactive oxygen species production and while the mutant animals are extremely susceptible to oxidative stress. Our results indicate that CLICs are involved in mechanisms protecting the aging heart from ischemia-reperfusion injury via the regulation of mitochondrial structural and functional integrity.

350 Downregulation of mTOR requires cysteine degradation and anaplerosis. P. Jouandin, A. Parkhitko, N. Perrimon Genetics, Harvard medical school, Boston, MA.

Organisms must optimize growth by balancing nutrient consumption and supply. This process involves the TORC1 (Target of Rapamycin Complex 1) pathway that uses a lysosomal nutrient sensor to integrate the availability of amino acids to control anabolism. In parallel, anabolism requires the mitochondrial tricarboxylic acid (TCA) cycle that provides the cells with carbons for the production of biomass. When nutrients become scarce, TORC1 is downregulated to adjust growth by slowing down anabolism and nutrient consumption. In addition, TCA cycle intermediates are extracted to maintain sufficient growth, which in turn challenges the cycle homeostasis. To compensate for this loss, specific metabolites enter the mitochondria to replenish the TCA cycle intermediates, a process termed anaplerosis. However, how TORC1 and anaplerotic metabolites coordinate their response to nutrient limitation is unclear. Here, we demonstrate that, upon starvation, cysteine anaplerosis controls the downregulation of TORC1 in the Drosophila fat body, an organ homolog to the liver and adipose tissue in mammals. We provide evidence that cysteine fuels the TCA cycle in the form of pyruvate, and that the cycle intermediate fumarate is necessary and sufficient to suppress TORC1 activity. Our work reveals that downregulation of TORC1 upon starvation is a process actively controlled by cysteine anaplerosis, and highlights an atypical growth-suppressive function of the degradation of an amino acid. Therefore, we demonstrate that a single amino acid mediates a crosstalk between cellular compartments to orchestrate a robust metabolic response to variations in diet at the organ level.

351 The Regulation of Lipid Metabolism by Heterogeneous Nuclear Ribonucleoproteins (hnRNPs) in Drosophila. J. Kanaskie1, J. Bhogal1, A. Nagengast2, J. DiAngelo1 1) Penn State Berks, Reading, PA; 2) Widener University, Chester, PA.

The storage of excess nutrients as triglycerides is essential for all organisms to survive when food is scarce; however, metabolic diseases may arise when triglyceride storage is altered. Yet, the mechanisms by which triglycerides are stored are not completely understood. Previous genome-wide RNAi screens in cultured cells have identified genes that are important in the regulation of triglyceride storage. One group of genes identified in these screens that our lab is interested in is those involved in mRNA splicing. Our lab has identified a number of splicing factors important for regulating triglyceride metabolism; however, the full complement of splicing proteins involved in achieving metabolic homeostasis is unknown. Heterogeneous nuclear ribonucleoproteins (hnRNPs), RNA binding proteins that inhibit the splicing of introns by preventing the assembly of splicing complexes, have no established metabolic functions. In this study, we identified specific
hnRNPs that have an effect on triglyceride storage when their levels are decreased. We knocked down hnRNP-K, glorund (glo), Hrb27C, Hrb98DE, and Hrb87F specifically in the adult fat body by inducing RNAi through the GAL4/UAS system. Interestingly, knockdown of hnRNP-K and glo showed a decrease in triglyceride levels, whereas inducing RNAi toward Hrb27C and Hrb98DE displayed an increase in triglyceride levels. A knock down of Hrb87F had no effect on triglycerides. In addition, the triglyceride phenotype in the Hrb27C-RNAi flies resulted from an increase in the number of cells per fat body as well as the amount of triglyceride stored per cell. Together, these results suggest that the hnRNP family of splicing factors have varying metabolic functions and may act on different metabolic genes to control their expression and processing.

Cancer cells proliferate rapidly by using an alternative metabolic system called aerobic glycolysis. Aerobic glycolysis, also known as the Warburg effect, uses carbons from carbohydrate metabolism to synthesize the proteins, lipids, and nucleotides needed for generating substantial amounts of biomass; however, the mechanism that regulates the switch to aerobic glycolysis remains poorly understood. Understanding how aerobic glycolysis and other metabolic pathways are regulated holds promise for development of new cancer treatments. Previous research has shown that the fruit fly Drosophila melanogaster also utilizes aerobic glycolysis to support rapid growth in larvae. Moreover, the onset of aerobic glycolysis in the fly is controlled by the Drosophila estrogen-related receptor (dERR), which encodes the only fly ortholog of the ERR nuclear receptor family. In order to further explore how dERR regulates biosynthesis and carbohydrate metabolism in the context of cell growth, we examined the role of this nuclear receptor in Drosophila oogenesis. Our analysis revealed that dERR is more highly expressed in stages 1 through 6 egg chambers compared to later stages of oogenesis (stages 7 through 14). Furthermore, dERR deficient egg chambers fail to grow past stage 5 size, suggesting that dERR is required to support cellular growth during this stage of development. Finally, we demonstrate that the dERR target gene Lactate Dehydrogenase (LDH) is also expressed during early oogenesis in a dERR-dependent manner. These findings suggest that dERR is required for activating a metabolic program that supports oocyte development and female reproduction.

353 Large Scale Genetic Screen to Identify Metabolic Regulators of Specification and Differentiation. R.C. Massey, S. Hardman, R. Lawson, A. Page, K. Tshiliiliwa, J. Morounfulou, J.M. Tennesen Indiana University Bloomington, Bloomington, IN.
Metabolism is an immense and intricate network of both catabolism and anabolism. Far more than producing ATP, metabolic pathways build nucleotides, create the fatty acids that allow signaling cascades to function, and may even drive the growth of certain tumors via the production of oncometabolites. Metabolism is an indispensable process, but is often overlooked as a cellular housekeeping function. This view of metabolism, however, is rapidly changing, metabolic genes have recently been implicated in nutrient sensing, signal transduction, and the regulation of cellular differentiation. This has motivated us to use the fruit fly to screen for factors that are involved in both metabolism and development. To investigate how metabolism influences the development of animal tissues, we conducted an RNAi screen to identify metabolic genes that are required for specification and differentiation of the Drosophila eye. By using the GAL4/UAS system, we systematically disrupted 93% of the genes associated with Drosophila metabolism in the developing fly eye, both before and after cellular specification. This screen was specifically designed to identify genes essential for early specification and differentiation of eye tissue while also allowing us to identify those genes that are simply required for cell viability. The screen implicates the electron transport chain (ETC) as playing an essential role in specification and differentiation independent of its role in ATP synthesis.

354 The Role of SR Protein Kinases in Regulating Lipid Metabolism. J. Mercier1, A. Nagengast2, J. DiAngelo1 1) Penn State Berks, Reading, PA; 2) Widener University, Chester, PA.
The survival of animals during periods of limited nutrients is dependent on the organism's ability to store lipids during times of nutrient abundance. However, the increased availability of food in modern Western society has led to excess storage of lipids resulting in a number of metabolic diseases. In order to better understand the genes involved in regulating lipid storage, a genome-wide RNAi screen was performed in cultured Drosophila cells and identified several groups of genes involved in controlling lipid droplet formation and storage. One group of genes of interest to our lab includes those involved in mRNA splicing. Our lab has previously shown that a group of splicing factors important for recognizing intron/exon borders known as SR proteins are involved in controlling lipid storage in Drosophila; however, how these SR proteins are regulated to control lipid storage is not fully understood. In Drosophila, three SR protein kinases (SRPKs) have been characterized: SRPK, darkener of apricot (Doa), and SRPK79D. We used the GAL4/UAS system to decrease the expression of SRPK, Doa and SRPK79D specifically in the adult fat body and then measured the storage of triglycerides and glycogen. SRPK-RNAi flies have lower levels of triglycerides when compared to control flies. In order to determine if the decrease in triglyceride stores in SRPK-RNAi flies was due to changes in feeding behavior, food consumption was measured using capillary feeding (CAFÉ) assays. Flies with decreased SRPK levels in their fat bodies eat less, which may be the cause of the decreased triglyceride phenotype. In contrast, Doa-RNAi flies store more triglycerides compared to the control animals. This is consistent with previous work from our lab showing that decreasing the SR protein 9G8 results in increased triglycerides as Doa directly
phosphorylates 9G8. Together, these findings provide evidence to support the hypothesis that lipid storage is controlled by the phosphorylation of factors involved in mRNA splicing.


Biochemical pathways often produce toxic side products that must be degraded to allow normal biological functions to continue. Phosphoglycolate phosphatase (PGP) is a highly conserved enzyme that has been shown in mammalian cells to dephosphorylate 4-phospho-erythronate and 2-phospho-L-lactate, side products interfering with the pentose phosphate and glycolytic pathways respectively. The ortholog in yeast, Pho13, functions similarly by dephosphorylating 4-phospho-erythronate as well as 2-phospho-L-glycerate. Another mammalian study has demonstrated that in addition to the metabolic function of the enzyme, PGP also plays a role in maintaining cell proliferation during oxidative DNA damage repair by controlling triosephosphate isomerase (TPI) activity and glycerolipid partitioning. Furthermore, it has been reported that PGP acts as a phosphatase to glycerol-3-phosphate (Gro3P), a molecule at the intersection of glucose and lipid metabolism, to control glycolysis, gluconeogenesis, lipogenesis, phospholipid synthesis, lipolysis, fatty acid oxidation, and ATP production. Currently, little work has been done on the function of PGP in animals outside of mammalian species and specifically in Drosophila melanogaster. Our study aims to test whether the potential PGP orthologs in flies, CG5567 and CG5577, have similar roles in metabolism as in mammals. We first generated CG5567 and CG5577 knockdown lines using RNAi and subjected those flies to stress tests. We found that both CG5567 and CG5577 knockdowns were more sensitive to ethylene glycol, an alcohol that causes large increases in phosphoglycolate. These data are consistent with the hypothesis that metabolic stressors in PGP deficient flies results in increased death. We are currently generating null mutants for CG5567 and CG5577 to further explore the functions of these genes in flies. In addition, we intend to perform metabolic tests for TPI activity and Gro3P, triglyceride, and phosphatidylcholine levels to gain a better picture of the metabolic importance of PGP in Drosophila.

356 Role of LanA in diet-induced Type-2 diabetes in Drosophila melanogaster. Younji Nam, Whitney Beavers, Laura K. Reed Biology, University of Alabama, Tuscaloosa, AL.

Type 2 Diabetes (T2D) and the correlated Metabolic syndrome (Met-S) in humans is a global health crisis. In the U.S. alone, prevalence of T2D and pre-T2D have grown from 10% in 1980 to a predicted 40% in 2020. There are two main factors that contribute to T2D: genetic predisposition and perturbing environmental effects. In particular, dietary regulation plays an important role in gene expression and function tightly associated with metabolism. Prolonged negative gene-diet interaction can lead to development of T2D by a loss of metabolic homeostasis. We know what factors are associated with T2D, but we do not fully understand the details of the gene-diet interaction in the T2D state. D. melanogaster is a powerful tool in which to model T2D exhibiting diet induced obesity, and insulin resistance, with a relatively short time to onset of diet-induced T2D in adult flies, and strong conservation with mammals of the insulin and stress response signaling pathways. Thus, the impact of genetic and diet manipulations on T2D in D. melanogaster can apply to the humans.

Laminins are relatively larger glycoproteins in the basement membrane of extracellular matrices, and the major functions of laminins are associated with cell-to-cell communication and tissue architecture. Defects in laminins are associated with diseases, such as early embryonic lethality and muscular dystrophy described in mice. Among the multiple subunits, earlier studies have shown that the function of Laminin subunit α5 (LAMAS) is closely associated with metabolism by regulating the intestinal morphogenesis of smooth muscle, but how LAMAS affects diet-induced T2D is not well understood.

In flies, the LanA locus is homologous to LAMAS. A previous study showed that female LanA mutant flies subjected to a normal level sugar diet had lower TAG storage, body weight, and total protein contents than control flies. In our study, we fed LanA null mutants and control flies with a normal (4%), intermediate (6%), and a high (12%) sugar diet, and measured their metabolic phenotypes such as sugars, TAG, and weight. From this, we found that the LanA null mutants are also more protected from a high sugar diet than the control flies by having a lower glucose, glycogen, TAG, and weight. Accordingly, we will also demonstrate whether LanA null mutants are also more resistant to diet-induced T2D phenotypes by restoring glucose tolerance and lifespan. In addition, we will assess the physiological tradeoffs of the loss of LanA to compare to physiological disorders in defective human laminins. Ultimately, the metabolic impact of LanA performed in this study will contribute to a better understanding of the gene-diet interaction in the development of T2D.

357 Detecting Drosophila SR splicing factor proteins by Western Blot. Y. Patel1, N. Kaur1, A. Nagengast2 1) Biology, Widener University, Chester, PA; 2) Biochemistry, Widener University, Chester, PA.

Obesity is a growing epidemic affecting over a third of the United States population yet the underlying causes are not very well understood. We use the model organism Drosophila melanogaster to study the regulation of genes related to triglyceride storage by manipulating gene expression in the fat body, a tissue similar to mammalian adipose tissue and the liver. Previous results have shown that the splicing factor 9G8 is critical for proper triglyceride levels in flies yet its protein function and splicing targets are not well known. Antibodies against 9G8 would be useful to understand its function yet are difficult to generate because of the repetitive nature of the SR (Serine and Arginine dipeptides) domain in the protein structure.
**358** **Metabolic characterization of the Drosophila E78 nuclear receptor.**  
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The alarming prevalence of metabolic disorders emphasizes the importance of characterizing how metabolism is controlled and how misregulation of metabolic pathways can lead to disease. Nuclear receptors (NRs) play critical roles in maintaining metabolic homeostasis as well as systemic physiology. My studies are focused on the Peroxisome Proliferator Activated Receptor (PPAR) subfamily of NRs that control lipid metabolism in the mammalian liver, adipose tissue, and muscle. This subfamily is composed of three paralogs with overlapping expression patterns and diverse functions (PPAR α, γ, δ). Members of the PPAR family regulate lipid homeostasis by stimulating fatty acid oxidation as well as promoting adipogenesis, regulating glucose sensitivity, fatty acid uptake and mobilization, and triglyceride synthesis. Transcriptional targets mediate the metabolic switches necessary to conserve energy during fasting. Phylogenetic studies have aligned a single Drosophila NR, designated E78, with the PPAR family, although this relationship is not orthologous. I am studying the regulation and function of E78 with the goal of defining the ancestral functions of the PPAR subfamily in the absence of genetic redundancy. E78 null mutants are fully viable and show no obvious growth defects or developmental delay. Genetic studies, however, have shown that E78 is required for the establishment of the somatic germline stem cell niche in the ovary. In addition, the E78 ligand binding domain is active within lipid-rich tissues, including the embryonic yolk, oenocytes, and fat body, consistent with PPAR function and suggesting a role in lipid metabolism. Here we show that E78 mutant adults have reduced motility, fecundity, and are resistant to starvation. E78 mutant males and females have sexually dimorphic metabolic defects, with females displaying increased carbohydrate stores and glucose, and males displaying reduced levels of triglycerides. Our current studies are focused on defining the molecular mechanisms by which E78 maintains adult physiology and metabolic homeostasis.

**359** **A Drosophila model of D-2-hydroxyglutaric aciduria.**  
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The oncometabolite D-2-Hydroxyglutarate (D-2HG) has emerged as a potent regulator of gene expression and cell fate decisions. While this compound is most commonly studied in the context of gliomas, where it is produced by neomorphic mutations in Isocitrate Dehydrogenase 1 or 2, D-2HG is also produced as a byproduct of normal animal metabolism. The production of D-2HG in healthy cells, however, is commonly considered a metabolic waste product and is rapidly degraded via the enzyme D-2-hydroxyglutarate dehydrogenase (D2HGDH). Mutations in the human D2HGDH homolog result in an inborn error of metabolism known as D-2-hydroxyglutaric aciduria, which is characterized by developmental delays and neuromuscular defects. In order to both better understand the role of D-2HG in cellular physiology and investigate the molecular mechanisms that lead to the pathophysiology of D-2HG aciduria, we used CRISPR/Cas9 to mutate the Drosophila homolog of D-2HGDH. Gas chromatography-mass spectroscopy (GC-MS) indicated that D-2HG levels were elevated in the mutant strains, demonstrating that this enzyme is required for D-2HG degradation in flies. We are now using these mutants to conduct an RNAi-based forward genetic screen, with the goal of identifying genetic interactions between D2HGDH and other metabolic enzymes. Our preliminary results suggest that D-2HG interacts with a number of metabolic pathways, and our future studies will examine the significance of these interactions.

**360** **Drosophila ceramide synthase Schlank regulates transcription according to lipid status.**  
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Ceramide synthases (CerSs) are a group of enzymes at the center of sphingolipids metabolism. Ceramides are now recognized as pivotal bioactive signaling molecules and many pathogenic changes such as diabetes, cancer, and degenerative diseases are associated with changes in ceramide levels. All CerSs contain a highly conserved catalytic Lag1p motif and many have a homeobox (Hox) domain. The homeodomain function is frequently not investigated due to the lack of animal models. In fact, previous studies doubted that the homeodomain of CerSs could act as transcription factor, focusing on the unpaired
enzymatic activity and on altered ceramides levels. We established an in vivo animal model and demonstrate an essential function of the Schlank homeodomain in the regulation of body fat metabolism and growth. ChIP and reporter assays show that CerS Schlank binds the promoter regions of lipases via the homeodomain and directly regulates transcription. Mutations of the nuclear localization site 2 (NLS2) within the homeodomain lead to loss of DNA binding and deregulated gene expression. This mechanism is conserved in mammalian CerS2. Furthermore, NLS2 mutants can no longer adjust transcriptional response to changing lipid levels. Ex vivo treatment of fat body and guts with fatty acids show that Schlank, with an intact homeodomain, can sense the status of cellular lipids and transduces the appropriate signal to the level of gene expression, shifting nuclear localization in fed and starved states. In summary, our study demonstrates a double role of CerS Schlank as enzyme and transcriptional regulator, sensing lipid levels and transducing the information to the level of gene expression.

361 Calcium Independent Phospholipase A2-beta Is Non-essential for Somatic Phospholipid Metabolism but Is Required for Maximal Lifespan and Fertility. J. Steinhauer1, M. Lubin1, A. Waklschlag1, S. Eizadshenass1, A. Dechter1, M. Ren2, M. Schlame2 1) Department of Biology, Yeshiva College, New York, NY; 2) Department of Cell Biology, NYU Langone Medical Center, New York, NY.

Phospholipases A2 (PLA2s) are important phospholipid remodeling enzymes that have been implicated in mitochondrial function, cell death, fertility, nervous system function, inflammation, metabolism, and cancer. PLA2s are conserved in Drosophila but have not been well characterized. We have generated a null mutation in the Drosophila calcium independent phospholipase A2 (iPLA2) -beta gene CG6718. Null mutants are viable, consistent with mouse knockout models, and show no major molecular species changes in cardiolipin or other phospholipids. In accord with the normal cardiolipin content, young iPLA2-beta mutants show no defects in locomotor activity. However, iPLA2-beta mutants develop severe locomotor defects with age and have reduced lifespan. We are testing the tissue specificity of this phenotype, using RNAi and transgenic rescue. In aged iPLA2-beta mutants, proteostasis can be compromised, as demonstrated by reduced processing of the ER stress transducer xbp1. Intriguingly, in wild-type adults, iPLA2-beta expression increases with age. Although iPLA2-beta also has been implicated previously in male fertility, our data do not support this conclusion. Still, iPLA2-beta mutants show reduced female fertility, with increased cell death in the female germline. Together, our results indicate that cardiolipin and glycerophospholipid remodeling do not strictly require iPLA2-beta, possibly owing to genetic redundancy amongst PLA2s or alternative phospholipid metabolism pathways. However, our work has uncovered two specific roles for iPLA2-beta, in female fertility and aging. iPLA2-beta is an emerging target for pharmaceutical intervention in humans, and investigations in Drosophila will shed light on its functions and mechanisms.

362 The identification of SR proteins crucial to the alternative splicing of the glucose-6-phosphate dehydrogenase coding gene by NADPH production assays. M. Tracewell, A. Nagengast Biochemistry, Widener University, Chester, PA.

The epidemic of obesity increases the likelihood of deadly diseases, increases health care costs, and decreases the quality of life. Although the regulation of genes related to obesity is not well understood, alternative splicing (AS) appears to play an important role. Drosophila is a powerful model to study obesity and gene regulation by AS, because metabolic pathways of flies are similar to humans. Specifically, the gene encoding for Glucose-6 Phosphate Dehydrogenase (G6PD) in the Pentose Phosphate Pathway (PPP) is alternatively spliced, resulting in two isoforms of the gene that codes for the enzyme. G6PD catalyzes the rate-limiting step in the PPP and converts Glucose-6 Phosphate (G6P) to 6-Phosphogluconate and generates NADPH required for fatty acid synthesis. Solving the splicing pattern of G6PD starts with understanding which SR (Serine-Arginine dipeptide repeat) proteins are regulating the gene. Different SR proteins are being knocked down through RNAi in the fat body of adult males using the gal4-UAS system. NADPH assays have been optimized in whole adult fly extracts to detect the reduction of NADP+ to NADPH at a wavelength of 562nm as a measure of G6PD activity. This will allow for the identification of the splicing factors critical for the AS of G6PD and correlate isoform to enzyme activity. Understanding the splicing pattern of G6PD could be a key factor in understanding a level of gene regulation related to lipid over/under storage and obesity.

363 Reduced lipogenesis alters lipid profiles and exacerbates type 2 diabetic phenotypes in Drosophila. B. F. Tuthill1, L. A. Searcy2, E. O’Hara3, C. J. Quaglia1, R. A. Yost1, L. P. Musselman1 1) Biological Sciences, Binghamton University, Binghamton, NY; 2) Department of Chemistry, University of Florida, Gainesville, FL.

Diets high in carbohydrates are associated with type 2 diabetes and comorbidities including hyperglycemia, hyperlipidemia, obesity, hepatic steatosis and cardiovascular disease. A high-sugar diet can be used to study the pathophysiology of diet-induced diabetes in Drosophila melanogaster. Lipotoxicity, or the accumulation of toxic lipids, may occur in peripheral tissues once dietary excess overloads the fat body beyond its maximum capacity. We are taking tissue-specific reverse genetic and metabolomic approaches to understand lipotoxicity in the fly fat body and heart. Our goal is to overload the heart by exceeding the storage capacity of the fat body in the presence of caloric overload. Preliminary data has shown numerous statistically significant changes in the lipid profiles of organs from flies aged on a high sugar diet as well as fat body Stearoyl CoA-desaturase-1 deficient flies when compared to controls. Assays for cardiac structure and function coupled with tandem
and imaging mass spectroscopy techniques provide insight into the relationship between potentially lipotoxic species and type 2 diabetes-associated cardiac pathophysiology. The ultimate goal is to further elucidate the endocrine mechanisms and molecular targets involved in metabolic disease.

364 Muscle directs diurnal energy homeostasis through a myokine-dependent hormone module in Drosophila. X Zhao, J Karpac Molecular and Cellular Medicine, Texas A&M University Health Science Center, College Station, TX.

Inter-tissue communication is critical to control organismal energy homeostasis in response to temporal changes in feeding and activity or external challenges. Muscle is emerging as a key mediator of this homeostatic control through consumption of lipids, carbohydrates, and amino acids, as well as governing systemic signaling networks. However, it remains less clear how energy substrate usage tissues, such as muscle, communicate with energy substrate storage tissues in order to adapt with diurnal changes in energy supply and demand. Using Drosophila, we show here that muscle plays a crucial physiological role in promoting systemic synthesis and accumulation of lipids in fat storage tissues, which subsequently impacts diurnal changes in circulating lipid levels. Our data reveal that the metabolic transcription factor Foxo governs expression of the cytokine Unpaired 2 (Upd2) in skeletal muscle, which acts as a myokine to control glucagon-like adipokinetic hormone (AKH) secretion from specialized neuroendocrine cells. Circulating AKH levels, in turn, regulate lipid homeostasis in fat body/adipose and the intestine. Our data also reveal that this novel myokine-dependent hormone module is critical to maintain diurnal rhythms in circulating lipids. This tissue cross-talk provides a putative mechanism that allows muscle to integrate autonomous energy demand with systemic energy storage and turnover. Together, these findings reveal a diurnal inter-tissue signaling network between muscle and fat-storage tissues that constitutes an ancestral mechanism governing systemic energy homeostasis.


The fruit fly, Drosophila melanogaster, is a useful model organism for examining the genetic and biochemical pathways of pigmentation. Cuticle pigmentation is a phenotypically plastic trait in flies that varies widely in patterning and intensity between and within species of Drosophila. Pigmentation can be affected by environmental factors including nutrition, which regulates insulin signaling. Previous studies have shown that flies reared on a low nutrient diet showed a significant decrease in the total amount and intensity of pigmentation of the abdominal cuticle. Although some research has looked at whether metabolic changes can be passed on to the next generation little is known about whether parental pigmentation patterns are inherited by their offspring. Our study seeks to examine the effects of parental nutrition on offspring cuticle pigmentation and looked at whether any pigmentation changes are passed down via maternal or paternal inheritance. Parental flies were reared on either a standard diet or a low nutrient diet and then crossed within and between groups. Flies reared on low nutrient food exhibited a significant decrease in levels of pigmentation compared to those reared on a standard diet. However, there were large variances of pigmentation among their offspring, with inconsistent results between trials. We were not able to make any concrete conclusions on the epigenetic changes that could have occurred. We are currently reworking our experimental design to answer this question of whether parental pigmentation is inherited by the offspring. However, this time we have considered a new experimental design that will have limited confounding variables and will analyze the important sources of variation at different scales: within individual segments of the abdomen, among segments within an individual fly, among flies of the same sex and between sex, and among and within experimental units.

366 Characterization of adult Drosophila melanogaster insulin pathway mutants. Jessica Alvarez, Juan Rafael Riesgo-Escobar Instituto de Neurobiologia, Universidad Nacional Autonoma de Mexico.

Diabetes encompasses a group of metabolic disorders generally characterized by hyperglycemia, resulting from defects in insulin signaling in the organism. We used Drosophila melanogaster to study the disease longitudinally, throughout the lifecycle of the organism. We characterized different metabolic parameters: chiefly, levels of lipids, sugars, and activity levels of InR (the insulin receptor fly homologue), Dp110 (the catalytic subunit of phosphoinositol 3 kinase fly homologue), or d56K (the ribosomal protein S6 kinase beta-1 fly homologue) heteroallelic mutants, as well as wildtype and heterozygotic controls from the same genetic background, at different ages of adult flies (at 1, 10, 20, and 30 days) in both sexes. The results obtained show a significantly decreased level of activity of some insulin mutants compared to wild flies. In addition to the well-known size decrease and higher levels of lipids and carbohydrates per milligram weight in the mutants at eclosion, we observed changing but significantly different metabolic phenotypes, more severe in InR mutants. The most common pattern observed across groups consisted of carbohydrate and lipid levels being at their lowest at the beginning and end of the adult life timepoints measured. These variations may influence the response to different treatments or challenges, as they vary with age in the diabetic organisms.

Financing: PAPIIT # IG200216 to JRR-E
367  A novel dye-based method for measuring solid media consumption in adult Drosophila. Brandon Shelles, Rebecca Schmitt, Kristen Lee, Scott Pletcher, Michael Grotewiel  1) Virginia Commonwealth University, Richmond, VA; 2) University of Michigan, Ann Arbor, MI.

Given the increasing global incidence of obesity-related health problems, studies in flies hold tremendous promise for continuing to reveal key mechanisms underlying diet-related phenomena that could ultimately translate into improved prevention and treatment of a multitude of diseases. One major hurdle to understanding the effects of diet on the physiology of flies, however, is that it can be very challenging to unambiguously measure the volume of standard agar-based media that flies consume under laboratory conditions. We addressed this challenge by developing a dye-based method called Consumption-Excretion (Con-Ex) for quantifying the intake of agar-based media in adult flies. In Con-Ex studies, flies consume solid food labeled with dye, and the total volume of food consumed is determined as the sum of the dye inside flies and the dye excreted by flies over the course of the experiment (hours to days). We found that flies in Con-Ex experiments consumed and excreted measurable amounts of FD&C Blue No. 1 (Blue 1), FD&C Blue No. 2 (Blue 2), FD&C Yellow No. 5 (Yellow 5), and xylene cyanol (XC). Blue 2, Yellow 5 and XC dose-dependently influenced the results of Con-Ex studies, decreasing their utility for measuring food consumption. In contrast, varying the concentration of Blue 1 (0.25-2.0% w/v) in Con-Ex studies had no discernable effect on consumption-excretion of agar-based media, making this dye the focus of the current project. In Con-Ex studies with Blue 1 as a food tracer, we found that (i) flies excreted >50% of their waste on the food medium, (ii) the total volume of consumed-excreted media increased linearly with feeding duration out to 24 h, (iii) measurements of total consumption were sensitive to starvation and genetic background, and (iv) flies altered their consumption of agar-based media in response to changes in some, but not all, food components. We also found that the volume of liquid medium (labeled with Blue 1) consumed from capillary tubes was indistinguishable from the volume of Blue 1 excreted by flies, suggesting that excreted Blue 1 is a reliable measure of consumed Blue 1. Our results indicate that the Con-Ex method with Blue 1 as a food tracer is well suited for measuring consumption of agar-based media in adult flies.

368  Assessing the genetic- and sex-specific interactions of adult exercise and poor larval diet in Drosophila. Kelsey Lowman, Brélahn Wyatt, Laura Reed  Dept of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Childhood obesity is a growing epidemic in westernized cultures that leads to lifelong adverse effects, even when corrective measures are taken later in life. Metabolic syndrome (MetS) research is centered on understanding the negative effects of obesity, such as increased risk of heart disease, diabetes, and stroke. Some underlying factors of MetS include the sex of the individual, excess calorie consumption, physical inactivity, and genetic factors. Our research aims to model these factors, their interactions, and their subsequent consequences. In particular, we focus on the negative effects that arise from eating a high-fat diet during childhood and the potential to ameliorate these effects with exercise in adulthood. Our previous work using the TreadWheel demonstrated that exercise substantially improves adult fly metabolic health in a sex- and genotype-specific manner. In this study, we explore further the effects exercise can have on adult fly health by comparing the outcomes for ten genetically distinct lines from the Drosophila Genetics Reference Panel (DGRP) fed a 1.5% high-fat or standard lab diet as larvae that then experienced an induced-exercise verses control treatment as adults. The daily exercise regime followed a five-day inverse pyramid protocol of alternating bouts of exercise and rest within a two-hour window. After protocol completion, we performed a negative-geotaxis climbing assay and measured dry weight, triglyceride storage, and survival. We found that climbing performance, adult weight, triglyceride storage, and survival showed interaction effects among sex, diet, exercise, and genotype. In future work, we will explore the impact of exercise on insulin signaling and sensitivity.

369  SIK2 coordinates animal growth via protein and carbohydrate sensing. L.May. Parsons, L Cauchi, J Kannangara, C Warr  School Biological Sciences, Monash University, Clayton, AU.

The regulation of tissue growth is perhaps the most important means by which the developmental programme generates an organism of a characteristic size and form. Four decades of intensive research has taught us a great deal about the molecular mechanisms by which a number of crucial cell signalling pathways regulate tissue growth and body size. However, we lack basic knowledge of the mechanisms that link both the growth signalling and metabolic networks to the nutrition status of the animal during development. We have identified the SIK2 nutrient sensing kinase as an exciting candidate for such a mechanism.

InR-TOR signalling senses and integrates a variety of environmental cues, including nutrient availability (amino acids and glucose/energy levels), to regulate organismal growth and homeostasis. Significantly, our data shows that SIK2 is sufficient to restore activity of the downstream networks required for TOR signalling to rescue animal survival. Specifically, inhibition of TOR signalling in the developing Drosophila wing by expressing a dominant negative isoform of TOR, or depleting a crucial mediator of TOR signalling, raptor resulted in lethality during pupal development. Strikingly, overexpression of dSIK2WT rescued lethality and generated adult animals. Thus, SIK2 is sufficient to activate the molecular networks required for organ growth and survival, downstream and/or parallel of TOR signalling.

SIK2 is a known regulator of glucose and lipid metabolism. However our finding that SIK2 can rescue inactivation of TOR signalling raises the possibility that SIK2 may also participate in protein and amino acid sensing. To determine which components of dietary nutrition SIK2 interacts with to regulate growth, we reared both SIK2 mutants and heterozygotes on a
range of different protein and carbohydrate diets in an approach called nutritional geometry. Measuring pupal size and adult weight clearly demonstrated that in addition to carbohydrate sensing SIK2 interacts with dietary protein levels to regulate animal growth. Taken together our dietary and genetic interaction studies suggest SIK2 is a novel component of TOR signalling and acts during development to co-ordinate dietary nutrition with animal growth.

370  Effect of dietary additives on intestinal permeability in *Drosophila*.  M Pereira, J Nostro  Biological Sciences, Binghamton University, Binghamton, NY.

Industrial food processing and food additive consumption have been on the rise along with an increase in the prevalence of diseases with intestinal complications, such as type 2 diabetes, Crohn's disease, celiac disease, multiple sclerosis, and irritable bowel syndrome. We used the Smurf assay to characterize the effects of food additives on gut function. Our results reveal that food additives can have a dramatic effect on intestinal permeability in *Drosophila melanogaster*. Diets with excess sugar and the surfactant Tween-20 caused the most drastic increases in intestinal permeability among the additives tested. We also quantified intestinal alkaline phosphatase activity for potential use as a biomarker of gut permeability. Data from our lab and others suggest that dietary additives could affect intestinal integrity both directly and via the microbiome. Future studies will use genetic and microbial manipulations to determine the mechanisms by which dietary additives control gut permeability.

371  Effects of Diet and Genotype on Cardiovascular Health in *Drosophila*.  Christopher Quaglia, Bryon Tuthill II, Eileen O’Hara, Laura Musselman  Binghamton University.

Obesity and related diseases can result from the over consumption of diets high in carbohydrates. In our work, we use *Drosophila* to study obesity-associated metabolic disorders such as cardiovascular disease and diabetes. We and others have shown diabetes-like biochemical and physiological phenotypes in flies fed high-sugar diets. Interestingly, some flies thrive on high-sugar diets, whereas other genotypes cannot tolerate them. Our goal is to understand the metabolic pathways controlling the ability to maintain metabolic health in the face of overconsumption. Obese flies may develop lipotoxicity, or abnormal fat accumulation, in peripheral tissues during high-sugar feeding. Using a combination of approaches to test exercise capacity, lipid content, and heart and excretory function, we will analyze the effects of diet and genotype on *Drosophila*. The long-term goal of this research is to determine how the diet contributes to metabolic diseases such as cardiovascular disease, obesity and diabetes.

372  GATA factor Pannier plays an essential role for proper sperm storage in adult spermathecae.  W. Shen1, J Sun1,2  1) Department of Physiology & Neurobiology, University of Connecticut, Storrs; 2) Institute for Systems Genomics, University of Connecticut, Storrs.

GATA-family transcription factors play essential and conserved roles in the formation of multiple organ systems including heart, immune system, and sensory organs; however, their roles in adult organ physiology have been sparsely studied. In this study, we found that *Drosophila pannier (pnr)* is specifically enriched in secretory cells of adult spermathecae and parovaria, two types of glandular organs in female reproductive tract. Previous study has showed that secretions from these glands are essential for sperm storage in the spermathecae and for the regulation of ovulation. Interestingly, we found that *pnr* knockdown in adult secretory cells of these glands leads to severe sperm storage defect in spermathecae but does not affect ovulation, indicating that *pnr* may play a role in controlling specific secretory productions regulating sperm storage. In addition, *pnr*-knockdown secretory cells in spermathecae show progressive hypertrophy and enlarged apical secretory cavity, which temporally stores secreted products and transports them into the central lumen through a cuticular end-apparatus and canal. The enlargement of secretory cavity is not due to the physical blockage of end-apparatus and canal according to electron micrography. We hypothesize that *pnr* regulate the composition of secreted products to ensure proper transferring of these products into the central lumen of spermathecae in order for them to attract sperm. Our study demonstrated a new role for *pnr* in regulating secretions for sperm storage.

373  Metabolic analysis of critical weight.  T. Fernando, P.C. Driscoll, A.P Gould  Physiology and Metabolism, Francis Crick Institute, London, GB.

The growth and development of all animals involves transitions between different physiological states. The key developmental transition of critical weight (CW) in *Drosophila* dramatically changes the growing larva's response to nutrient restriction (NR). NR arrests developmental progression before but not after CW. It is known that the timing of CW is regulated by the steroid hormone ecdysone but it is unclear how metabolism changes at CW and how larvae sustain NR-resistant developmental progression. To address these issues, we developed an NMR method (volume determination with two standards-VDTS) and used it to measure changes in the polar metabolomes when NR is applied either side of CW. We find that progression past CW correlates with the ability of NR larvae to sustain a substantial increase in the hemolymph concentration of tyrosine. A large post-CW increase in concentration is also observed for O-phospho-tyrosine (OPT), a putative storage form of tyrosine. We have identified two genes involved in OPT metabolism and find that their knockdown delays
374 Identification of novel FOXO-binding partners regulating oxidative stress response in Drosophila melanogaster.  
A. Birnbaum, K Arndt, H Bai Genetics Development and Cell Biology, Iowa State University, Ames, IA.

The insulin signaling pathway in Drosophila melanogaster is responsible for cell growth and development, but has also been shown to regulate cellular response to multiple stressors. Response to these cellular changes stimulates dFOXO, a transcription factor that localizes in the nucleus upon depletion of insulin or under stress conditions. The FOXO protein regulates a wide array of cellular functions including apoptosis, metabolism, cell cycle arrest, stress resistance, and aging. dFOXO acts as a hub of protein interactions, and through this network it is able to control transcriptional activity of many target genes including those involved in stress response and maintenance of cellular reactive oxygen species (ROS). To further characterize the role of the dFOXO protein network (interactome) in the process of oxidative stress resistance, we have conducted a mass spectrometry analysis to characterize dFOXO binding partners under both normal and paraquat treated conditions in Drosophila KC167 cells. Through this, candidate interacting proteins have been identified that are either unique, resistant, or susceptible in their interaction with dFOXO under paraquat treatment. These candidates were knocked down using RNAi and screened for response to paraquat-induced stress through a mortality assay. Lines with significant lifespan alteration post knockdown were confirmed to interact with dFOXO via co-immunoprecipitation. Ongoing experiments are being carried out to further examine the transcriptional co-regulation of stress response genes by dFOXO and the identified co-factors. Thus, our studies have identified a new dFOXO interacting protein network and uncovered novel dFOXO co-factors that facilitate dFOXO's transcriptional control of cellular homeostasis and stress resistance in Drosophila melanogaster.

375 Expression and localization of superoxide dismutase (SOD) enzymes in mammalian cell systems.  
J.D. Parker, M. Valentine, K. Ckless, A. Ryan, S.G. Segarra, Bio 490 student group D Dept of Biological Sciences, SUNY Plattsburgh, Plattsburgh, NY.

Superoxide dismutases (SOD) are antioxidant enzymes thought to be involved in lifespan determination of the fruit fly Drosophila melanogaster. SOD has been found to be expressed in cytoplasmic, mitochondrial, and extracellular forms in all animals and it has been hypothesized that several forms of SOD may interact with each other. The Cu Zn forms of SOD are SOD1, which is cytoplasmic, and two different sized forms of SOD3; a long form with a membrane binding region and another that is secreted. The purpose of this study is to determine if the two variants of SOD3 are membrane bound or secreted and to gather evidence supporting that there are interactions occurring between the cytoplasmic CuZn-SOD1 and the two differently sized forms of extracellular CuZn-SOD3. An undergraduate group in Investigative Biology Experience (Bio 490) will be presenting their work creating fluorescently-tagged DNA constructs of the various forms of Cu Zn SOD. These constructs will be tested in mammalian tissue culture and cloned into vectors to make UAS transgenic flies.

376 The mitochondrial proteome of flies expressing the alternative oxidase under different dietary conditions.  
M.M. Chiodo, E.A. McKinney, T.S. Balbuena, M.T. Oliveira Departamento de Tecnologia, FCAV, Universidade Estadual Paulista UNESP, Jaboticabal, SP, Brazil.

The mitochondrial alternative oxidase (AOX) is an enzyme that provides an alternative electron transport pathway to the respiratory chain, bypassing complexes III and IV and modulating reactive oxygen species formation. Drosophila melanogaster and humans do not naturally express AOX, since a redundant proteins for AOX expression and control flies in HCD, and 542 and 565 in RD, representing 50-80% of the predicted total of mitochondrial proteins. The spectral counts for the subunits of the respiratory chain complexes are equivalent for both dietary conditions in AOX-expressing and control flies. Surprisingly, the levels of citrate synthase, the first enzyme in the Krebs cycle, is decreased 1.5-2.0 fold in the AOX-expressing pupae in both diets, but this is apparently not accompanied by a decrease in mitochondrial mass. More analyses are underway to help us explore this abundant dataset, aiming at elucidating the changes in the mitochondrial molecular mechanisms and metabolic pathways involved with AOX function in distinct dietary conditions. Our work will provide basic mechanistic support to the

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### 377 Drosophila tafazzin mutants have impaired exercise capacity.

**Deena Damschroder**, Robert Wessells Wayne State University School of Medicine , Detroit , MI.

Cardiolipin (CL) is a mitochondrial phospholipid that helps maintain the curvature of the mitochondrial membrane and stabilize the protein complexes of the electron transport chain to promote efficient ATP synthesis. Tafazzin is an acyltransferase required for synthesis of the mature form of CL. Mutations in the tafazzin (Taz) gene are associated with a human disorder known as Barth syndrome (BTHS). Symptoms of BTHS often include muscle weakness and exercise intolerance. Previous work demonstrates Drosophila Taz mutants exhibit motor weakness resulting in reduced flying and climbing abilities. However, Drosophila Taz mutants' response to exercise has not been examined. In this study, we examined the baseline exercise capacity of Taz mutant flies, and their ability to adapt to exercise training. Prior to training, Taz mutants demonstrated reduced endurance, flight, and climbing capabilities relative to control flies. After training, exercised Taz mutants’ endurance and flight ability did not improve. Although cardiac phenotypes are observed in human patients, no obvious cardiac phenotype was observed in Drosophila Taz mutants. In the future, we hope to use endurance as a novel screening tool to identify genetic modifiers of Taz.

### 378 The mitochondrial alternative oxidase mitigates the effects of cold stress in Drosophila melanogaster.

**G.S. Garcia**, M.T. Oliveira Departamento de Tecnologia, FCAV, Universidade Estadual Paulista UNESP, Jaboticabal, SP, Brazil.

Alternative oxidases (AOX) are non-proton-pumping enzymes which can bypass complexes III and IV of the mitochondrial respiratory chain (RC), transferring electrons directly to oxygen. Vertebrates and arthropods independently lost the AOX-coding gene throughout evolution, but, its transgenic expression in Drosophila melanogaster showed amelioration in the conditions associated with mitochondrial dysfunctions. Considering that AOX has a thermogenic role in plants, we aimed at investigating the effects of different temperatures on the development of AOX-expressing fly lines, including conditions of thermal stress. We used a wild type strain (w1118), and five transgenic lines in which the Ciona intestinalis AOX gene is expressed under the control of the α-tubulin promoter (tubAOX lines): tubAOX12 (chromosome X), tubAOX11 (chromosome 2), tubAOX2 (chromosome 3), 2XtubAOX (chromosome 2 and 3) and 3XtubAOX (chromosomes X, 2 and 3). Developmental assays were conducted at 12, 15, 18, 25 and 29°C and the number of eggs, pupae and adults were counted to calculate the viability rates of eggs/larvae and pupae, and development time. No significant differences among AOX and control lines at 18 and 25°C were observed for the analyzed parameters. At 15 and 12°C, lines with higher levels of AOX expression had higher viability rates, and development was more accelerated. Although all flies died at the late pupa stage at 12°C, egg/larval viability of 2XtubAOX and 3XtubAOX was 2 fold higher and the pupae developed on average 8-10 days faster. At 29°C, development time among all the lines did not differ, however, the egg/larval viability of the AOX lines was higher than that of w1118. Our findings indicate a possible thermogenic role of AOX in Drosophila at low temperatures, so we measured mitochondrial oxygen consumption in larvae at 25 and 15°C. No apparent differences in the ratio of antimycin A-resistant oxygen consumption (provided by AOX, ~30%) at both temperatures were identified for 3XtubAOX, and AOX inhibition by propylgalate (in the absence of antimycin A) did not cause a significant decrease in respiration by the RC complexes III/IV. At 25°C, maximum respiration for 3XtubAOX driven by pyruvate, proline, malate, glycerol-3-phosphate and ADP was 3 fold higher than for w1118; however, this difference was not detected at 15°C, which is not consistent with the hypothesis of thermogenesis by AOX. Infrared thermographic analyses will be performed for a direct measurement of heat production, but it is very likely that the advantages that AOX provides to flies at cold temperatures are attributable to its thermogenic function.

### 379 Mitochondrial Genotype Alters Metabolic and Transcriptional Regulation by TOR Signaling in Drosophila.

**J.C. Santiago**, D. Rand Cell Biology and Biochemistry, Brown University, Providence, RI; 2) Ecology and Evolutionary Biology, Brown University, Providence, RI.

The target of rapamycin (TOR) acts as a regulator of cellular metabolic functions in response to changes in nutrient availability. This process is necessary in order to protect the cell from exhausting cellular energy reserves. Mitochondrial function has been shown to both regulate, and be regulated by, TOR signaling but the underlying mechanisms responsible for this regulation are poorly understood. Our lab has previously demonstrated that TOR signaling is altered by genes encoded in the mitochondrial genome (mtDNA). This project seeks to identify how transcriptional responses following TOR inhibition are modified by mtDNA genotype. Using RNA-seq analysis I have been able to show mitochondrial genotypes alter the expression of nuclear encoded metabolic genes in response to rapamycin implicating them as important factors that mediate how TOR signaling and mitochondrial function regulate one another.

### 380 The role of the ribonucleoprotein Clu in mitochondrial health and protein import.

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Mitochondria are very dynamic organelles in the cell and are the major source of cellular ATP. Functional, healthy mitochondria are essential for proper cellular function. Although in humans the mitochondria genome encodes for only 13 polypeptides, there are hundreds of nucleus-encoded proteins that are required for the myriad of biochemical functions mitochondria carry out. While the majority of these proteins are imported into mitochondria using the canonical post-translational pathway involving cytoplasmic chaperons, there is increasing evidence that nucleus-encoded mRNAs localize to mitochondria to undergo co-translational import. However, we have a poor molecular understanding of what this mechanism may be. The Drosophila protein Clueless (Clu) is required for proper mitochondrial structure and function. clu mutant flies are sick, uncoordinated, sterile and have damaged nonfunctional mitochondria. These defects are a direct effect of lack of Clu because Clu also interacts with outer mitochondrial membrane proteins such as Tom20, Porin and Pink1. Recent observations from our lab and others have shown that Clu and the mammalian homolog Cluh are ribonucleoproteins that preferentially bind nucleus-encoded mitochondrial mRNAs. In addition, we have shown Clu binds ribosomal proteins at the mitochondrial outer membrane, and Clu genetically and physically interacts with the major mitophagy pathway components Pink1 and Parkin. Our model is that Clu binds mRNAs for co-translational import into mitochondria and may act as a sensor for mitochondrial quality through Clu's interaction with the mitophagy machinery. Here we will present Clu's role as a ribonucleoprotein in further detail and explore the function of Clu in mitochondrial co-translational protein import.

381  **A soma to germline signaling relay triggers OXPHOS biogenesis during early oogenesis essential for Drosophila mitochondrial inheritance.** Z-H. Wang, Y. Liu, H. Xu National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Mitochondrial genome (mtDNA) encodes key components of electron transport chain (ETC) for oxidative phosphorylation (OXPHOS), and thereby are vital for life. Mitochondria are transmitted exclusively through maternal lineage in most metazoan, which demands mothers to furnish mature oocytes with massive amount of mitochondria to power the early embryogenesis. Our lab previously reported that, mtDNA replication is quiescent in the early gerarium stages, but commences at the region 2B. Additionally, mtDNA replication in germanium relies on mitochondrial ETC activity, which is critical for selective inheritance against severely mutated mtDNA. Nonetheless, the signals triggering mitochondrial/OXPHOS biogenesis and mtDNA replication during oogenesis remain elusive. In this study, we uncovered that the spatial pattern of mtDNA replication in germanium is coincided with the mitochondrial ETC activities/biogenesis judged by a cytochrome c oxidase/succinate dehydrogenase histochemical staining. Targeted RNAi screenings reveal a novel follicle cells (FC) to germ cells (GC) signaling relay that induce OXPHO biogenesis in region 2B. In FCs, wingless signaling regulates transcription of TNF-α converting enzyme (TACE) to release TNF-α/Eiger to moderately stimulate JNK signaling in region 2B GCs. Intriguingly, insulin pathway is downstream of JNK signaling in GC to support ETC biogenesis and post-transcriptionally regulates dMyc levels. Indeed, dMyc OE, but not dPgc-1 the master of mitochondrial biogenesis, is sufficient to promote ectopic mitochondrial import, ETC biogenesis and mtDNA replication. Our study identifies that a metabolic shift into OXPHOS accompanying a transition from initial germline stem cell differentiation to follicle development is mediated by a follicle to germline signaling relay, essential for mitochondrial inheritance in Drosophila. This mechanism suggests that new neighbors stimulate a mild stress response in differentiated cells to trigger a survival and growth pathway leading to a metabolic transition from glycolysis to OXPHOS for further development and function.

382  **A mitochondrial rescue of a nuclear defect in starvation resistance and lipid levels in Drosophila.**  S. Williams, B. Franklin, D Rand Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI.

Disruptions in mitochondrial function underlie several metabolic disorders. Although multiple studies have identified mutations in mitochondrial or nuclear genomes which independently give rise to disease, very little is known about the role of genetic interactions between the two genomes (mito-nuclear interactions). To model the contribution of mito-nuclear interactions in complex disease, we utilize Drosophila lines where different mitochondrial genomes (mtDNA) are substituted into different nuclear DNA (nDNA) backgrounds. As a proxy for metabolic function, we measured resistance to starvation reasoning that an impairment in nutrient processing and storage will increase sensitivity to starvation. This approach identified a case where the introduction of a specific mtDNA rescues a nuclear defect in starvation resistance (sil mtDNA on DGRP 765 nDNA background), but has no effect in other nDNA backgrounds. Using chromosome mapping, we determined that a locus on chromosome 3 modifies this mtDNA dependent resistance to starvation. In addition to this, the sil;765 mito:nuclear combination has increased triacylglycerol levels compared to another mito-nuclear combination (Zim53 mtDNA on DGRP 765 nDNA background) which suggests that the differences seen in starvation resistance may result from changes in lipid metabolism. This finding highlights a novel genetic interaction between mtDNA encoded genes and lipid metabolism.

383  **Effects of Hemocytes and Hemocyte Signaling on Longevity in Drosophila.**  Allyson Sams, Matthew Giedd, Anton Bryantsev Molecular & Cellular Biology Department, Kennesaw State University, Kennesaw, GA.

In flies, hemocytes are universal immune cells that circulate in the hemolymph and participate in cellular and humoral immune responses. In this work, we used hemocyte-specific Hml driver to affect properties of hemocytes in adult flies. Expression of proapoptotic gene rpr resulted in a ten-fold reduction of hemocyte counts without causing apparent adverse
effects to flies. Unexpectedly, the experimental flies with depleted hemocytes lived substantially longer than their control counterparts. An alternative approach to reduce hemocyte amounts by knocking down expression of anti-apoptotic gene Rbf1 also resulted in lifespan extension. Although in this case lifespan extension was less profound, it was in a good agreement with the lesser extent of hemocyte count reduction. Conversely, expression of constitutively activated oncogene mutant RasN17 (but not dominant-negative mutant RasN17) moderately increased hemocyte counts and shortened lifespan of the experimental flies. Collectively, our data strongly suggest that the amount of hemocytes in adult flies inversely correlates with their longevity.

In order to probe for the molecular mechanism that enables hemocytes to affect longevity, we addressed the central molecule in hemocyte humoral immune signaling - Rel (Drosophila homolog of NF-kB). Experimental flies with hemocyte-specific knockdown of the Rel gene demonstrated an extension in their lifespan. Conversely, when hemocytes expressed the activated truncated form of Rel (Rel68), it shortened fly lifespan. We conclude that Rel plays a role in hemocyte-dependent longevity, potentially via stimulating expression of secretory peptides.

Our study adds up to the growing evidence that the immune system plays a significant role in longevity.

384 Exploring the role of histones in replicative and organismal ageing in Drosophila melanogaster. S. Chari, A. Amodeo Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

Ageing is a complex biological phenomenon that affects all organisms and is influenced by genetic, epigenetic and environmental factors. Ageing can be classified into two non-mutually exclusive and broad categories. Replicative ageing is the process where mitotically active cells are unable to divide further and become senescent. Organismal ageing refers to the general lifespan and senescence of an entire organism. The link between replicative and organismal ageing is unclear, but may be due in part, to tissue dysfunction caused by loss of replicative capacity and cellular senescence. Specific chromatin changes are associated with ageing, including a general loss of histone protein content on DNA in aged yeast. In S. cerevisiae increased histone levels extends replicative lifespan, and in C. elegans and M. musculus, certain lifespan extension regimes are accompanied by increased histone expression. To test the role of histone loss in the aging of Drosophila melanogaster we generated mutant lines that conditionally overexpress both H3 and H4 histone subunits either ubiquitously or in a tissue specific manner and examined their effects on adult lifespan. Preliminary data from ubiquitous histone overexpression suggest that the overexpression extends adult lifespan in these flies. We will extend these results to multiple phenotypes associated with replicative and organismal ageing and address if replicative tissues, such as the gut, are the primary contributors to this effect. Further, we will explore if histone levels influence the fecundity-lifespan trade-off.

385 RiboTag Profiling of aging and oxidative stress responses in adult hepatocyte-like cells. K. Huang, H. Bai Iowa State University, Ames, IA.

Background: During aging, multicellular organisms undergo metabolic and cellular changes. Liver as an important metabolic organ is often challenged with oxidative stress during aging. Age elevated reactive oxygen species (ROS) has been implicated in many human diseases, including nonalcoholic fatty liver disease. Yet, a clear mechanism for liver aging and its interplay with oxidative stress is lacking.

Method: Oenocytes are specialized hepatocyte-like cells in Drosophila that are primarily responsible for lipid metabolism. Here, we performed ribosome-associated mRNA profiling using RiboTag-seq technique to characterize the differential gene expression in oenocytes extracted from aging and paraquat-treated flies. We have identified high-resolution translatonin profile on aged oenocyte and paraquat-fed oenocyte.

Results: Our RiboTag-Seq results show that young oenocytes show greater variation in gene expression upon oxidative stress compared to old oenocytes. Aged flies exhibit similar gene expression, but not inclusive to flies under oxidative stress. Pathway analysis revealed that aging suppresses the expression of genes involved in oxidative phosphorylation, peroxisome, and lipid metabolism. Both aging and oxidative stress elevate DNA repair pathway. Interestingly, paraquat induces the expression of genes involved in Toll and Imd pathways at young age, while suppresses them at old age.

Conclusion: Although both aging and oxidative stress regulate a similar set of genes in oenocytes, old adult oenocytes exhibit reduced transcriptional activation upon oxidative stress than that found in young oenocytes. Furthermore, many pathways (e.g. innate immunity) show distinct stress responses at different ages. Thus, oenocyte-specific transcriptional activities and tissue homeostasis are likely influenced by integrated signals from aging and environmental stresses.

386 Circadian environmental cues modulate aging in Drosophila melanogaster. J. Johnson, H. Richardson, S. Pletcher Molecular Integrated Physiology, University of Michigan, Ann Arbor, MI.

Across taxa sensory perception modulates health and aging. Manipulations of only a few sensory neurons can improve health and increase lifespan. However, the manner and modalities by which sensory neurons orchestrate changes throughout the organism to promote beneficial effects on health and longevity remain unknown. One largely unexplored perceptual system in the context of longevity is time perception. In humans perturbations of the circadian system leads to
increased incidence of obesity, diabetes, and metabolic syndrome. This is of particular importance as the number of shift workers, a group susceptible to circadian disturbance, is on the rise. We used *Drosophila melanogaster* to study how environmental zeitgebers interact with biological rhythms to influence longevity. Here we test the hypothesis that greater environmental synchrony, and number of perceived days lived contributes to longevity and health-span. We find *Drosophila melanogaster* to be not affected by environmental light cycles. Our results establish time perception as a potentially modulatable cue to influence physiological state and longevity.


Resveratrol is the most widely known compound due to its ability to extend the lifespan of several model organisms. In our present study, we investigated the anti-aging effects of resveratrol-enriched rice callus DJ526. As we found from our previous work that the resveratrol-enzymed enriched rice DJ526 had expectedly beneficial health effects in mice. Using the fruit fly, *Drosophila melanogaster*, as a model organism, we demonstrated that the resveratrol-enriched rice callus DJ526 significantly extended lifespan surpassing the introduced genetic characteristic of resveratrol synthetic ability. The synergistic effect of its innate and transgenic properties not only ameliorates physical deterioration in advanced aged but also significantly extends lifespan. We analyzed locomotive activity, body weight, eye phenotype of wild-type *Drosophila melanogaster*. We found that resveratrol-enriched rice callus DJ526 is effective in extending lifespan and also maintaining healthy levels of body weight, locomotion and eye phenotype.

Keyword: Resveratrol; Lifespan; Callus; *Drosophila melanogaster*; synergistic

388 De novo retrotransposon insertions mediated by Myc affect lifespan and aging-associated phenotypes in *Drosophila melanogaster*. S. Lightcap¹, J. Secombe¹, J. Vigg¹, N. Neretti² 1) Albert Einstein College of Medicine, Bronx, NY; 2) Brown University, Providence, RI.

Increased age is a major risk factor for many of the leading causes of death in the U.S., including cancer, heart disease, and neurodegenerative disorders. As such, it is important to understand the mechanisms of aging in order to improve lifespan and health span (the period of time when a person is healthy). In understanding these mechanisms, we will identify potential targets for therapies to treat the process of aging and the diseases that arise as humans age.

Our goal is to define the contribution of one possible mechanism of genetic aging, accumulation of transposable element (TE) insertions. Our studies focus on one subclass of TEs, retrotransposons, which replicate via a copy-and-paste mechanism that increases the copies of the TE over time. Using a single cell whole genome sequencing approach, we have generated preliminary data showing that de novo TE insertions increased with age, and that some retrotransposons were more active than others. To understand the impact of new TE insertions on aging, we are characterizing their genomic locations (e.g. promoters, introns, exons). We are also defining the contribution of TE mobilization to aging by using inducible shRNA transgenes to knock down (KD) the expression of specific TEs individually or in combination. Consistent with TE insertions contributing to aging, ubiquitous KD of one TE, 412, extended the lifespan of males and females. We will be analyzing aging-associated phenotypes in TE-KD flies to determine the impact of retrotransposons on health-span.

Another missing piece in the literature is the mechanism by which TEs are transcriptionally activated and inserted, especially in relation to possible age-associated increases in both of these activities. We posit that the transcription factor Myc is important for this process. Previous studies demonstrated that decreasing Myc expression extends lifespan in mice and flies. Conversely, higher levels of Myc resulted in a shortened lifespan and increased expression levels of several retrotransposons. We are interested in elucidating the mechanism by which Myc activates a subset of transposons, and whether it involves a direct binding interaction with TEs or occurs via an indirect mechanism. Using inducible TE knockdown strains, we will also address the contribution that TE activation plays in the lifespan effects mediated by Myc.

389 How can the anti-senolytic drug combination of Dasatinib and Quercetin extend lifespan in *Drosophila melanogaster*? J.D. Parker, M. Valentine, P. Bejo, F. Capobianco III, Bio 490 student group C Dept of Biological Sciences, SUNY Plattsburgh, Plattsburgh, NY.

Cellular senescence is a state of metabolically active cells that experience an irreversible growth arrest upon some type of stress. Recently cellular senescence has been tied to age related diseases (Zhu et al, 2015). Physiological aging is also associated with increased levels of ROS, but it is not understood how senescence and oxidative stress react to induce aging. We previously found that a combination of senolitics (Dasatinib and Quercetin) increase the lifespan of one wild type line *Drosophila melanogaster*, with no effect on the other. Previous experiments of our lab have shown a difference in oxidative stress response between these two genetic backgrounds. An undergraduate group in Investigative Biology Experience (Bio 490) will be presenting their work extending these findings by testing for differences in how the anti-senolitics affect oxidative damage accumulation and autophagy gene activation in the lines with these lifespan response differences.
390  **How does social environment affect aging in Drosophila melanogaster?**  J.D. Parker¹, M. Valentine¹, T. Segre¹, J. Tromblee¹, J. Binnsteam, Bio 490 student group A ¹) Dept. of Biological Sciences, SUNY Plattsburgh, Plattsburgh, NY; 2) Dept. of Biological Sciences, University of Southampton, Southampton, UK.

Ruan (2008) discovered that social environment (specifically the presence of young individuals) can rescue the phenotype of a cytoplasmic superoxide dismutase (SOD) mutant. We replicated this effect in another SOD mutant, and additionally found that the presence of young helpers extended lifespan on one of two wild caught lines cleared of Wolbachia. The line (Plattsburgh) where lifespan was extended was also rescued from oxidative stress by the presence of Wolbachia suggesting that both social environment and Wolbachia infection can compensate for oxidative stress limiting lifespan on some genetic backgrounds. An undergraduate group in Investigative Biology Experience (Bio 490) will be presenting their work extending these findings by testing for differences in oxidative damage accumulation and autophagy gene activation in the presence and absence of helpers in the lines with lifespan differences.

391  **Toward a Genome-Wide Association Study of Diet Related Mortality in Drosophila melanogaster: High Sugar Diet.**  S.Provinbhai. Patel, Bridget Konadu, Matthew Talbert Biology, University Of Louisiana Monroe, Monroe, LA.

Diet-induced obesity elevates risk of diabetes, cardiovascular disease, neurological decline and cancer, which increases the risk of mortality. Their short lifespan, sequenced genome and analogous physiological systems with mammals makes them a good model for the genomic study of obesity-induced mortality via a high sugar diet (HSD). The HSD state is induced by providing sucrose in a disproportionate amount to yeast in their solid diet, providing sucrose as a nutrient source compared to protein in a ratio of 5:1 w/v in our case. Using the Drosophila Genetic Reference Panel (DGRP), a genome-wide association study (GWAS) of lifespan on HSD will be performed. The DGRP is a population of 200 repeatedly inbred, wild caught flies: their whole genomes sequenced, their existing variation genotyped and publicly available. The DGRP has been previously used to identify many genes underlying lifespan, triglyceride storage and climbing reflex on normal dietary conditions. In this study, 20 synchronously mated adult females from each of the 193 DGRP lines were subjected to our HSD and average lifespan of each line was determined. Lifespan on a HSD showed significant variation across lines, with DGRP 911 surviving the longest for an average for 74.3 days and DGRP 832 the shortest for an average of 10.2 days. The DGRP analysis pipeline was utilized, determining association between polymorphisms and lifespan on a HSD after adjustment for Wolbachia infection status, inversion status, and cryptic relatedness. Wolbachia infection and the five major inversions: (In(2L)t, In(2R)NS, In(3R)K, In(3R)P, and In(3R)Mo had no significant effect on lifespan, p>0.05. Polymorphisms with the most significant p-values ranged from 1.7 X10⁻³ to 1.0X10⁻¹ and they identify genes involved in neural processes, behavior, development, and apoptosis, among other functions.

392  **Dh31 signaling regulates Drosophila oogenesis.**  T. Ma, M. Markwei, D. Drummond-Barbosa Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

Neuropeptides are evolutionarily conserved signaling molecules that lie at the intersection between the environment and our physiology and regulate many aspects of biology, including reproduction. In mammals, previous studies have shown that changes in nutritional status, as transmitted by hormones such as leptin and insulin, lead to the release of neuropeptides such as kisspeptin and neuropeptide Y (NPY). These neuropeptides then affect the release of gonadotrophin releasing hormone to regulate many aspects of reproduction, including stimulating ovulation in females and promoting germ cell maturation in males. NPY signaling has also been shown to modulate the survival and proliferation of some cancerous tumors. However, many questions still remain about how neuropeptides affect reproduction and, more generally, stem cells and their progeny. For example, can neuropeptides act as endocrine hormones? Can neuropeptides signal directly to stem cells? What other neuropeptides regulate reproduction, and what environmental or physiological changes do those neuropeptides convey? Are there other neuronal circuits involved in the regulation of reproduction? We use Drosophila melanogaster as our model system to probe some of these questions. Through three RNAi screens, we identified the neuropeptide diuretic hormone 31 (Dh31) and its cognate receptor Dh31-R as candidates that may be involved in the regulation of oogenesis. Adult-specific knockdown of Dh31 pan-neuronally led to a decrease in the number of eggs laid compared to controls. Adult-specific somatic knockdown of Dh31-R produced similar results. When we examined the ovaries of these genotypes, we found a statistically significant decrease in the number of early germline cysts. Additionally, Dh31 hypomorphic ovaries look normal upon eclosion but rapidly deteriorate, with death seen in the early stages of oogenesis in most ovarioles. We are currently working on fully characterizing the phenotypes observed upon loss of Dh31 signaling using both Dh31 and Dh31-R mutants. In addition, we are using neuron- and other tissue-specific knockdowns to determine what neurons produce Dh31 to regulate oogenesis and what organs require Dh31-R to relay that signal to the germ cells. Our results will lead to a better understanding of the various mechanisms through which neuropeptides affect reproduction.

393  **Neprilsyn 4 modulates SERCA activity via cleavage of Sarcolamban A.**  R. Schiemann¹, M. Stuke¹, P. Ferrero², A. Paululat¹, H. Harten¹ ¹) Zoology and Developmental Biology, Osnabrueck University, Osnabrueck, Lower Saxony, DE; 2) Cardiovascular Research Center, National University of La Plata, La Plata, Argentina.

Muscle contraction represents a highly conserved and well characterized molecular process that requires proper regulation
of the intracellular Ca\(^{2+}\) concentration. By transporting Ca\(^{2+}\) from the cytosol into the sarcoplasmic reticulum (SR), the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) constitutes a major player regulating this concentration. Dysregulation of SERCA severely affects initiation of muscle relaxation, which renders tight regulation of SERCA activity crucial to proper heart and muscle function.

As known for vertebrates, SERCA activity is regulated by binding of certain peptides, e.g. Phospholamban or Sarcolipin. In *Drosophila melanogaster*, at least two peptides are known to mediate this functionality, Sarcolamban A (SclA) and Sarcolamban B (SclB). By binding to SERCA, the Scl peptides significantly reduce its activity (Magny et al., 2013). Consequently, Scl Loss-of-function mutants exhibit impaired Ca\(^{2+}\) transients in heart cells, concomitant with severe heart arrhythmia. Up to now, it is largely unknown how turnover of such peptides within membranes of the SR is regulated. Interestingly, we found that increased expression of the peptidase Nepriylsin 4 (Nep4), which also localizes to the SR membrane, phenocopies these effects on heart physiology. In addition, SERCA-activity is considerably elevated in corresponding animals, which presumably represents the physiological explanation for the phenotypes. Ectopic expression of catalytically inactive Nep4 is without consequences, which confirms that impaired catalytic activity, and thus abnormal peptide hydrolysis, represents a causative factor.

In order to characterize the molecular mechanism by which Nep4 regulates SERCA activity, we initially assessed orientation of Nep4 in the SR membrane. Based on the results we performed cleavage assays, which confirmed that Nep4 is able to hydrolyze SclA at its C-terminus. Heart-specific expression of the resulting truncated peptide will reveal the physiological relevance of the cleavage event. In addition, simultaneous overexpression of SclA and Nep4, combined with super resolution microscopy and pull-down assays, will help to further elucidate the Nep4-Scl-SERCA interaction.

Our detailed analysis of the Nep4 mediated regulation of SERCA activity will significantly advance the current understanding of muscle physiology and functionality.


394 Octopamine drives endurance exercise adaptations in *Drosophila*. Alyson Sujkowski, Divya Ramesh, Axel Brockmann, Robert Wassels 1) Physiology, Wayne State University, Detroit, MI; 2) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India.

Endurance exercise is an effective therapeutic intervention with substantial pro-healthspan effects. Male *Drosophila* respond to a ramped daily program of exercise by inducing a conserved suite of physiological responses similar to those seen in mice and humans. Female flies respond to an exercise stimulus, but do not experience the adaptive training response seen in males. Here, we use female flies as a model to demonstrate that differences in exercise response are mediated by differences in neuronal activity. The activity of octopaminergic neurons is specifically required to induce the conserved cellular and physiological changes seen following endurance training. Additionally, we find that all 4 octopamine receptors are required for at least one aspect of the exercise response, but only one, Octβ2R, is required for all of them. Excitingly, either intermittent, scheduled activation of octopaminergic neurons, or octopamine feeding, is able to fully substitute for exercise, conferring a suite of pro-healthspan benefits to sedentary *Drosophila*. These experiments indicate that octopamine is a critical mediator of adaptation to endurance exercise in *Drosophila*.


Seminal fluid proteins (Sfps) are male reproductive molecules that are transferred to the female during mating, and enhance female fertility. However, the pathways through which Sfps act to alter female post-mating physiology remain elusive. We focus here on ovulin, a 264 amino acid pro-hormone-like Sfp that stimulates short-term increases in ovulation. We are employing complementary genetic and biochemical approaches to identify ovulin’s receptor (OvR). OvR candidates were initially identified through an evolutionary rate co-variation (ERC) screen. The list was further narrowed by removing genes not expressed in the nervous system or female reproductive tract, and checking the remaining genes for egg-laying effects upon knockdown. We are currently testing two candidates for interaction with ovulin. Ovulation assays will determine whether females with a deletion (or knockdown) of either candidate OvR locus phenocopy wild-type females mated to ovulin-null males. Cell-culture based calcium assays and the *in vivo* Tango assay are being used to test for direct ovulin/OvR binding. Identifying OvR will allow us to determine where ovulin acts, and how this Sfp and its receptor co-evolve.

396 PERIOD O-GlCNacylation regulates its interaction with CLOCK to prevent premature initiation of circadian repression phase in the *Drosophila* clock. Y. Li, X. Liu, J. Vaneslow, A. Schlosser, H. Zhang, J. Chiu 1) Department of Entomology, University of California Davis, California; 2) Rudolph Virchow Center, University of Wurzburg, Wurzburg, Germany; 3) Department of Molecular Biology and Biochemistry, Rutgers, the State University of New Jersey, New Jersey.

Circadian clocks coordinate time-of-day specific metabolic and physiological processes to maximize organismal
The Tribbles (Trib) family of pseudokinases are adaptor proteins for E3 ubiquitin ligase-mediated proteolytic and non-proteolytic functions. Notably, human Trib3 binds Akt to block insulin signaling and hence represents a potential drug target to treat Type II diabetes. To better understand the conserved developmental and physiological functions of Trib family members, we have combined a structure-function analysis of Trbl with genetic screens of candidate regulators and targets. We previously showed that fly Tribbles (Trbl) inhibits Akt phosphorylation to promote tissue catabolism. To test the interaction between Trbl and Akt further, we misexpressed a membrane-targeted form of Akt (myr-Akt) in the fat body and observed strong Trbl accumulation to the inner leaf of the cell membrane, demonstrating that Trbl-Akt co-localization occurs in vivo. To determine whether insulin pathway components promote Trbl trafficking to the membrane, we used RNAi knockdown to show that reduction of TSC2 resulted in striking cortical accumulation of WT Trbl. To identify the domains in Trbl that mediate TORC1 pathway regulation of Trbl trafficking, we used mutagenesis to identify a conserved motif (ESLE286) that resembles WT Trbl, misexpression suppresses Neur wing phenotypes and blocks Neur-dependent macrochetae patterning. In an ovarian tissue model, Trbl stabilizes Neur to activate Notch signaling. In vivo functional characterization indicates that O-GlcNAcylation at PER(S942) reduces interactions between PER and CLOCK (CLK). We propose that PER(S942) O-GlcNAcylation in the day time functions to prevent premature initiation of circadian repression phase. Taken together, our results support that clock-controlled feeding activity provides metabolic signals to reinforce light entrainment to regulate circadian physiology via intricate balance between clock protein O-GlcNAcylation and phosphorylation.

The core functions of the insulin signaling (IIS/TOR) pathway are in nutrient sensing, energy homeostasis and growth. Insulin signaling is known to interact directly and indirectly with sex determination, and often plays a role in regulation and development of sexually dimorphic traits. For example, in Drosophila the IIS/TOR pathway is required during development for body size dimorphism and in adults for activity level dimorphism and modulation of mating behaviors. To understand the degree to which the insulin signaling pathway contributes to sexually dimorphic gene expression in adult animals, we examined the effect of perturbation of the pathway on gene expression in male and female Drosophila. A dominant negative insulin-like receptor transgene (InR<sup>dn</sup>) was expressed in adults using the drug inducible, ubiquitously expressed, Gene-Switch GAL4 system. Expression was assessed by RNA-seq of head tissues, in replicate for each sex expressing the dominant negative allele, and for genetically matched controls. Our data reveal that males and females have a shared regulatory response to knock-down of InR function by Gene-Switch. This shared response is heavily enriched for genes and pathways involved in metabolism. However, a large number of genes also show striking sex differences only under the perturbation conditions; conditions that drastically decrease the ability of the organism to respond to insulin signaling. Interestingly, this includes sex-differences in expression of immune, defense and stress response genes primarily driven by male-specific effects of the perturbation. Although, female specific effects, predicted to be associated with lifespan extension, are also
observed. Finally, a subset of genes are dimorphically expressed only when insulin signaling functions normally. These include energy homeostasis genes regulated by insulin signaling, including those already known to be sex-differentially expressed in Drosophila (e.g. sxe2). Collectively our results suggest that insulin signaling is important for sex differences related to energy homeostasis and may also mediate differences between males and females in stress responses, including defense responses and potentially the response to infection. Collectively, these results have broad implications for the role of insulin signaling in the physiological underpinnings of trade-offs, sexual conflict and sex differences in expression variability.

Identification of transcriptional mechanisms that locally and distantly control cell and whole animal size in response to fat body Toll signaling. M. Suzawa, N. Muhammad, B. Joseph, M. Bland Pharmacology, University of Virginia, Charlottesville, VA.

In Drosophila, Toll signaling is activated in response to Gram-positive bacterial or fungal infection and drives synthesis of anti-microbial peptides (AMPs) that carry out the humoral arm of the immune response. We previously reported that Toll signaling inhibits insulin signaling and fat body cell growth by interfering with phosphorylation of Akt by Pdk1. Interestingly, activating Toll signaling in the larval fat body also reduces whole animal growth. However, it remains unclear what molecules act locally in the fat body and distantly in the whole animal to control growth in response to Toll signaling in fat body. To address this question, we first undertook RNA sequencing (RNA-Seq) to identify genes that are induced or repressed in response to Toll signaling in the fat body. Total RNA for RNA-Seq was isolated from third instar larval fat bodies with or without adult, 12 hour induction of a constitutively-active Toll (Toll10s) transgene. Predictably, the top genes up-regulated by Toll signaling were AMPs. Intriguingly, Insulin-like peptide 6 (ilp6) was significantly reduced by Toll signaling in fat body. Second, we focused on two major NF-kB homologs, Dif and dorsal, that act downstream of Toll to drive AMPs. Dif and dorsal transcript and protein expression were up-regulated by Toll signaling in fat body. We examined how Dif and dorsal affect fat body cell and whole animal and adult wing size by over-expressing or knocking down Dif or dorsal in the larval fat body with or without expression of Toll10s. Interestingly, Dif was necessary and sufficient to decrease fat body cell growth and both whole-animal growth and adult wing size. On the other hand, dorsal was sufficient to reduce whole animal and adult wing size but not fat body cell size. Furthermore, dorsal was not necessary for Toll signaling to inhibit fat body cell growth. In summary, Dif and dorsal may drive expression of distinct sets of genes to control growth locally in the fat body (Dif targets) and distantly in the periphery (Dif and dorsal targets).

Characterizing a null mutant for spargel/dPGC-1, a homologue of mammalian PGC-1 gene supports its essential requirement in embryonic development. Mohammad Basar, Atanu Duttaroy Biology, Howard University, Washington, DC.

Peroxoxome Proliferator Activated Receptor g (PGC-1) is a key transcriptional coactivator in mammals that is widely involved in many physiological processes, including mitochondrial biogenesis, oxidative metabolism, adaptive thermogenesis, fiber type switching in skeletal muscles, antioxidant defense and heart development. The single Drosophila melanogaster orthologue of PGC-1, known as spargel/dPGC-1 is also known to play a diverse role in Drosophila including ovarian growth and development, intestinal stem cell homeostasis, nutrient sensing and mitochondrial OXPHOS. The neonatal death of double knockouts PGC-1a/b mice indicates PGC-1 is important in mammals during early development. Homozygous spargel hypomorph (srl/srl) and ubiquitous down regulation of spargel through RNAi leads to delayed eclosion of adults with smaller body size and shortened adult life span. These observations call for a spargel null mutant for further study of this gene. We created a spargel null mutant (srl-/-) using CRISPR/Cas9 whereby major part of the spargel's coding region has been deleted including exon 2 to 6. Homozygous srl-/- null mutants die in the embryonic stage as no homzygous larvae were recovered. We believe maternally contributed spargel helps through the initial stages of embryogenesis but lack of zygotic spargel expression impedes larval hatching. Available in situ hybridization data from Flyexpress 7 supports that spargel transcripts are expressed heavily during embryogenesis explaining the early lethality of spargel nulls. Currently we are analyzing the spargel expression during early development using a spargel monoclonal antibody. Finally, with the help of same CRISPR/Cas9 technology we now removed the RRM (RNA Recognition Motif) and the Serine/Arginine (RS) repeats of spargel. A complete biological characterization of various spargel alleles will be presented.

Activin-Beta/TGF-Beta signaling in skeletal muscle controls insulin/TOR signaling, metabolism and final body size. L. Moss-Taylor, X. Pan, M. O'Connor GCD, University of Minnesota, Minneapolis, MN.

Inter-organ communication is essential for regulating both development and homeostasis. Mutations in the gene coding for the Drosophila TGF-Beta ligand Activin-Beta (Act-Beta) cause accelerated pupariation and reduced final body size. To determine how Act-Beta affects size and timing, we first looked at which cells express Act-Beta and found expression in the Insulin Producing Cells (IPCs), neuroendocrine cells, and motor neurons. In Act-Beta mutants, overexpressing Act-Beta in motor neurons or skeletal muscle rescue body size but not developmental timing, indicating Act-Beta regulates these processes independently. Accordingly, the growth rate is reduced in Act-Beta mutants, demonstrating the size phenotype is not simply due to early growth termination and precocious pupariation. Muscle-specific knockdown of the TGF-Beta signaling transducer/transcription factor dSmad2 also reduces body size, identifying muscle as a target tissue of the Act-Beta signal. Additionally, levels of phospho-dSmad2 are reduced in skeletal muscle samples of Act-Beta mutants and increased in
animals overexpressing Act-Beta from motor neurons. Levels of phospho-S6K in Act-Beta mutants are correlated with phospho-dSmad2 levels, suggesting TGF-Beta signaling regulates insulin/TOR signaling. Because insulin signaling controls metabolism, we used GC/MS analysis to identify and quantify levels of metabolites in whole-larval samples of Act-Beta mutants. We found intermediates of the energy-producing steps of glycolysis and lactic acid are reduced in Act-Beta mutants. Overall, this indicates neuronally-derived Act-Beta signals to the skeletal muscle to regulate levels of insulin/TOR signaling, metabolism and growth to control body size. We have identified over 300 downstream targets of dSmad2 using RNA-seq of Act-Beta mutant skeletal muscle and are testing the function of certain target genes in skeletal muscle to determine how Act-Beta/dSmad2 signaling regulates insulin/TOR signaling, metabolism and body size.

402 Ribosome synthesis and the control of growth and development in Drosophila. Lisa Deliu1,2,3, Savraj Grewal1,2,3, Abhishek Ghosh1,2,3, Clark H Smith Brain Tumour Centre, Arnie Charbonneau Cancer Institute, Calgary, Alberta, CA; 2) Alberta Children’s Hospital Research Institute, Calgary, Alberta, CA; 3) Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, CA.

Drosophila final body size is determined by the growth rate and duration of the larval developmental period. These processes are influenced by the rate of protein synthesis and ribosome biogenesis. Recent findings suggest that the inter-organ signaling required for larval growth is regulated by protein synthesis. We are exploring these links between ribosome function and development further, using minutes, a class of of ribosomal protein (rp) heterozygote dominant mutants. We analyzed three minutes (rpS13+/+, rpS26+/+ and rpL38+/-) and found that each display the classic minute developmental delay phenotype. We also observed that they had altered growth rates and that they showed increased final pupal size, each to variable extents. However, we found that the minutes showed little or no change in either global ribosome numbers (as measured by 18S and 28S rRNA levels), and no change in overall protein synthesis rates, compared to wild-type controls. Recent work in other model organisms has raised the idea that ribosomal proteins may play a role in selective mRNA translation in various tissues. We are therefore investigating a) whether these minutes may exhibit selective changes in mRNA translation, and b) the contribution of cell-autonomous vs. non-autonomous effects on the overall organismal growth and development phenotypes. In particular, we are examining possible roles for ribosomal proteins in neuroendocrine tissues important for controlling larval growth and development.

403 A role for takeout and juvenile hormone in the high fat diet obesity state of Drosophila melanogaster? Zachary Palowsky, Caleb Green, Sumit Patel, Matthew Talbert. Department of Biology/School of Sciences, University of Louisiana at Monroe, Monroe, LA.

After we observed an increased head capsule expression of takeout during an RNA sequencing and expression microarray in adult flies exposed to high fat diet (HFD), we were led to further explore the topic. In our case, the HFD is a 10% w/v sucrose/yeast medium supplemented with 20% w/v coconut oil. There are known links between takeout and longevity, foraging behavior, and food intake in adult Drosophila melanogaster. There is also a known binding interaction between takeout and juvenile hormone. By using existing Met mutant strains, GAL4-UAS lines, as well as the juvenile hormone analogue methoprene, we can begin to appreciate their respective roles in the context of a HFD and the obesity-like state in Drosophila. We observed triglyceride content, negative geotaxis reflex, feeding quantity, and average lifespan for mated female Canton-S flies that were administered methoprene dissolved in ethanol. In our negative geotaxis assay, the obesity-like state induced by HFD caused an expected decline in climbing performance, but consistent methoprene administration for adults resulted in a significant increase in climbing pass percentage for HFD flies after 7 days of adult diet exposure (P=.0024, as per ANOVA). The climbing performance of HFD/methoprene exposed flies was not significantly different from normal diet/methoprene exposed flies. Any effect of methoprene on climbing was not discernable at 14 days of consistent drug and diet exposure, although the effect of HFD remained. HFD expectedly increased triglyceride content of whole flies, but methoprene exposure had no effect on triglyceride content within either diet. Neither HFD or methoprene exposure impacted feeding quantity significantly. Lifespan decreased as expected with HFD, but the observed impact of methoprene on lifespan will be later reported.

404 Identification and functional characterisation of two putative ecdysteroid kinases in Drosophila. J. Scanlan1, R. Gledhill-Smith1, P. Batterham2, C. Robin1 1) School of BioSciences, The University of Melbourne, Parkville, VIC, AU; 2) Bio21 Institute of Molecular Science and Biotechnology, Parkville, VIC, AU.

The activity of ecdysteroids, the arthropod moulting hormones, can be negatively regulated by conjugation reactions, the most widespread of which in insects is phosphorylation. The physiological functions of specific phosphate conjugates, and the identities of the kinases that synthesise them, however, are almost entirely unknown. Ecdysteroid 26-hydroxylation is an intermediate step in a developmentally essential carboxylation pathway mediated by the cytochrome P450 Cyp18a1, and in the Drosophila melanogaster S2 cell line, this irreversible 26-carboxylation has previously been shown to be blocked by constitutive phosphorylation of the 26-hydroxyl group. We hypothesised that CG13813, a member of the poorly characterised EcdKine/DUF227 gene family, may encode the ecdysteroid 26-kinase responsible for this reaction, due to a collated body of
published data, including its positive regulation by 20-hydroxyecdysone and ecdysteroid-response pathways, enrichment in the prothoracic gland, and co-expression with Cyp18a1. Our phylogenetic analyses of insect EcKinasases demonstrate that CG13813 and its paralog CG1561 are Dipteran orthologs of a known Lepidopteran ecdysteroid 22-kinease, BmEc22K. RNAi knockdown of CG13813 suggests it is essential for larval and pupal development, and its ectopic misexpression produces many tissue-specific phenotypes, including embryonic lethality, larval moulting defects, cuticle and tracheal melanisation, extended larval wandering, and defects in metamorphosis. Knockdown of CG1561 results in head eversion defects, pharate adult lethality and a "drowning in quicksand" phenotype upon eclosion, while its ectopic misexpression specifically arrests the L2/L3 molt. Many of these phenotypes are reminiscent of those seen upon perturbation of ecdysteroid signalling. We are currently testing possible genetic interactions between these putative ecdysteroid kinases and known ecdysteroid catabolic pathway genes, including Cyp18a1 and a putative ecdysteroid-phosphate phosphatase (CG13604), as well as components of ecdysteroid signalling pathways. These results may lead to deeper insight into the developmental roles of ecdysteroid conjugation in Drosophila melanogaster and other insects.

**405 Disruptions in fried/CG31320 cause precocious larval wandering, delayed pupariation, and larvaal lethality.** Zelie Anner, Kalliopi Chatzis, Abigail Cross, Siwen Xie, Jason Morris  Natural Sciences, Fordham University, New York, NY.

Drosophila fried alleles were initially identified in an ovary germline clonal screen for mutations that disrupt oogenesis (Morris et al., 2003). We have shown that fried alleles disrupt CG31320, which encodes HEATR2, a HEAT Repeat protein required for cilia assembly in the mechanosensory neurons of the embryo (Diggle et al., 2014).

Fried mutants frequently undergo precocious wandering and they arrest as larvae rather than pupariating. They die within seven days after egg deposition following a progressive darkening of the trachea. In order to study Fried/HEATR2 protein expression and subcellular localization during larval stages, we are employing CRISPR-Cas9 to tag Fried protein with a V5 amino acid sequence at the C terminus of the protein.


**406 Novel Microbe-Regulated Host Genes, Proteins, and Traits Identified Through Transcriptome and Proteome Analysis of the Drosophila Head.** Scott Keith, Malachi Blundon, Rolly Eatsey, Jennifer Huang, Stacie Oliver, Brad Solomon, Heewook Lee, Carl Kingsford, Jon Minden, Luisa Hiller, Brooke McCartney  Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

Microbial symbions profoundly impact many physiological systems of their animal hosts to promote health and homeostasis. Yet our understanding of the exact molecular mechanisms by which the microbiota confer these beneficial effects on metazoans is limited. Using Drosophila and its bacterial microbiota as a tractable model system, we are delineating key components of the host-microbe molecular interface that impact host physiology and behavior via transcriptomics and proteomics of the fly head. To identify microbiota-dependent host molecular changes, we used these unbiased techniques to compare transcript and protein levels in the heads of germ free (GF) flies to levels in conventionally reared (CV), microbe-associated flies. Our motivation for examining the head transcriptome and proteome was to identify molecular changes occurring in the brain due to the accumulating evidence that symbiotic microbes can modulate fly behavior. In addition, the head contains a fat body, an endocrine organ that regulates metabolism and modulates behavior. RNA-seq profiling and Nanostring nCounter transcript analysis revealed several classes of genes differentially transcribed in the heads of GF flies, including genes involved in immunity, oxidative stress responses, and metabolic function. A limited, gnotobiotic bacterial community consisting of two *Acetobacter* and two *Lactobacillus* species was sufficient to revert these gene expression changes to conventional levels. We also used 2D Difference Gel Electrophoresis (2D-DIGE) to compare GF and CV head proteomes, and found 22 proteins with either increased or decreased abundance in GF heads. Two host proteins elevated in GF heads were Alcohol Dehydrogenase (ADH; ethanol metabolism) and Senescence Marker Protein 30 (*Drosophila* cold acclimation (SMP-30/Dca). These candidates were intriguing given their roles in a fly's responsiveness to ethanol and low temperature exposure, respectively. These physiological responses have not been shown previously to be influenced by the microbiota. Corresponding to these molecular alterations, we found that several parameters of a fly's physiological response to ethanol are dramatically altered by elimination of the microbiota, and that GF flies are more resistant to chronic cold exposure than microbe-associated flies. Our ongoing investigations seek to uncover mechanistic links between the observed gene expression and protein level changes, identified behavioral and physiological phenotypes, and the activity of specific bacterial members of the microbiota.
A dominant modifier screen to identify novel pH-sensitive proteins. Jeremy Middleman1,2, Daniel Orozco1, Donia Momen1, Donna Boucher1, Bree Grillo-Hill1 1) San José State University; 2) Vassar College.

The aging of an organism is associated with progressive diseases. These diseases, such as Limb-Girdle Muscular Dystrophy (LGMD), are characterized partly by the formation of protein aggregates in the cells. One of these cellular mechanisms which regulates aggregation and protein homeostasis in a cell is Chaperone Assisted Selective Autophagy (CASA). The CASA complex consists of Hsc70, HspB8, and starvin (stv/BAG-3). We find that the p38 MAPK regulates protein aggregation through the CASA complex as the fly ages. However, the targets of p38 MAPK and the CASA complex are unknown. In order to identify possible targets of p38 MAPK and the CASA complex, we have performed quantitative proteomics. Interestingly, one of these targets is lamin, a protein that makes up the nuclear lamina, part of the nuclear envelope. Another interesting aspect to lamin is that mutations in the gene cause LGMD along with Hutchinson-Guilford Progeria, a disease related to rapid aging. Since previous studies have shown that the CASA complex regulates protein homeostasis of a cell in regards to aging, we decided to test if lamin was a direct target of the CASA complex because it is related to both aging and disease. We found that lamin levels accumulate in both p38 MAPK mutants and stv knockdown muscles. In addition, we find that lamin co-immunoprecipitates with all the members of the CASA complex. These data suggest that p38 MAPK and the CASA complex may influence aging through the turn over lamin.

Muscle Atrophy in Cancer Cachexia. Ruth Silimon, Mary Baylies Weill Cornell Medical College, New York, NY.

Cancer cachexia is a severe muscle wasting syndrome often seen in patients with advanced stage tumors. Inflammatory cytokines, secreted by the tumor into the bloodstream, are well established mediators of the progressive loss in muscle mass. However, little is known about the temporal changes to muscle cell (myofiber) metabolism, organelle number and function in this pathological environment.

To address these gaps in our knowledge, we are using a Drosophila model of tumor-induced organ wasting (YkiACT) to investigate muscle in an environment that mimics cachexia in humans. In this model, adult flies conditionally expressing activated Yorkie (Yki) in intestinal stem cells rapidly develop intestinal tumors, and organ wasting subsequently ensues. We are using the YkiACT model to test the hypothesis that the perturbations in muscle function, morphology, and physiology are primarily due to early changes in autophagy. We will determine temporal changes in YkiACT muscle and test how genetic alterations in autophagy affect the wasting process.

Functional and physiological perturbations to the muscle have been measured over time using functional, imaging, and molecular analysis. The YkiACT flies show progressive decrease in muscle function, accumulation of ubiquitin aggregates, and loss of nuclei as the muscle wastes. This indicates that there are increased levels of proteins designated to be degraded and potentially enhanced macro-autophagy. These findings correlated with early increases in gene expression associated with autophagy. Further characterization will include measurements of muscle glycogen, triglyceride, and mitochondrial respiration.

Altogether, preliminary characterization of the YkiACT muscle indicate that perturbations in autophagy are early drivers of muscle wasting in cancer cachexia. Future directions include genetically manipulating effectors of autophagy in the YkiACT fly muscle to determine how they alter the wasting state. This information may aid in the development of biomarkers and early strategies for preventing cancer cachexia.

Genome-wide association analysis reveals novel regulators of basal autophagy in Drosophila. Ping Kang, Hua Bai Genetics, Development, and Cell Biology, Iowa State University, Ames, IA.
Macroautophagy (hereafter autophagy) was originally discovered as a cytoplasmic degradation process when cells are stressed and injured. Due to its important role in recycling cellular damage and maintaining tissue homeostasis, autophagy has been implicated in pathological and disease conditions, as well as organismal aging. Although basal autophagy remains at low levels in most of the tissues, impairments of basal autophagy often lead to disruption of tissue homeostasis and are associated with many human diseases. Currently it remains unclear whether basal (constitutive) and stress-induced autophagy are regulated by common or distinct mechanisms.

To examine the role of identified candidate genes in autophagy regulation, we selected 55 candidate for functional validation. Out of 55 genes tested, knockdown of the expression of 37 candidate genes (using loss-of-function mutants or RNAi) induced lysosomal activity or autophagosome number. Abelson tyrosine kinase (Abl) mutants are among those with the highest induction. To examine whether the autophagy induction in Abl mutants was caused by enhanced autophagic flux, we measured the autophagosome number before and after the treatment of bafilomycin A1 (Baf A1), a lysosome inhibitor. RNAi against Abl resulted in elevated autophagic flux, as indicated by the accumulation of autophagosome after the Baf A1 treatment. Taken together, our GWA analysis identified many novel basal autophagy regulators, and revealed an important role of Abl kinase in the regulation of lysosomal activity and autophagic flux. Importantly, our study represents the first evidence that high basal autophagy exists in genetically diverse natural populations of Drosophila.

411 High-Throughput mRNA Sequencing to Identify Components of the Polyamine Transport System. Michael Haney, Laurence von Kalm Biology, University of Central Florida, Orlando, FL.

Polyamines are components of the cell that control many vital cellular processes including cell growth and proliferation. Cancer cells have high levels of polyamines to maintain their growth and polyamine depletion is considered to be a attractive chemotherapeutic strategy. Cellular polyamine homeostasis is maintained by a balance between biosynthesis, import and export. Polyamine biosynthesis can be blocked with the FDA approved drug, difluoromethylornithine (DFMO), however most cancer cells circumvent this therapy by upregulating polyamine import from the extracellular environment. Thus, a therapy that simultaneously targets biosynthesis and import is desirable. While the polyamine biosynthetic pathway is well understood, the components of the polyamine transport system (PTS) remain poorly defined in multicellular eukaryotes. This gap in knowledge is remarkable given that almost all organisms have the ability to transport polyamines. To identify components of the PTS, we conducted an mRNA-sequencing assay in which we compared control gene expression with two different treatment conditions. Drosophila larvae were grown on media supplemented with polyamines alone (basal transport), or media containing DFMO and polyamines (obligate transport). Under obligate transport conditions animals must transport to survive because the biosynthetic pathway is blocked by DFMO. We identified 36 genes with human orthologs that were significantly up-regulated under obligate transport conditions, and an additional 16 genes with human orthologs that were significantly down-regulated under the same conditions. Surprisingly, several genes known to be associated with polyamine metabolism and transport were not among the genes we identified, suggesting that the transport system may be in-part regulated through post-transcriptional mechanisms.

412 Genetic analysis of rapid tracheal fluid absorption and air filling during Drosophila ecdysis. J.V. Alvarez, J.
Ewer Centro Interdisciplinario de Neurociencia de Valparaiso, Universidad de Valparaiso, Valparaiso, CL.

In insects, the tracheal respiratory system is the branched tubular network that brings air directly to the organs, and has served as a useful model for understanding how vascular systems develop and function. One process that is vital for insect survival is to maintain the trachea filled with air and free of liquid fluids. However, during each molt a new larger tracheal lining is secreted, which is initially liquid filled. The new tracheal system must rapidly be filled with air as the animal transitions to the next developmental stage. Air-filling is one of the first events of the ecdysial sequence, which culminates in the shedding of the remains of the old tracheal lining together the external cuticle from the previous stage. A failure to rapidly clear the new trachea of fluids and replace it with air is invariably fatal.

At ecdysis the liquid that fills the new trachea is rapidly absorbed during a process that includes the collapse of the tubular system (tracheal collapse; TC) followed by its expansion, as the trachea fills with air (air filling; AF). How TC-AF occurs at ecdysis is poorly understood. Nevertheless, it is disrupted when the peptidergic system that controls ecdysis is defective, as
occurs in Drosophila mutants for 2 key ec dysial neuropeptides, Ecdysis Triggering Hormone (ETH) and Eclosion Hormone (EH). We have previously suggested that EH plays a major role in orchestrating the TC-AF process during Drosophila ecdysis. Consistent with this, we found that the EH receptor (EHR), and not the ETH receptor, is expressed in the tracheal epithelium. In addition, knockdown of EHR in these cells causes a defective TC-AF phenotype in which tracheae seem unable to completely reabsorb the liquid at TC, causing tracheae to be partially liquid-filled by the end of AF. The absorption of liquids involves the uptake of ions from the fluid, which is mediated by ion channels such as sodium and potassium channels, as well as by water channels. A specific sodium channel family is expressed in tracheae and their actions, as well as those of AQP, a water channel, are mediated by cGMP signaling, which is also the second messenger of EH. We are using an RNAi based screen to identify effectors of TC-AF downstream of EH. To date, our results implicate specific sodium, potassium and AQP channels in this process. Interestingly, TC-AF defects are most intense when a potassium channel is knocked down. On the other hand, reducing expression of some sodium channel genes caused regions of the trachea to appear permanently collapsed.

Finally, and supporting the link between EH action and ion and water channel activation, we found that knockdown of a cGMP-dependent protein kinase (PKG) in the tracheae produces defective TC-AF. These results suggest that the motor program of ecdysis and tracheal air-filling are coordinated through a common neuropeptide trigger.

413 Complete deletion of the endogenous white gene using CRISPR/Cas9 to utilize a novel interhomolog recombination reporter assay. H.C. Bloomer, J.R. LaRocque. Human Science, Georgetown University, Washington, DC.

The white gene plays a vital role in Drosophila melanogaster genetic research for several reasons. First, the white gene has been well characterized molecularly. Second, mutations and modifications in the white gene are easy to distinguish phenotypically. Third, the white gene is not essential, and thus can be utilized in reporter assays measuring a range of genetic events. Despite these benefits, a complete deletion of the white gene is not widely available in the Drosophila community. To address this need, a complete deletion of the white gene (wd) was achieved using CRISPR/Cas9. Two guide RNAs (gRNAs) were designed and cloned into a Drosophila expression vector and subsequently injected into Cas9-expressing Drosophila embryos using standard CRISPR/Cas9 technologies. The gRNAs corresponded to sequences directly upstream and downstream of the white gene coding sequences. This led to Cas9 nuclease-mediated DNA double-strand breaks that resulted in the complete deletion of the white gene. The wd line has many potential applications, including utilization of the previously established DR-white and newly developed inwhite2 DNA double-strand break repair assays to determine the preference between using the sister chromatid or the homolog as a template for homologous recombination repair.

414 Characterization of exercise response genes in Drosophila melanogaster. N.C. Riddle, L.P. Watanabe. Biology, University of Alabama at Birmingham, Birmingham, AL.

Obesity affects approximately 25% of adults in the United States, and one of the most common treatments for obesity is an increase in physical activity or “exercise”. Despite the popularity of exercise as a treatment for obesity and as part of healthy living, little is known about how genetic background impacts the response to exercise. Drosophila melanogaster is a relatively new model system in the exercise field, but due to the large number of resources available, it has great promise. In this study, we use the Drosophila Genetics Reference Panel 2 (DGRP2) to carry out a genome-wide association study to identify genes controlling a) basal animal activity levels and b) activity levels during rotational induced exercise. We find significant variability in basal activity and exercise activity levels within this population, with activity levels differing by at least a factor of ten. In addition to a strong effect of genetic background, we also detected a significant impact of sex on activity levels. The GWAS identified approximately 100 genetic variants that significantly contribute to exercise-induced activity levels, some of which showed sex-specific impacts. Gene Ontology (GO) analysis revealed that many of the genes linked to exercise-induced activity levels have membrane-associated functions and contribute to neuromuscular junctions, response to stimuli, and metabolism. Approximately one-third of the candidate genes are currently uncharacterized, but they also include several loci which have been linked to locomotion (i.e. cac and Spn). These results demonstrate that we were successful in identifying genes that impact exercise-induced activity levels. Thus, this study validates the use of rotation-based exercise systems for Drosophila and demonstrates the potential of Drosophila to serve as a model in the field of exercise genetics.

415 Gut Microbiome Effects on Desiccation Resistance in Drosophila melanogaster. A. Darby1, S. Patton2, A. Gibbs1 1) School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV; 2) Nevada State College, Las Vegas, NV.

The microbiome is the collection of microorganisms that occupy an individual's skin and intestines, and it has many potential effects on an animal's physiology. Changes in the microbiome affect an organism's ability to tolerate certain stressors such as starvation. To our knowledge, no study has yet examined whether gut bacteria have any impact on an organism's ability to tolerate dry conditions, which is important to understand how animals may react to a drier climate. The Gibbs lab has selected for desiccation resistance in replicated populations of Drosophila melanogaster for over 225 generations, resulting in desiccation-selected (D) flies that survive desiccation ~50% longer than fed control (F) flies. D and F flies had similar numbers of gut bacteria. We generated axenic flies by washing embryos with bleach and rearing them on sterile media. Axenic D and F flies survived desiccation stress ~20% longer than non-sterile controls. Axenic flies did not lose
water more slowly than non-sterile controls; instead they were larger and contained more water. Our results suggest that the gut microbiome may affect insect survival in arid environments. Supported by the McNair Scholars Institute at UNLV and an REU supplement to NSF award IOS-1355210.

416 Determination of the Lethality of Thujone and its Derivatives on Adult Drosophila melanogaster. A.V. Mykytyn, Matthew Dickson, Emily Castner, Genieve Henry, Pavithra Vivekanand Chemistry, Susquehanna University, Selinsgrove, PA.

Thujone is a monoterpenoid that is present naturally in white cedar and a few other plant species. We investigated the insecticidal effects of cedar oil, thujone and a number of ester derivatives of thujone on *Drosophila melanogaster*. We also determined whether lethality required direct contact with the compounds. Male and female adult melanogaster were placed in 50-mL centrifuge tubes containing a small vial with 10 µL of the test compound or water as a control. To test lethality by contact, the vial was left uncovered, and to test the effects of volatility, a piece of mesh was secured over the vial. During the contact trials, commercial cedar oil and thujone were the most lethal, killing 100% of the flies. Thujol acetate was also very lethal and killed 100% of the flies in two of its trials, and the average number of flies killed across three trials was 87%. Thujol and thujol acetate were less lethal, killing only 72.2% and 50% of flies, respectively. Interestingly, thujol iso-nicotinate, an isomer of thujol nicotinate, was the least lethal, killing only 2.7% of the flies tested. The remaining derivatives will be investigated in the future, as well as the sub-lethal effects such as fertility of both males and female flies.

417 Maintaining cell identity by a single transcription factor and nuclear laminas. E. Bitman, N. Flint-Brodsly, O. Boico, M. Monastirioti, M. Gessler, C. Delidakis, H. Rincon-Arano, A. Orian 1) Rappaport Research Institute and Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; 2) Institute of Molecular Biology and Biotechnology (IMBB) Foundation for Research and Technology, Crete, Greece; 3) Biocenter of the University of Wu?rzburg Developmental Biochemistry Am Hubland Wu?rzburg, Germany; 4) Div. of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle WA, USA.

Maintaining the differentiated identity of adult cells and tissues requires active supervision. This regulation is essential for ensuring normal tissue physiology and homeostasis, and serves as a barrier against pathological reprogramming. Indeed, the ability of cells and tissues to maintain the differentiated identity is compromised during aging. In a search for aging-related identity regulators we found that the transcription factor Hey together with nuclear laminas supervise the identity of differentiated enterocytes (ECs). During aging, Hey protein levels decline and EC identity is lost. Moreover, expression of Hey in ECs restores cell identity, and gut integrity of aged flies.

G-TRACE Lineage tracing of post-mitotic ECs established that aged gut or young gut where Hey is targeted, are unable to maintain their unique nuclear organization. Genomic studies including Dam1D profiling and RNA-seq, unveiled that Hey and Lamins orchestrate this organization, establishing and maintaining enhancers’ activity, shifting from a stem-cell nuclear organization s into a differentiated one. Indeed, genetic ablation of Hey or ectopic-expression of stem-cell-related Lamin override EC identity programs, mimicking aging, resulted in loss of EC identity, reduced epithelial integrity and organismal survival. Thus, a single transcription factor concomitantly supervises chromatin and nuclear organization, safeguarding cell identity.

418 Carbonic anhydrases mediate respiratory activation in Drosophila melanogaster. N. Maziok, S Sweet, G. Jean, J. D. Baker, Biology, University of Miami, Coral Gables, FL.

The insect respiratory system is activated late in embryonic development via a process termed tracheal filling. During filling, the liquid of the tracheal lumen is replaced with a gas; this permits free gas exchange with tissues to meet oxygen demand and eliminate excess CO2. During this event two sub-processes must occur: liquid must leave the tracheal lumen (liquid absorption) and gas must replace the liquid (gas filling). Tracheal filling also occurs independent of contact with outside air, indicating that gas production is endogenous. Little is known about the cellular and genetic mechanisms that mediate this process. Interestingly, carbonic anhydrases, a well characterized class of enzymes that convert HCO3− and H+ to CO2 and H2O, can effectively supersaturate solutions with CO2 while promoting osmosis. We have found that *Drosophila* mutants for either Cah2 (homozygous lethal) or CG6074, two tracheally-expressed carbonic anhydrase genes, exhibit filling defects. However, we have yet to observe the phenotype of a double-mutant for these genes. CRISPR/Cas9 mutagenesis was used to target Cah2 and CG6074 simultaneously. Progeny of 12 independent lethal or semi-lethal lines from this mutagenesis are being evaluated for genotypes and phenotypes. To study the regulation of filling, we sought to manipulate the substrates of carbonic anhydrase by abrogating the functions of rotary ATPases (proton pumps), and the bicarbonate transporter NDAE1. Expression data reveal that five of these ATPases are enriched in the trachea and are expressed at the time of filling. Our data shows that mutants for these ATPases and NDAE1 share phenotypes with carbonic anhydrase mutants reinforcing our hypothesis that carbonic anhydrases function to promote tracheal filling. To better understand the dynamics of pH change we plan to monitor the pH of the tracheal lumen during filling using red shifted pH sensitive dyes. In sum, our studies aim to
clarify a novel role for carbonic anhydrases in respiratory system activation, and to elaborate the diverse mechanisms that come to play in this process.

419  **Roles for two novel genes in post-meiotic mitochondrial shaping during *Drosophila* spermatogenesis.**  *Katherine Copenhagen,* Karen G. Hales  Department of Biology, Davidson College, Davidson, NC.

Homozygous males from the ZZ-2588 strain of *Drosophila melanogaster,* from the Zuker collection, are male sterile and have defects in nebenkern morphology and mitochondrial elongation during spermatogenesis. We characterized the ZZ-2588 mitochondrial defect by phase contrast and fluorescence microscopy, rhodamine 123 staining to test membrane potential and Hoechst staining to screen for nuclear shaping defects during spermatogenesis. We used deficiency mapping to localize the ZZ-2588 mutation within two overlapping deficiencies on the second chromosome that failed to complement the mutation. Within the overlapping region we selected the testis specific and highly expressed genes, CG5043 and CG5050 for sequencing in ZZ-2588 homozygous males. We identified a single base pair mutation resulting in a premature stop codon in CG5043. To further confirm CG5043 as the gene altered in the mutant, we demonstrated phenocopy via RNAi knockdown in the testis. We observed separate mild abnormalities in nebenkern morphology in CG5050 knockdowns as well. We designed a guide RNA for CG5050 and will use CRISPR/Cas9 mutagenesis to generate loss of function mutants and screen them for mitochondrial defects. The gene products of CG5043 and CG5050 have 31% sequence identity and are completely novel, with no recognizable motifs.

420  **Phosphoinositides modulate specific nuclear morphogenesis events during spermiogenesis.**  *L. Fabian*, J.A. Brill1,2  1) Cell Biology, The Hospital for Sick Children, Toronto, Ontario, CA; 2) Molecular Genetics, University of Toronto, Ontario, CA.

Phosphoinositides (PIPs) and their regulatory enzymes are involved in many diverse biological processes, such as cell morphogenesis and polarity, membrane trafficking, RNA localization, and cytoskeleton reorganization. Here we show that PIPs are also required for nuclear morphogenesis, a process essential for male fertility and which requires interactions between nuclear membrane, nuclear matrix, chromatin and cytoskeleton. Sperm head formation in Drosophila involves major changes in the shape and size of the nucleus and also in the state of chromatin condensation. As the sperm nuclei elongate, chromatin is reorganized and tightly condensed by replacing histones with transition-like proteins and then with protamines, small basic DNA-binding proteins. These processes are impaired in phosphatidylinositol 4,5-bisphosphate (PIP2)-deficient flies; nuclei do not elongate, nuclear matrix structure and organization is defective and the males are sterile. Posttranslational modifications of histones are impaired and their removal is delayed. Protamines get incorporated into nuclei despite histones not being completely removed. Localization of inner nuclear membrane proteins is defective, sumoylation is impaired, and repair of double-stranded DNA breaks is incomplete. Our present data suggest that normal levels of PIPs and their regulatory enzymes are required to coordinate interactions between the nuclear membrane, chromatin and the cytoskeleton to drive nuclear morphogenesis.

421  **Phenocopying the spermatid individualization defect of *mulet* using germline-specific RNAi.**  *James J. Fabrizio,* Erin Dailey, Justin Pronovost, Victoria Siracusa, Simon Innabi  Natural Sciences, College of Mt St Vincent, Bronx, NY.

Proper spermatid individualization requires the coordinated movement of 64 actin-based investment cones along the spermatid flagella. This coordination is severely disrupted in *mulet* mutant testes, resulting in a failure of spermatid individualization and male sterility. *mulet* encodes Tubulin-binding cofactor E-like (TBCEL), a chaperone responsible for the disassembly of microtubules. Indeed, both fluorescence and electron microscopy revealed the persistence of inter-flagellar microtubules in *mulet* mutant testes suggesting that TBCEL may be responsible for removing these microtubules as a prerequisite for spermatid individualization. Recently, the *mulet* mutant phenotype was rescued by driving expression of wild-type TBCEL using a *tubulin*-GAL4 driver.  *tubulin*-GAL4 preferentially drives expression in the male germline, suggesting that *mulet* is required in the germline syncytiun for spermatid individualization. The success of these rescue experiments prompted the use of the GAL4/UAS system in RNAi experiments. If *mulet* is required in the germline for spermatid individualization, then driving expression of double-stranded *mulet* RNA in the germline should also produce the discoordinated investment cones characteristic of *mulet* mutant testes. Multiple GAL4 drivers were tested for their ability to drive expression of double-stranded *mulet* RNA. While *tubulin*-GAL4, *hsp*-GAL4 and *nanos*-GAL4 were ineffective, RNAi driven by *bam*-GAL4 successfully phenocopied *mulet,* confirming that *mulet* is required in the germline for spermatid individualization. The effects of *bam*-GAL4 were enhanced by incubation of flies at higher temperature (28°C) and by including UAS-Dicer to further enhance RNAi. Indeed, the expression of double-stranded CG12214 RNA under *bam*-GAL4 control phenocopied the hypomorphic *mulet* mutations. Interestingly, the enhancement of RNAi using UAS-Dicer successfully duplicated the near-wild-type phenotype of the null mutation, consistent with our previous findings. Taken together, these results confirm the germline requirement for *mulet* and reveal *bam*-GAL4 as a potent driver of post meiotic RNAi induced defects.

Spermatid individualization is accomplished by an F-actin based Individualization Complex (IC) composed of a coordinated array of 64 investment cones. ICs become discoordinate into a scattered array of investment cones in mulet mutant testes, indicating that mulet is required for the coordinated movement of the IC. Additionally, the arrangement of spermatid mitochondria, detected using don-juan-GFP, also appear discoordinate, indicating that the defect is not restricted to the arrangement of the investment cones. mulet encodes Tubulin-binding cofactor E-like (TBCEL), suggesting a role for microtubule dynamics in individualization. Indeed, a population of ~100 inter-flagellar microtubules fails to disappear in mulet mutant testes. The persistence of these microtubules in the mutant, detected using both epi-fluorescence and electron microscopy, suggests that removal of these microtubules by TBCEL is a prerequisite to spermatid individualization. Thus, absent TBCEL, microtubules remain between the sperm tails and disrupt the structure of the IC. Consistent with this proposed role for TBCEL, immunofluorescence reveals TBCEL expression in elongated spermatid cysts. In addition, testes from mulet mutant were rescued to wild-type using tubulin-Gal4 to drive expression of TBCEL in mulet testes, indicating that the mutant phenotype is indeed caused by the lack of TBCEL and TBCEL is specifically required for spermatid individualization. Finally, RNAi driven by bam-GAL4 successfully phenocopied mulet, confirming that mulet is required in the germline for spermatid individualization. The effects of bam-GAL4 were enhanced by incubation of flies at higher temperature (28°C) and by including Dicer to further enhance RNAi. Indeed, the expression of double-stranded CG12214 RNA under bam-GAL4 control phenocopied the hypomorphic mulet mutations, while the enhancement of RNAi using UAS-Dicer successfully duplicated the near-wild-type phenotype of the null mutation. We propose a model in which the inter-flagellar microtubules may serve as an alternate track for the investment cones to travel down the length of the sperm tails. Thus, in the complete absence of TBCEL, these microtubules persist and successfully guide the investment cones to the ends of the sperm tails, while when TBCEL levels are reduced, fragments of microtubules may remain between the sperm tails, resulting in derailed investment cones that never complete their task.

423 Characterization of putative testis-specific sugar transporters in Drosophila melanogaster. Emily Fontenoy, Stephanie Hrabar, Mark Hiller Goucher College, Baltimore, MD.

Spermatid development is a complex process that reshapes undifferentiated germline cells into mature sperm. Spermatogenesis requires energy for the production of sperm, and mature sperm require energy for motility and fertilization. The Drosophila genome contains twenty-five genes characterized as Solute Carrier Family 2 (SLC2) encoding genes that are believed to encode sugar transporters. Five of these genes, sut3, sut4, Glut3, Tret1-2 and CG14605, appear to be expressed primarily or exclusively in males and are candidates for testis-specific sugar transporters. Since the energetic requirements necessary to produce functional sperm have not been extensively characterized, we are characterizing the gene expression of these putative transports by RT-PCR in an attempt to verify tissue-specific expression. Several Transposable Element insertions in these genes have been generated by the BDGP gene disruption project, but flies homozygous for insertions are viable and fertile. We will determine if these some of these genes are functionally redundant.

424 Importin α1 is required for maintenance of male germline stem cells. J. Heaney, N. Siddall, F. Casagranda, G.R. Hime Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, AU.

Importin α (Impα) proteins are required for transporting proteins from the cytoplasm into the nucleus via interaction with Impβ in all tissues but also have roles in transcriptional regulation and organisation of chromatin. They have been suggested to act as developmental switches during germline development. The Drosophila melanogaster genome encodes four Impα genes. We have identified a specific requirement for Impα1 (Kapa1) in maintenance of male germline stem cells and spermatogonial differentiation. Loss of function Kapa1 mutants are male sterile. They lose germline stem cells (GSCs) and this loss can be rescued by germline specific expression of Kapa1 but the rescued animals are still infertile indicating a secondary role in spermatogenesis. The GSC loss is accompanied by development of germ cell cysts that contain aberrant numbers of germ cells suggesting that cysts may be differentiating prematurely. The pre-meiotic Kapa1 mutant phenotype can be phenocopied by germline expression of a dominant-negative Kapa1 protein that lacks the Impβ binding domain. We identified genes known to be expressed in the testis that produce proteins containing a consensus nuclear localisation sequence and screened for the ability of the dominant-negative allele to prevent protein transport into spermatogonial nuclei. From this screen we identified Cyh4 and CG12909 as potential targets of Kapa1. Germline specific knockdown of CG12909 produces a phenotype similar to that of Kapa1 alleles. The mammalian ortholog of CG12909, LYAR, has been associated with recruitment of the methyltransferase PRMT5 to specific regions of chromatin and symmetric dimethylation of histone H4 Arg3 suggesting that Kapa1 may influence epigenetic regulation of male germ cell gene expression via control of CG12909 nuclear importation.
Sex chromosome pairing can be mediated by euchromatic homology in male meiosis. C.A. Hylton, J.E. Tomkiewicz

Drosophila males are an exception to the canonical process of meiosis in that they lack crossing-over and have evolved separate mechanisms to ensure disjunction of sex chromosomes and autosomes. Three proteins comprise a putative conjunction complex required for bivalent integrity. Modifier of Md4 in Meiosis (MNM) and Stromalin in Meiosis (SNM) are required for all chromosomes, whereas Teflon (Tef) is only required by autosomes. These proteins may mediate conjunction rather than pairing per se, and little is known about the mechanism of pairing. “Pairing sites” have been defined as sequences required for bivalent integrity in late prophase to metaphase but may be more important for conjunction, as pairing in early prophase has not been directly examined. These autosomal pairing sites are euchromatic and distributed throughout the arms, whereas sex chromosome pairing sites correspond to intergenic spacer (IGS) repeats within the heterochromatic rDNA clusters on the X and Y. Here, we examine the requirements for pairing versus conjunction, and the role(s) these sequences play in each process. Several unanswered questions about pairing in this system remain: Why is sex chromosome pairing/conjunction confined to the rDNA intergenic spacers? Could other sequences participate in pairing? Does the specificity of Tef reflect a euchromatin- or an autosome-specific function? To address these questions, we devised a pairing assay between the X chromosome and Y chromosomes bearing duplications of X euchromatin. Segregation frequencies of an rDNA- deleted X chromosome from Dp-bearing Y chromosomes were quantified. X euchromatin homology was capable of restoring >90% disjunction of the X and Y at meiosis I. No relationship between homology length and disjunction was observed, however there was a direct correlation between centromere proximity and disjunction. Deletion derivatives of a single duplication revealed that as few as 120 kb could direct segregation. This euchromatin-mediated XY disjunction was independent of Tef, suggesting that Tef is autosome- rather than euchromatin-specific. To differentiate between pairing and conjunction, we directly examined pairing between euchromatic X sequences by FISH in early prophase both in the presence and absence of the X rDNA. Our observations suggest that X euchromatin homologies are capable of mediating sex chromosome pairing and disjunction independently of the endogenous rDNA pairing sites.

Knockdowns in Protamine A and Protamine B can imitate the abnormal function of Sd-RanGAP. J.R. McLean, S. Sheltz Kempf, L. Kritzman

The Segregation Distorter (SD) system in Drosophila melanogaster results in the transmission of one chromosome (typically the SD homolog) at the expense of the SD homolog, a phenomenon known as Segregation Distortion. This happens through the interplay of the SD gene and its enhancers and the Rsp locus during spermatid development, impairing sperm-specific chromatin condensation in half of the developing spermatids. SD encodes RanGAP, which is normally found at the periphery of the nucleus and is required for normal Ran-mediated nuclear transport. The Sd mutation produces a truncated RanGAP protein lacking one of two nuclear export signals and resulting in active RanGAP protein in the nucleus, interfering with nuclear transport. While nuclear transport is presumably affected in all cells, only one phenotype has been observed in SD flies: spermatids carrying the Rsp locus fail to develop properly. One hypothesis is that proteins needed for sperm-specific chromatin condensation are unable to enter the sperm nuclei in sufficient amounts to allow development, and that Rsp-bearing chromosomes require more of these proteins than their Rsp homologs. To test this hypothesis we have used genetic knockdowns of Protamine A, Protamine B, and Mst77f, proteins involved in sperm-specific chromatin condensation. Our findings show that reducing the amount of Protamine A or Protamine B, but not Mst77f, causes significant distortion.

Defects in spermatogenesis underlying hybrid male sterility. Colin Meiklejohn, Kathleen Gordon

Between Drosophila species, the earliest intrinsic postzygotic incompatibilities to evolve cause sterility in hybrid males. This pattern can be attributed to the rapid evolution of X-linked genetic factors that cause interspecific hybrid male sterility. To determine why the X chromosome accumulates hybrid incompatibilities faster than the rest of the genome, we are investigating the genetic and developmental basis for X-linked hybrid male sterility between Drosophila simulans and Drosophila mauritiana. To identify developmental defects associated with hybrid male sterility we examined spermatogenesis in eight genotypes of D. simulans that carry small segments of the D. mauritiana X chromosome. We visualized DNA and the expression of a Protamine B-GFP transgene. In all eight sterile 2P introgression genotypes, the seminal vesicles were empty, and individualized sperm were absent. In six genotypes, sperm bundles were present, and GFP-positive spermatid nuclei in the distal portion of the testis initiate but fail to complete nuclear reshaping. The other two genotypes show additional disruptions to spermiogenesis. All eight 2P introgression lines appear to have defects that manifest between meiosis and sperm individualization, a phenotype that is also frequently observed among male-sterile mutations within D. melanogaster.

The Dlg-module and clathrin-mediated endocytosis regulate EGFR signaling levels and cyst cell-germline coordination in the Drosophila testes. F. Papagiannouli, M.T. Fuller

A fundamental question in biology is how cell-cell communication and exchange of short-range signals from the local tissue
microenvironment regulates cell proliferation and cell fate for setting up functional tissues. In all adult tissues harboring stem cells, tissue homeostasis and repair relies on the proper communication of stem cells and of their differentiating daughter cells with the local tissue microenvironment. The microenvironment of the male testis cysts, built by the intimate connection of the squamous epithelial-type cyst cells and the germline, provides an ideal model system to investigate how cell polarity, cyst cell growth and expansion are coordinated with cellular trafficking and exchange of short-range signals. Setting up a functional cyst microenvironment is a prerequisite for cyst cell-germline coordination in terms of signaling exchange and co-differentiation, yet it remains a mystery how these tightly packed cysts coordinate cortical polarity with cellular trafficking and signaling at their contact sites. Primary candidates for this intimate interaction are Dlg, Scrib and Lgl, highly conserved polarity and scaffolding proteins called the Dlg-module, that localize at the cortical side of the testis cyst cells and are critical for setting up functional testicular cysts. Cell type-specific knockdown of dlg, scrib or lgl in Drosophila testes cyst cells results in cyst cell autonomous and germ cell non-autonomous defects that severely impact cyst cell-germline coordination and testis homeostasis. Using cell-type specific knockdowns and genetic interactions we show that Dlg, Scrib and Lgl cooperate with endocytic components involved in clathrin-mediated endocytosis to regulate EGFR signaling levels. Loss of dlg, scrib or lgl function in the cyst cells results in loss of cortical localization of clathrin adaptor proteins and increased EGFR activation, while lowering EGFR levels can rescue the observed defects, restore testicular cyst function and spermatogenesis. Our work provides new insights into the role of Dlg, Scrib and Lgl polarity components in endocytosis, cyst cell-germline cross-communication and signaling regulation. Elucidating the cooperation of the Dlg-module with endocytosis and EGFR regulation provides significant insights on the fine regulation of receptor firing under physiological conditions along the progressive steps of spermatogenesis, and provides paradigms of how polarity cues cooperate with different cellular events to achieve a coordinated cellular output and testis homeostasis.

**429 Nmd regulates peroxisome biogenesis and mitochondrial shaping in Drosophila spermatogenesis.** M. Ummer Qureshi, Karen G. Hales Department of Biology, Davidson College, Davidson, NC.

At the onion stage of Drosophila spermatogenesis, two large mitochondrial derivatives intertwine to form the spherical Nebenkerne adjacent to the nucleus. The AAA ATPase Nmd plays a critical role in the formation of this structure, as mutants show mitochondrial aggregation failure, vacuolated Nebenkerne, and cytokinesis failure. Msp1, the Saccharomyces cerevisiae ortholog of Nmd, traffics peroxins from mitochondria to peroxisomes, and therefore we hypothesized that Nmd may regulate the morphology and development of both organelles in the specialized context of spermatogenesis. We examined the subcellular organization of peroxisomes in developing spermatids and are the first to show that the organelle, in part, interacts with microtubule organizing centers (MTOCs), sites associated with Nmd localization. Pex19 RNAi knockdown disrupted peroxisome biogenesis and reduced Nmd localization to MTOCs in early spermatids, confirming that Nmd interacts with peroxisomes. In addition, testes from nmd mutant males showed impaired peroxisomal matrix protein import, and mutants of the Nmd testis-specific paralog CG4701 show peroxisome fragmentation. We are examining peroxisome membrane formation in these mutants to further dissect how Nmd regulates peroxisome biogenesis. Our work reveals a type of inter-organelle regulation that may underlie and connect mitochondrial dynamics to peroxisome biogenesis disorders.

**430 Small Ubiquitin-like Modifier (SUMO) posttranslational modifications mediate critical regulatory events required for proper sperm development and transfer to seminal vesicles during Drosophila spermatogenesis.** J.E. Rollins1, J. Steinhauser2, S. Brown1, T. Chin1, N. Desouza1, S. George1, W. Lang1, G. Reyes1, J. Reyes1, G. Russo1, P. Morris3 1) Natural Sciences, College of Mount Saint Vincent, Riverdale, NY; 2) Biology Department Yeshiva University, New York, NY; 3) Center for Biomedical Research, Population Council, New York, NY.

Drosophila spermatogenesis is a dramatic, temporally-orchestrated developmental stage-specific process. Sperm production includes marked changes in mitosis and meiosis, chromosomes, transcription, translation, and posttranslational modifications, with striking nuclear remodeling during spermiogenesis. The posttranslational modifier (SUMO) protein has been shown to play diverse roles in many highly conserved cellular processes such as spermatogenesis in various species. The purpose of this study was to define the precise stage-specific timing of Smt3 (Drosophila SUMO)-mediated events during germ cell development, determine whether Smt3-deficiency affects sperm production in heterozygotes and germline knockdown of Smt3 gene. For bioimaging, unconjugated Smt3 and Smt3-modified proteins were detected by immunofluorescence using both whole mounts and squash preparations of testis from wild-type and heterozygous Smt3-deficient mutant stocks as well as in flies expressing Smt RNAi. In wild-type flies, Smt3-SUMOylated proteins show strikingly different patterns in most stages of spermatogenesis including spermatogonia undergoing mitosis, resting and meiotically active spermatocytes, and round and elongating spermatids in various stages of nuclear condensation during spermiogenesis as well as the head cyst cells. The tests of heterozygotes showed reduced levels of Smt3 and an altered SUMOylated protein profile compared to wild-type. Interestingly, the reduction of Smt3 signals was readily observed in meiotic spermatocytes; no change for mitotic spermatogonia was apparent. Heterozygote males exhibited a reduced fertility and their testes show a marked defect in sperm translation to the seminal vesicles.

SUMO-modifications were confirmed using human and rodent tests with normal spermatogenesis. When expression was knocked down in the germline no mature sperm were found. Nuclei fail to condense do not condense properly in post-meiotic spermatids and actin cones are formed and scattered throughout the tests but are not bundled around the nuclei. Our data are suggestive that 1) precise timing of SUMOylation events in developing fly germ cells is required for normal spermatogenesis; 2) Smt3-deficiency can result in failure of spermatids to properly undergo spermiogenesis and sperm transfer, findings consistent with marked reduction in fertility. Taken together, our results indicate important roles for Smt3 and SUMOylation during and after meiosis in Drosophila testes.

**431 Fertility and sperm storage in aged Drosophila males.** Y. Schwartz, S. Friedman, J. Steinhauler Department of Biology, Yeshiva University, New York, NY.

Age related declines in fertility are observed in humans and other animals. Reproductive senescence in females has been well studied, but less is known about males. In Drosophila males, germline stem cell number and proliferative capacity
decrease with age. Consistent with this, our lab has observed that germline cyst production declines steadily throughout the life of the fly. Surprisingly, our initial measurements of male fertility did not show a coincident drop with age, even in 30 day old males. This was determined to be due to sperm storage throughout the lifetime of the males. When males were mated throughout the course of their life to deplete sperm storage, fertility was seen to decline at 30 days, but not at 15 days of age. Seminal vesicles of unmated males were significantly larger than those of mated males, confirming that sperm were stored. In males aged for 15 days, average germline cyst number per testis was significantly reduced compared to younger flies but was similar between mated and unmated males, despite large differences in seminal vesicle size, indicating that seminal vesicle size and sperm storage do not feed back on cyst production. We conclude that sperm production in Drosophila males declines steadily with age under the influence of an intrinsic gonadal clock. Our current experiments use temperature sensitive spermatogenesis mutations to block sperm production at distinct age points. These experiments will reveal whether sperm made by younger males (and stored) are equally viable to sperm made by older males. We are interested in the regulation of sperm storage and potential age related declines in sperm viability.

432 An evolutionarily conserved protein required for sperm motility in Drosophila. R. Snow, H. Florman, G. Findlay. 1) Department of Biology, College of the Holy Cross, Worcester, MA; 2) Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA.

Sexual reproduction in internally fertilizing animals often requires sperm to swim from the site at which they are deposited in females to a distant site of fertilization. This swimming is regulated by proteins on the sperm, as well as by female- and male-derived molecules present in the female reproductive tract. In mammals, a number of sperm proteins required for motility have been identified. The functional analyses of such proteins could be advanced by the development of equivalent mutants in the Drosophila model system. Here, we report the initial characterization of Drosophila sperm gene 1 (dsg1), which encodes a sperm motility protein conserved in Deuterostomes. Male mice homozygous for a null allele of the orthologous gene showed greatly reduced fertility, and sperm from these males swam inefficiently toward the oviduct. In D. melanogaster, testis-specific RNA interference of dsg1 reduced gene expression below detectable levels and caused similar phenotypes. When mated to wild-type females, dsg1 knockout males were severely sub-fertile. Using a protamine-GFP marker, we observed that levels of sperm production and sperm transfer to females were normal, but few sperm from knockout males were stored successfully in the female seminal receptacle, suggesting a motility defect. We have recently replicated these phenotypes with a putative null allele of dsg1 generated by CRISPR/Cas9, and we are using microscopy to visualize and quantify patterns of sperm motility in these mutants. These results demonstrate a broadly conserved role of dsg1 in Deuterostome sperm motility and suggest that further insights into the function and interactions of its encoded protein may be gleaned from experiments in the tractable Drosophila system.

433 The PNG kinase activator GNU interacts with RNP granule components in mature Drosophila oocytes. E. Aviles Pagan, T. Orr-Weaver. 1) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; 2) Whitehead Institute for Biomedical Research, Cambridge, MA.

The transition from oocyte to embryo marks the onset of development for most metazoans, whereby the differentiated oocyte becomes a totipotent egg. Egg activation serves as the trigger of the oocyte-to-embryo transition, initiating a cascade of events that lead to completion of meiosis and restoration of potency. As the oocyte is transcriptionally silent, the global changes in gene expression that take place during egg activation occur by posttranscriptional mechanisms, such as changes in mRNA translation. In Drosophila, the PNG kinase complex is a major translational regulator that is active exclusively during egg activation. The complex is composed of a catalytic ser/thr kinase subunit, PNG, and two activating subunits, PLU and GNU. Activity of the complex is regulated in part by the binding of GNU (Hara, Petrova and Orr-Weaver, 2017, eLife, e22219). Prior to egg activation, the PNG complex is kept inactive by inhibitory CycB/CDK1 phosphorylation of GNU, which prevents its association with the complex. As egg activation occurs, and CycB/CDK1 is inactivated as meiosis is completed, GNU is dephosphorylated, thus is able to bind and activate PNG before being degraded shortly thereafter. This restricts the translational control of maternal mRNAs by the PNG complex to a tight developmental window. The mechanisms by which GNU is kept from binding PNG before egg activation, or how it regulates the activity of the complex remain unclear. Our goal is to understand how GNU is kept from activating the PNG complex in mature oocytes and to determine whether GNU has additional roles independent of PNG in the mature oocyte. We performed a pull-down/mass spectrometry analysis to identify interactors of GNU. Using this approach, we identified components of RNP granules, membrane-less organelles involved in regulating mRNA translation, stability and localization, as potential interactors with GNU. In addition, using a GFP-tagged construct, we observe a granular localization for GNU in the cytoplasm of mature oocytes, a localization that is dependent on the sterile-alpha-motif (SAM) domain of GNU. Together, our observations suggest a relationship between GNU and RNP granules. Currently, we aim to understand the relationship between GNU and RNP granule components, and how this could affect regulation of PNG complex activity by GNU during the oocyte-to-embryo transition.

434 orb functions in the initial specification and maintenance of oocyte identity. J. Barr, R. Gilmutdinov, Y. Shidlovskii, P. Schedl. 1) Molecular Biology, Princeton University, Princeton, NJ; 2) Institute of Gene Biology RAS, Moscow.
The orb gene codes for a cytoplasmic polyadenylation element binding protein (CPEB) that functions in the translation of mRNAs localized to the oocyte. Orb binds to the 3'UTR of orb mRNA, and promotes its own accumulation within the oocyte. Previous studies have shown that this positive autoregulatory feedback loop is critical for maintaining sufficient levels of Orb protein in the oocyte during midstages of oogenesis. When the orb autoregulatory feedback loop is disrupted, this interferes with orb regulation of key target mRNAs like gurken. We wondered if orb autoregulation is also important for the early development of the germline. Previous studies showed that orb is also required early in oogenesis. orb null alleles fail to form a 16-cell germline cyst while strong loss of function alleles do not correctly determine an oocyte. The orb mRNA and protein are localized to the oocyte prior to the formation of an egg chamber, and thus orb mRNA and protein are among the earliest markers of oocyte fate. Here we take several approaches to better understand the role of orb and the orb autoregulatory loop in the initial specification of the oocyte and the subsequent maintenance of oocyte identity.

We first asked if orb is required to maintain oocyte identity. To address this question we knocked down orb after the initial specification of the oocyte. We found that loss of orb after oocyte specification leads to a failure to maintain oocyte identity. Instead all cells in the cyst differentiate as nurse cells and development arrests early. Next we asked if the orb autoregulatory loop is important for the initial specification or maintenance of oocyte identity. If orb autoregulation is required for early orb function, we would predict that deletion of the orb 3'UTR would disrupt oocyte specification or maintenance. In contrast to wildtype in which Orb protein and mRNA concentrate in the oocyte, in the 3'UTR deletion the mRNA and protein are unlocalized and are uniformly distributed throughout the cyst. Apparently as a consequence, the oocyte is not properly specified; instead all of the cells assume a nurse cell identity. In addition, we found that the cyst fails to completely exit the mitotic cycle. While these findings argue that orb autoregulation is necessary for oocyte specification, we are currently testing the possibility that orb autoregulation is also sufficient for oocyte identity.

**435 Spargel/dPGC-1 is essential for nutrient-mediated ovarian growth.** Mohammad Basar, Atanu Duttaroy Biology, Howard University, Washington, DC.

Dietary proteins are crucial for oogenesis. The Target of Rapamycin (TOR) is a major nutrient sensor controlling organismal growth and fertility, but the downstream effectors of TOR signaling remain largely uncharacterized. We previously identified Drosophila spargel/dPGC-1 as a terminal effector of the TOR-TSC pathway, and now report that spargel connects nutrition to oogenesis. Here, we first reported endogenous spargel's subcellular localization with a monoclonal antibody which we found mostly restricted in the nucleus contrary to previous report of cytoplasmic localization. We found that spargel is expressed predominantly in the ovaries, and germline spargel knockdown caused degeneration of egg chambers as well as female sterility. Unexpectedly, dietary protein quantity affected spargel/dPGC-1 expression, and spargel/dPGC-1 is required to transmit nutrient-mediated signals into ovarian growth. We found that spargel knockdown disrupts endosomal trafficking, leading to cytoplasmic aggregation of the Notch transcription factor and disruption of Notch signaling in follicle cells. We propose that potentiating spargel/dPGC-1 expression in the ovary is instrumental in nutrient-mediated regulation of oogenesis.

**436 small ovaries negatively regulates ovarian transposable element expression.** L. Benner1,2, C. Whitworth3, K. Cook3, E. A. Castro4, B. Oliver1, D. A. Lerit1 1) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 2) Department of Biology, Johns Hopkins University, Baltimore, MD; 3) Department of Biology, Indiana University, University, IN; 4) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA.

The piRNA pathway is a small RNA defense mechanism that has evolved to protect the genome by attenuating transposon activity by both piRNA directed cleavage of transposon mRNA and silencing of heterochromatic regions where transposons become trapped over evolutionary timescales. Failure to repress transposable element activity disrupts genomic integrity resulting in somatic and germline cell death. We have evidence that small ovaries (sov), acts within this pathway. sov was first identified as a set of non-complementing recessive female sterile and lethal alleles that resulted in a range of dystrophic ova

**437 Defining the function of p53 isoforms in Drosophila melanogaster.** A. Chakravarti1, B Zhang2, H Thirimanne3, B Calvi1 1) Biological sciences, Indiana university, bloomington, IN; 2) Current address: Advanced Cell Diagnostics, CA ; 3) Current address: University of Washington, Seattle, WA.

The human p53 gene is well-characterized as a tumor suppressor. It is now known that p53 and its paralogs, p63 and p73,
regulate a wide array of processes, including metabolism, stem cell division, and germline integrity. In addition, each paralog encodes over 10 protein isoforms that may have distinct functions that have yet to be fully defined. We have been analyzing the single p53 family member in Drosophila melanogaster as a tractable genetic system to investigate the evolution and function of different p53 isoforms.

The single Drosophila p53 gene encodes at least three different protein isoforms; p53A, p53B, and p53E. Our previous analysis of isoform-specific mutants showed that the p53A isoform is both necessary and sufficient for the apoptotic response to radiation. Although the longer p53B isoform was not required for radiation response, when over-expressed it was a much more potent than p53A at inducing transcription and cell death. We are currently exploring whether the longer transactivation domain of p53B recruits chromatin modifiers and transcription elongation factors to activate a paused RNA Pol II at pro-apoptotic gene promoters.

To address the normal physiological function of p53B, we examined expression of GFP-p53A and mCherry-p53B in genomic BAC transgenes. While GFP-p53A localized to discrete sub-nuclear foci in the soma and germline, mCherry-p53B was largely restricted to the germline. Importantly, our analysis of isoform-specific mutants suggests that p53B has a specialized function during female meiosis. Our results for p53 isoforms in Drosophila are consistent with other evidence that p53 germline function likely predates its function in the soma. Defining the function of different p53 isoforms will provide insight into what developmental cues may have shaped the ancestral p53 network and the functions of the more complex p53 family in humans.

438  **Nuclear lamina dysfunction triggers of a novel germline stem cell-specific checkpoint.** Tingting Duan¹, Rebecca Cupp¹, Lacy Barton², Pamela Geyer¹ ¹) Biochemistry, University of Iowa, Iowa City, IA; 2) Department of Cell Biology, Howard Hughes Medical Institute, and Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY 10016.

Drosophila gametogenesis depends upon maintenance of adult germline stem cells (GSCs). Mutations in a nuclear lamina (NL) protein, Otefin (Ote), causes an early block in GSC differentiation followed by GSC death. Ote is a member of the conserved LEM-domain protein family which interacts with a histone and DNA binding protein Barrier Against Autointegration Factor (BAF), which promotes tethering of chromatin to the nuclear periphery. Loss of Ote causes GSC-specific defects in nuclear architecture, including thickening of the NL and a coalescence of heterochromatin. These defects activate ATM- and Rad3-related (ATR) and Checkpoint kinase 2 (Chk2), two kinases in the DNA damage response (DDR) pathway. Strikingly, loss of ATR or Chk2 completely rescues germ cell death and differentiation defects in ote mutants, yet nuclear architecture defects persist. Although the germline checkpoint involves DDR components, the triggers and targets of this novel checkpoint differ from those in the canonical DDR pathway. Canonical triggers, such as DNA damage and replication stress, are absent in ote mutant GSCs, and GSC death is independent of the canonical target, p53. To identify the novel trigger(s) for ATR/Chk2 activation, we are conducting genetic and cytological studies. Although loss of ATR/Chk2 restores oocyte production, the rescued oocytes do not support embryogenesis. Based on these findings, we suggest that NL dysfunction triggers a novel germline checkpoint that is required for maintenance of high quality gametes to ensure fitness of the next generation.

439  **A Proteomic Analysis of Me31B Interactome in Drosophila Germ Granules.**  H. DeHaan¹, A. McCambridge¹, B. Armstrong¹, C. Cruse¹, D. Solanki¹, J. Trinidad², A. Arkov³, M. Gao¹ ¹) Biology Department, Indiana University Northwest, Gary, IN; 2) Department of Chemistry, Indiana University, Bloomington, IN, USA; 3) Department of Biological Sciences, Murray State University, Murray, KY, USA.

Germ granules are large RNA-protein complexes (ribonucleoproteins, RNP) essential for the development of the germline cells. Me31B, a DEAD-box ATP-dependent RNA helicase, is a conserved protein component of Drosophila germ granules. During oogenesis, Me31B confers post-transcriptional regulation of mRNAs by interacting with other germ granule proteins. Therefore, mapping the molecules that complex with Me31B and determining how they interact with Me31B is critical to understand the role and mechanism of Me31B in germ granule functioning. In Drosophila ovaries, we chemically crosslinked Me31B with its in vivo interacting partner proteins, isolated the Me31B complex by immunoprecipitation, and identified the proteins in the complex by mass spectrometry. We found that Me31B interactome contains polypeptides from four groups: RNA regulation proteins, glycolytic enzymes, cytoskeleton/motor proteins, and germ plasm components. We surprisingly found conserved germ plasm proteins Tudor, Vas, and Aub in the Me31B interactome, which is consistent with immunostaining experiments showing that the three proteins likely colocalize with Me31B in the nuage and germ plasm of developing egg chambers. Furthermore, we provide biochemical evidence that Me31B may directly bind to Tudor in a symmetrically dimethylated arginine-dependent manner. Our study revealed a dynamic interacting network of Me31B in Drosophila germ granules, supporting Me31B’s role in RNA post-transcriptional regulation and indicating its new functions in germ granule assembly and germline development.
The Surprising composition and biophysical properties of a synaptonemal polycomplex. Elizabeth Hemenway, Stacie Hughes, R. Scott Hawley, J. Young, J. Sun. Stowers Inst Med Res, Kansas City, MO; University of Missouri-Kansas City, Kansas City, MO; University of Kansas Medical Center, Kansas City, KS.

Loss of an E3 ubiquitin ligase results in large proteinaceous structures (known as rods) during oogenesis. Rods contain proteins found in the synaptonemal complex (SC), a structure required for proper segregation at meiosis I. Rods vary in size up to 5 microns in length, and persist much longer than does wild-type SC. Surprisingly, we observe that cohesin proteins are components of some, but not all, of the rods. Thus, some rods contain not only SC proteins but critical chromosome axis proteins as well. Recent findings by others suggest the SC may be a liquid-crystalline structure whose assembly and disassembly may be mediated by phase-separation. We asked whether the addition of 1,6-hexanediol, which is known to induce liquid-crystal phase transitions of the SC, would force a similar transition of the rods into liquid phase. We find that in the presence of 1,6-hexanediol, rods in early meiosis are far less frequent, suggesting their polymerization is impaired or they are disaggregated. However, some rods persist after treatment, suggesting a high stability of at least a population of rods. From these data, we conclude rods are not uniform in composition nor stability. Further study of this mutant may help elucidate the mechanisms of SC assembly and disassembly.

The NR5A nuclear receptor Hr39 functions in both reproductive glands and mature follicles to regulate ovulation. E. Knapp, J. Sun. Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT.

The NR5A nuclear receptor Hr39 is essential for development of Drosophila female reproductive glands (spermathecae and parovaria), and its expression in secretory cells of these glands in adult females is critical for proper ovulation, yet the mechanism remains largely unknown. Our recent work demonstrated that Drosophila ovulation, resembling mammalian ovulation, involves the degradation of posterior follicle cells, the rupture of mature oocytes into the oviduct, and the formation of corpus luteum by the residual follicle cells. This rupture process is induced by the octopamine (OA) signaling in mature follicle cells that elicits an increase in intracellular calcium to activate matrix metalloproteinase 2 (Mmp2) enzymatic activity. In this study, we demonstrated that Hr39-regulated secretory products are required for OA-induced follicle rupture during ovulation. Particularly, we found that these secretory products regulate components upstream of intracellular calcium rise in mature follicle cells during OA-induced follicle rupture. In addition, we found that Hr39 is also required within the mature follicle cells to regulate follicle rupture/ovulation; however, Hr39 in mature follicle cells regulates components downstream of intracellular calcium rise to regulate Mmp2 activation. This follicle cell-specific role of Hr39 in ovulation is reminiscent to its mouse counterpart Liver receptor homolog 1 (LRH-1) in granulosa cells to control ovulation. Our data strongly suggest that Hr39 plays dual and non-redundant roles in the ovary and reproductive glands to regulate ovulation and that such roles are likely conserved in mammals.

A role for the axon guidance receptor frazzled in Drosophila oogenesis. K.M. Laws, S.A. Russell, G.J. Bashaw. Department of Neuroscience, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

During embryogenesis, combinations of axon guidance receptors and their ligands generate the stereotyped pattern of axon connections in the nervous system. For example, Netrin secreted at the embryonic midline acts through the guidance receptor Frazzled (Fra) to induce cytoskeletal rearrangements in developing neurons, leading to attraction toward the ligand source. Recent work in our lab demonstrated that, in addition to this canonical, Netrin-dependent signaling, Fra also controls axon guidance through the transcriptional activity of its C-terminal intracellular domain (ICD). The only known transcriptional target of the Fra ICD, commissureless (comm), also plays an integral role in axon guidance at the midline. By downregulating the Roundabout (Robo) receptor in axons, Comm makes cells refractory to repulsive Slit/Robo signaling, allowing them to cross the midline. The majority of this Netrin-independent pathway, however, remains poorly understood.

Several axon guidance pathways, including Slit/Robo signaling, have documented roles outside of neuronal development. Indeed, a previous report suggests that Netrin is required for Drosophila oogenesis, although its precise role is unclear. We generated negatively marked clones to investigate the intrinsic requirement for Fra in various cell types throughout oogenesis. Preliminary genetic mosaic analysis indicates that fra is required for egg chamber survival during oogenesis. Intriguingly, when C-terminally GFP-tagged fra is expressed under the control of its genomic regulatory elements, GFP signal accumulates in nurse cell nuclei of the ovary. This is consistent with the translocation of the Fra ICD to the nuclei of these cells, suggesting that Fra could act transcriptionally in the germline. Using transgenes that distinguish between the Netrin-dependent and -independent functions of Fra, we will determine the mode(s) of Fra signaling required for egg chamber survival during oogenesis. Additionally, complementary studies will investigate cell-type specific roles for Netrin and comm in oogenesis. The size and tractability of the ovary make it a valuable tool for the investigation of Fra signaling, and future work in the tissue may uncover novel constituents of both Netrin-dependent and -independent signaling pathways.

NADPH oxidase-generated reactive oxygen species in mature follicles are essential for Drosophila ovulation. W. Li, J. Young, J. Sun. 1) Department of Physiology & Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs, CT.
Ovulation, a critical step for reproduction, is conserved from insects to humans at the cellular and molecular level such that both mammalian and *Drosophila* ovulation requires matrix metalloproteinase (Mmp) for follicle wall breakdown and steroid signaling for activation of Mmp and follicle rupture. Ovarian reactive oxygen species (ROS) are indispensable for mammalian ovulation; however, it is unclear how ROS are produced in mammalian ovaries and whether ROS play conserved role in *Drosophila* ovulation. In the current study, we investigated the role of NADPH oxidase (Nox), an essential enzyme for superoxide production, in *Drosophila* ovulation. Nox is highly enriched in mature follicle cells, and Nox knockdown in mature follicle cells leads to reduction of superoxide production and defective ovulation. We then showed that extracellular superoxide dismutase 3 (SOD3) converts superoxide to hydrogen peroxide, which acts as a key signaling molecule for ovulation control, independent of Mmp activation. Furthermore, we showed that Nox enzymatic activity depends on intracellular Ca^{2+} concentration, which is strongly elicited by octopamine (OA)/octopamine receptor in mushroom body (Oamb) signaling in mature follicle cells. Considering the fact that Nox homologs are also expressed in mammalian follicle cells, our work strongly suggest that Nox-dependent hydrogen peroxide plays a conserved role in regulating ovulation.

444 Towards understanding the effects of insulin/insulin-like growth factor signaling (IIS) and IIS-dependent pathways on ovarian number and other fitness aspects in the specialist species *Drosophila sechellia*. Aracely A. Newton, Cassandra G. Extavour. Harvard University, Cambridge, MA.

In *Drosophila*, fecundity is directly proportional to the number of ovarioles, structures in the ovary that house developing oocytes at progressing stages of maturity. Ovariole number can exhibit considerable variation both within and between *Drosophila* species. *Drosophila sechellia*, a close relative of *D. melanogaster* and specialist of the toxic fruit Morinda citrifolia, consistently displays few ovarioles, typically harboring between seven to ten per ovary, which is less than half the number of ovarioles of most *D. melanogaster* populations. *D. sechellia* ovariole number also exhibits low ovariole plasticity in response to nutritional modification. Low ovariole number coupled with low nutritional plasticity of ovariole number appears to have evolved more than once in specialist species, suggesting an involvement of nutrition-dependent pathways in the evolution of ovariole number variation.

Our lab previously reported that insulin/insulin-like growth factor signaling (IIS) positively regulates ovariole formation in *Drosophila*. Indeed, female *D. sechellia* display lower insulin signaling than *D. melanogaster*, partly accounting for the lower ovariole number of *D. sechellia*. However, the molecular mechanisms and IIS-dependent pathways mediating ovariole formation have yet to be elucidated. Moreover, IIS has previously been reported to affect behavior, suggesting a connection between low IIS and other fitness traits in *D. sechellia*. Our ultimate goal is two-pronged: (1) to elucidate the effects of altered signaling on other aspects of *D. sechellia* fitness and (2) to uncover genetic pathways that mediate reduced IIS and ovariole number in *Drosophila*. Uncovering the causes and consequences of reduced IIS will aid in understanding the evolutionary biology of specialization in Drosophilids.

Here we show that *D. sechellia* display potential indicators of lower fitness compared to other *Drosophila* species endemic to the Seychelles region, based on laboratory assays of mating behavior and flight response in females. Both of these behaviors are enhanced upon supplementation with octopamine, an insect neurotransmitter that has previously been suggested to decrease IIS. This indicates a possible molecular regulatory connection between different fitness traits in *D. sechellia*.


The guidance receptor Frazzled (Fra) has two reported functions: to respond to its ligand Netrin to regulate cytoskeleton and plasma membrane dynamics, and to regulate transcription independently of Netrin. Currently, Fra is only known to regulate transcription in the embryonic ventral nerve cord to control axon guidance. However, Fra is also expressed and required during oogenesis. Preliminary evidence suggests Fra may act as a transcription factor during *Drosophila* oogenesis: the c-terminal GFP tag of a Fra-GFP fusion protein expressed from a BAC is detected in the nuclei of nurse cells in egg chambers. In addition, fra and netrin mutant germlines appear to have different phenotypes. These observations suggest that, like in the nerve cord, the intracellular domain of Fra may be able to enter the nucleus to regulate transcription, and that Fra may have a Netrin-independent function during oogenesis.

While we know that Fra can activate transcription of commissureless in the ventral nerve cord, and the conserved P3 motif is the activation domain, we still have a very limited understanding of the mechanism Fra uses to regulate transcription. Of particular interest is how Fra interacts with DNA, since Fra lacks known DNA-binding motifs. To answer this question, we completed a yeast-two-hybrid screen to identify proteins that interact with the Fra intracellular domain, and found multiple DNA-binding proteins and transcriptional cofactors. We then used an RNAi approach to knockdown proteins identified in the yeast two hybrid in the germline, and analyzed egg-laying. Positive hits from this secondary screen include the DNA-binding protein Clawless/C15 (Cll), and the co-repressor Groucho (Gro). In addition, a third protein identified from the yeast two hybrid screen, Pleiohomeotic-like (Phol), a DNA-binding protein, has previously been reported to function in *Drosophila*.
446  **The protein kinase CK2 substrate Jabba regulates lipid metabolism during Drosophila oogenesis.**  E. McMillan, S. Longo, M. Smith, S. Broskin, B. Lin, N. Singh, T. Strohich  Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA.

Lipid metabolism plays a critical role in female reproduction. During oogenesis, maturing oocytes accumulate significant levels of neutral lipids that are essential for both energy production and for the synthesis of other lipid molecules. Metabolic pathways within the ovary are partially regulated by protein kinases that link metabolic status to oocyte development. While the functions of several kinases in this process are well established, the roles that many other kinases play in coordinating metabolic state with female germ cell development are unknown. Here, we demonstrate that the catalytic activity of casein kinase 2 (CK2) is essential for Drosophila oogenesis. Using an unbiased biochemical screen that took advantage of a unique enzymatic property of CK2, we identified a novel CK2 substrate in the Drosophila ovary, the lipid droplet-associated protein Jabba. We found that both Jabba and CK2 are essential for modulating ovarian lipid metabolism and regulating female fertility in the fly. Our findings shed light on a CK2-dependent signaling pathway governing lipid metabolism in the ovary and provide insight into the long-recognized but poorly understood association between energy metabolism and female reproduction.

447  **In vivo evidence for the role of CG15436 during endoreplication within ovary follicle cells.**  Rachel Williamson, Katelyn Karalic, M. Logan Johnson  Notre Dame College, South Euclid, OH.

Endoreplication is a process where a cell undergoes multiple rounds of replication without cellular or nuclear division. Often this occurs in terminally differentiated cells that are required for specialized processes, such as mass production of proteins. Within the Drosophila ovary the follicle and nurse cells undergo endoreplication. Specifically, the follicle cells become highly polyplid at specific regions to boost the production of chorion proteins needed for the egg shell. This type of replication, or endocycling, is distinct because only specific regions undergo DNA replication rather than the whole genome. This event occurs when stage 6 follicle cells enter a rapid series of endocycles, enabling these cells to produce a high number of chorion proteins. One protein previously demonstrated to play a critical role in endocycle replication in follicle cells is SuUR (Suppressor of Under-Replicated). Furthermore, studies have also identified similar chromatin localization and protein interactions between SuUR and CG15436, a relatively unstudied gene. The current study examines CG15436 in vivo to determine if a phenotypic link can be established between CG15436 and SuUR. Using FLP-FRT recombination, a null allele of CG15436 has been generated and is homozygous viable. Further analysis, both of the null allele and an insertion allele of CG15436, demonstrate a reduction of egg production that is also exaggerated at a less permissive temperature. Moreover, upon examination of the ovaries it is noted that CG15436 mutants lack normal ovariole structure between developmental stage 6 and stage 10, which corresponds to endocycle replication within the follicle cells. Additionally, eggs from CG15436 mutants, that did reach maturity, exhibit slightly smaller appendages on the egg a phenotype that has been previously demonstrated with SuUR mutants. Taken together, this data suggests that the previous molecular associations between SuUR and CG15436 have in vivo relevance during endoreplication within ovary follicle cells. This newly assigned role of CG15436 better defines which molecular members participate in endocycling within the follicle cells; therefore, elucidating the mechanisms which control differential DNA amplification, not only in follicle cells, but other species that undergo endoreplication.

448  **Regulation of Germline Sexual Identity in Drosophila melanogaster.**  Pradeep Bhaskar1, Shu Yuan Yang2, Sheryl Southard3, Mark Van Doren1 1) Department of Biology, Johns Hopkins University, Baltimore, MD; 2) Department of Biomedical Sciences, College of Medicine, Chang Gung University, Kweishan, Taoyuan, Taiwan; 3) Carnegie Institution for Science, Washington, United States.

Sexual dimorphism is common throughout the animal kingdom, with males and females exhibit phenotypic characters specific for their sex. While a great deal is known about the establishment of sexual identity in somatic cells, this process is much less well understood in the germline. Germline sexual identity is critical for sex-specific development of germine stem cells and production of sperm vs. eggs. Thus, it is an essential aspect of animal sexual reproduction and human fertility. Germ cells depend on both signals from the somatic gonad as well as their own sex chromosome genotype to determine their sex. Therefore, when the “sex” the germline fails to match the “sex” of the soma, germline development is severely disrupted. How somatic signals and germ cell intrinsic cues act together to regulate germline sex determination is a key question about which little is known in any organism. We have previously identified the JAK/STAT pathway as a key male determining signal from the soma to the germline; evidence exists for a similar signal in females, but this signal has not been identified. Further, the RNA-binding protein Sex-Lethal (SXL) and the chromatin factor PHF7 have been identified as key components promoting female vs. male germline identity, respectively. We propose that the JAK/STAT pathway is a direct activator of PHF7 expression.
in the male germline, and that this pathway is inhibited by SXL in the female germline. Further, male-specific expression of PHF7 appears to be regulated at the levels of both transcription and translation to ensure its proper male-specific expression. Both the 5' and 3' UTR of Phf7 seems to be regulated sex specifically. Additionally, we have evidence suggesting signals from the soma can affect PHF7 function, whereas SXL function in the germline seems to be exclusively regulated by intrinsic factors. We are investigating the regulation of the key germline sex determination factors PHF7 and SXL and how they are controlled by both somatic signals and germ cell intrinsic factors. Our understanding of mechanism of male specific expression of PHF7 will help to understand how cellular sexual identity is maintained for successful gametogenesis and fertility.

449 Postmatting modification to the Drosophila melanogaster sperm proteome. E. Whittington1, A. Singh2, M. Wolfner2, S. Dorus1  
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Sperm undergo several stages of modification within the female reproductive tract. These modifications are often essential for progression through postmatting events, such as storage and capacitation, which culminate in fertilization. Furthermore, interacting male and female proteins involved in these modifications are frequently shown to coevolve rapidly. These interactions may underlie species differences and act as potential barriers to reproduction. However, our current understanding of post mating sperm modification is founded upon isolated studies of particular gene candidates and reproductive events. The breadth and ubiquity of modifications, and what they entail on a broad scale, is unknown. Here we characterize the Drosophila melanogaster sperm proteome from the female bursa and sperm storage organs half an hour, 2 hours, and 4 days post mating. This allows us to survey how the sperm proteome varies over time in the female reproductive tract, with particular relation to initial interactions in the female reproductive tract, sperm storage entry, and long term sperm storage. We apply bioinformatic and comparative proteomic approaches to assess changes in proteome composition, protein abundance, and functional and molecular pathway enrichment across time points, characterizing broad suites of modifications to the sperm proteome in the female reproductive tract. Additionally, we identify likely candidates for proteins associated with sperm whilst in storage and further across time points. This work is the first to characterize the sperm proteome from the female reproductive tract at different time points, to track post mating sperm modifications at the molecular level. This data will substantially improve our comprehension of sperm modifications beyond that of isolated cases, and to a broader understanding of their breadth and constitution. Proteins identified as involved in sperm modifications, for example by their addition to the sperm proteome, are potential key players in the post mating interactions essential to reproduction. We discuss the data in the context of interactions between sperm, seminal fluid, and female contributed proteins.

450 Guidance of stem cell niche assembly, position, and architecture. Lauren Anllo, Lindsey Wingert, Stephen DiNardo  
Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

Stem cells are required for renewal and regeneration of damaged or aging tissue. Intricate signaling between the niche and its resident stem cells is necessary to accomplish these tasks. Signaling requires intimate contact with a well-designed niche: one positioned accurately in the tissue, and with its constituent cells well-organized. Unfortunately, there are few cases where niche morphogenesis can be studied at the necessary resolution. For this reason I have chosen to study niche morphogenesis in the male gonad. The eventual function of this niche is well defined, and has served as a paradigm in niche stem cell biology. Furthermore, our lab recently pioneered live-imaging morphogenesis of the niche during embryogenesis. We capitalized on visualizing niche assembly to uncover candidate tissues that could be the source for signals that organize niche formation. Imaging revealed that the niche was located near the trachea, an alary muscle, and the visceral mesoderm, all of which are known to send developmental regulatory signals. We genetically ablated each of these tissues in turn, and discovered that niche assembly is disrupted when the visceral mesoderm is removed. Thus, the visceral mesoderm (Vm) produces a guidance cue to direct niche placement. To identify the potential cue, I screened an extant database (http://flyfish.ccb.rutontoca/) for signaling genes expressed within the Vm, and identified candidates such as Netrin, Wunen, and Neurotrophic factor. I am using mutants and misexpression experiments to test such candidates for roles in guiding niche placement. In summary, I have identified a tissue source which guides niche assembly, and I have identified candidate signals that might be used for this purpose. This research represents the first work describing how the stem cell niche is positioned correctly during its development, and thus will bridge an important gap in our knowledge of stem cell-niche biology.

451 The role of prostaglandins in collective, invasive cell migration. E. Fox, T. Tootle  
Anatomy and Cell Biology, University of Iowa, Iowa City, IA.

Collective cell migration – the coordinated movement of tightly or loosely associated cells – is important for both normal development and tumor invasion. While prostaglandins (PGs), short-range lipid signaling molecules, regulate cell migration, their mechanisms of action are poorly understood in both single and multicellular migration contexts. To address this knowledge gap we use the collective, invasive, epithelial migration that occurs during Drosophila oogenesis. The Drosophila ovary contains chains of developing follicles composed of 15 germ line derived nurse cells and 1 oocyte surrounded by a layer
of somatic epithelial cells. During Stage 9 of oogenesis, a cluster of 6-8 of these somatic cells delaminate from the outer epithelium and migrate invasively between the nurse cells to the oocyte border; this migration is termed border cell migration. To study the roles of PGs in border cell migration, we utilize genetic mutations in pxt, the Drosophila cyclooxygenase-like enzyme, which is responsible for all PG synthesis. Using quantitative analyses, I find that loss of Pxt causes aberrant border cell migration. Loss of Pxt results in both a significant delay in border cell migration and an increase in cluster length compared to wild-type controls. We hypothesize that both the delay and alteration in cluster morphology are due to changes in among the border cells and/or between the border cells and the surrounding nurse cells. While E-Cadherin appears to be unaffected by the loss of Pxt, integrin levels on the interface between the border cells and the nurse cells is reduced. As integrin-based adhesion is essential for correctly timed border cell migration and cluster cohesion, our data supports that model that PGs regulate integrins to control border cell migration and cluster morphology. Future work will further investigate this model as well as the role of PGs in the border cell cluster vs the nurse cells. Our studies on PG signaling during border cell migration provide insights into the conserved mechanisms by which PGs regulate collective, invasive cell migrations. Indeed, high levels of PGs and integrins are independently associated with cancer migration and metastasis.

452 Role of rib in Gonad Development and Function. Danielle E. Talbot, Manuel Alvarez, Usama Khan, Sana Moqeeet, Jennifer Jemc 1 1) Department of Biology, Loyola University Chicago, Chicago, IL; 2) University of Illinois at Chicago, Chicago, IL.

During organogenesis, cell signaling plays a critical role in the regulation of cell migration, proliferation, and the establishment of cell-cell interactions. Misregulation of any of these processes can lead to organs that fail to form and/or execute their functions properly, resulting in disease, disorders, and even lethality. Many genes regulating cell proliferation, differentiation and apoptosis during organogenesis are also required for the maintenance of adult organ structure and function. The gonad has proven an excellent model to study how signaling pathways that function early in organ development act to maintain adult organ homeostasis. Previous studies identified the gene *rib* (rib) as a critical regulator of embryonic gonad development. Immunohistochemistry reveals that *rib* continues to be expressed in the somatic cells and germline of the adult testis, suggesting that Rib may play a role in the maintenance of gonad structure and function. In order to explore Rib function in later stages of development and in the adult, rib overexpression and knockdown experiments were performed in germline and somatic gonadal cells. *rib* overexpression in the male and female somatic cells results in severe morphological defects, while germline overexpression yields a milder effect on male gonad development. Knockdown of *rib* in somatic cells results in mild gonad defects in the male and no effect on the female gonad. Current studies are ongoing to understand the basis of the severe morphological defects observed upon *rib* overexpression, to examine the effects of reducing *rib* levels, and to identify the molecular context in which Rib functions. Understanding the role of Rib in the context of the gonad will allow us to understand how it functions in other tissues to promote development and organ homeostasis.

453 Signalling interactions in hippo-dependent somatic cell number regulation during *Drosophila melanogaster* ovarian morphogenesis. T. Kumar1,2, CG Extavour1,2 1) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Ovarioles are the functional units of the Drosophila ovaries, consisting of a germarium and an ontogenetic series of developing ovarian follicles. The development of the ovary begins in the embryo with the formation of the gonadal primordium. During larval development, the ovarian cells undergo proliferation and differentiate into its constituent cell types. Regulation of somatic cells in the ovaries is necessary and sufficient to define the number of ovarioles in an adult ovary.

Ovariole number is very stable both within and across species suggesting a stereotypic developmental pathway and regulated signalling cascades. Hippo signalling has been implicated in the regulation of somatic cell number and therefore, ovariole number.

Here, we present a detailed map of the interactions of all the known *Drosophila* developmental signalling pathways in the hippo-dependent regulation of somatic cell numbers. In particular, we focus on the roles of cell proliferation and apoptosis in the specification and regulation of the different somatic cell types in the ovary.

We have identified a novel role for dark, a key regulator of apoptosis, in the regulation of ovariole number. Our preliminary results also show that Hedgehog signalling is required for the increase in ovariole number caused by the abrogation of hippo signalling.

Ovariole number is, a plastic phenotype, strongly correlated with ecological changes like nutrition, altitude and temperature. We visualise this phenotypic malleability, as a series of dials which impinge on different signalling pathways and are regulated by external feedback. With our comprehensive understanding of the signalling pathways in ovarian development, we aim to better understand the regulation of phenotypic plasticity.

454 Sex-specific specification of the follicle stem cells in the developing *Drosophila* ovary. A. Fuchsman, M. Van Doren Biology Department, Johns Hopkins University, Baltimore, MD.
Sexual dimorphism is crucial for the propagation of a sexually reproducing species and the formation of an oocyte vs. sperm. We are interested in how the sex determination pathway controls sexual dimorphism, including how the conserved transcription factor Doublesex (DSX) regulates sex-specific development of the somatic gonad. Follicle cells are female-specific cells that surround and nurture the developing oocyte and are found in diverse animals, including flies and mammals. The germarium of the Drosophila ovary contains the stem cells that give rise to the follicle cells, but how these stem cells are specified remains unknown. Recent work from the Kalderon lab suggests that there is a single type of somatic stem cell in females, which can give rise to both escort cells (which nurture the germline early in differentiation) and follicle cells (which nurture the germline later). One hypothesis is that these female somatic stem cells (SSCs) are equivalent to the cyst (somatic) stem cells (CySCs) in males, and that the role of the sex determination pathway is to differentiate between these two types of SSCs. We are investigating the developmental origin of the female SSCs, their relationship to CySCs, and the role of DSX in controlling their sex-specific development. The best-known marker for FSCs is the transcription factor Castor, which labels female SSCs in addition to pre-follicle cells, and stalk cells. We have conducted a time-course immunostaining of pupal ovaries examining Castor expression as a readout for SSC specification. Castor is not observed at 2 hrs through 7 hrs after pupal formation (APF). The earliest Castor expression can be seen at 9 hrs APF in cells intermingled with the germ cells in the middle of the developing ovarioles. At 24 hrs APF, Castor expression is seen primarily in the basal stalk cells posterior to the germline. We are currently using lineage analysis to study the origins of the SSCs and if they are related to basal stalk cells. Additionally we have determined that SSC specification is dependent on the JAK/STAT pathway, similar to what is thought for CySC specification in males. Knockdown the JAK/STAT pathway in the somatic cells of the ovary results in a loss of follicle cells and Castor expression without seemingly affecting any other cell type. We are investigating the mechanisms that control female SSC specification and how the JAK/STAT pathway may intersect with information about sexual identity regulated by DSX.

455  “Survival of the fittest”: Understanding hypercompetition in the follicle stem cell niche.  S.D. Tatapudy, M Cook, T Nystul
UCSF, San Francisco, CA.

The germarium, a structure at the tip of the ovariole of a Drosophila ovary, contains two follicle stem cells (FSCs) that are lost and replaced regularly by daughters of neighboring stem cells. However, some mutations can confer a competitive advantage or disadvantage to a mutant stem cell relative to the neighboring wild type stem cell. A genetic screen conducted in the Nystul lab through a collection of 126 mutants in essential genes on the X chromosome, identified candidate hypercompetitive and hypocompetitive alleles that increase and decrease stem cell replacement in the FSC niche respectively. Since hypercompetition mutations, by definition, enhance certain cellular features that are selected for by the competition process, understanding hypercompetition phenotypes will provide insight into underlying mechanisms that regulate niche competition. This study aims to elucidate mechanisms by which certain mutations confer a hypercompetition phenotype upon FSCs. By using FRT mediated mitotic recombination, we made stem cell mutant clones and measured the rate of stem cell replacement at six, twelve and eighteen days after clone induction. Our results confirm that BenA, an allele of an E2 Ubiquitin ligase Bendless, is a strong hypercompetitor that exhibits a significantly higher stem cell replacement rate as compared to the control. Given that Bendless plays a crucial role in cellular signaling, ongoing experiments are working towards identifying signaling pathways that are differentially regulated in BenA mutant and wild type stem cells. Additionally, using BenA and other candidate hypercompetitive alleles, we will investigate the role of differentiation, proliferation and apoptosis during stem cell replacement in the FSC niche.

456  Small ovary regulates germline stem cell survival and differentiation by promoting heterochromatin formation.  F. Jankovics1, M. Ben61, Z. Takács1, B. Szarka-Kovacs1, B. Laurinyecz2, L. Bodai2, A Pettko-Szandtner1, R. Sinka2, M. Erdélyi1 1) Institute of Genetics, Hungarian Academy of Sciences, Szeged, HU; 2) University of Szeged, Szeged, HU.

In a large scale RNAi screen, we have identified small ovary (sov) to be essential for Drosophila germ line development. By FLP/FRT based mitotic recombination and subsequent complementation analysis, loss of function sov alleles were isolated and tested for germ cell defects. Mutants displayed rudimentary female gonads suggesting a pleiotropic role for sov in germ line stem cell (GSC) niche function. In sov mutant niches, GSCs are lost and the differentiation of the GSC daughter cells is not initiated. As a consequence, permanent proliferation of the GSC daughter cells generates small-sized stem cell tumours. Cell-type specific silencing and analysis of mutant mitotic clones revealed that Sov functions simultaneously in GSCs and somatic niche cells to ensure stem cell survival and differentiation. We show that Sov maintains niche integrity and function by regulating piRNA-mediated transposon silencing. Sov plays an essential role in piRNA production by promoting generation of the long piRNA precursors which are derived from the genomic piRNA clusters. The majority of these clusters reside in the pericentromeric regions of the genome and require a heterochromatic context for their transcription. We demonstrate that Sov enhances heterochromatin formation which in turn enables efficient transcription of the piRNA clusters.

457  Germline stem cell maintenance control by adipocyte collagen in adult Drosophila females.  L. Weaver, D. Drummond-Barbosa
Biochemistry and Molecular Biology Department, Johns Hopkins University, Baltimore, MD.
Stem cells reside in specialized niches that are a source of local signals and also receive a variety of systemic inputs. In adult female *Drosophila*, germline stem cells (GSCs) are physically attached through E-cadherin adhesion to a somatic niche composed primarily of cap cells. Local signals produced by cap cells, including bone morphogenetic protein (BMP) signals, are required for proper regulation of GSC function. In adult females, a prominent collagen IV-containing extracellular matrix is maintained around the GSC niche; however, the cellular source of collagen IV in the adult GSC niche or whether it affects GSC function is unknown. In the adult female fat body, collagen IV proteins are abundant and regulated by diet, leading us to ask whether collagen IV function in adipocytes is required for GSC function. Adipocyte-specific knockdown of collagen IV in adult females leads to increased GSC loss over time. Interestingly, we found that collagen IV produced in adult adipocytes is transported to and incorporated into the extracellular matrix in the established GSC region during adulthood. Although BMP signaling from the niche is not perturbed, E-Cadherin levels are decreased at the GSC-niche interface upon collagen IV knockdown in adipocytes. In addition, collagen IV genetically interacts with integrin and focal adhesion kinase (FAK) to influence GSC maintenance. Furthermore, GSC loss caused by adipocyte collagen IV knockdown is dominantly enhanced by removal of one copy of FAK. Together with the knowledge that collagen IV is a ligand for integrin in other contexts, these results suggest that collagen IV transported from adipocytes directly stimulates integrin signaling in the niche to regulate E-Cadherin levels. To our knowledge, this is the first example of an extracellular matrix component produced in adult adipocytes being transported to a stem cell niche in a distinct, fully established adult tissue. These findings are a major step in advancing our understanding of the wide range of mechanisms for how adipocytes control the function of other organs, and are widely relevant considering the devastating impact of the current global obesity epidemic on our health.

458 Molecular mechanisms of neuroblast reactivation in *Drosophila*. **J. Huang**, H. Wang 1,2,3 1) Neuroscience & Behavioural Disorders, Duke-NUS Graduate Medical School, Singapore, SG; 2) NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, SG; 3) Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, SG.

Stem cells have the ability to undergo states of self-renewal, differentiation or reversible quiescence. Understanding the mechanisms controlling the switching between these states will aid in the development of stem cell-based therapies and cancer treatment. In the central nervous system (CNS) of Drosophila, neuroblasts enter quiescence at the end of embryogenesis and re-enter the cell cycle later after larval hatching. Therefore, neuroblasts have become an excellent model for studying stem cell quiescence and reactivation (i.e. the exit of quiescence and the re-initiation of proliferation). Neuroblast reactivation is simulated by dietary amino acids and insulin-PI3K pathway. However, the mechanisms that control this pathway are largely unknown. We identified Hsp83 (Heat shock protein 83), a well-known chaperone, as a novel intrinsic regulator in neuroblast reactivation. Knockdown of Hsp83 by RNAi in neuroblasts results in neuroblast quiescence. The level of components of insulin-PI3K pathway decrease in the brain upon hsp83 knockdown. Both Hsp83 and its co-chaperone Cdc37 physically interact with insulin receptor (InR). Remarkably, hsp83 knockdown phenotype is rescued by overexpressing a constitutively active form of InR. Our study reveals that Hsp83 affects neuroblast reactivation via interacting with InR of insulin-PI3K pathway. Both Hsp83 and the pathways governing neuroblast reactivation in Drosophila are highly conserved in mammalian systems. Our results will not only broaden the understanding of neuroblast reactivation regulation in Drosophila, but also provide insights in understanding mammalian neural stem cells.

459 Cullin4-RING ubiquitin Ligase (CRL4) complex regulates reactivation of *Drosophila* neural stem cells. **P. Thao Ly**, Chwee Tat Koe1, Yingjie Zhang1, Hongyan Wang1,2,3 1) Neurosciences & Behavioral Disorders, Duke-NUS Medical school, Singapore, Singapore, SG; 2) National University of Singapore Graduate School for Integrated Sciences and Engineering, Centre for Life Sciences, SG; 3) Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.

The balance between quiescence and reactivation of stem cells is critical for tissue homeostasis and tumorigenesis prevention, but the underlying molecular mechanisms are largely unknown. The *Drosophila* larval neural stem cell (neuroblast) is a powerful model to study reactivation in vivo. Currently, only four pathways are known to regulate neuroblast reactivation intrinsically: (i) insulin signaling, (ii) transcription factor Prospero, (iii) Hippo signaling, and (iv) Spindle matrix protein Chromator. Based on a genetic screen, we uncover the highly conserved Cullin4-RING E3 ubiquitin ligase complex (also known as CRL4 complex) as a novel regulator for neuroblast reactivation. The loss-of-function of CRL4 core subunits, DDB1 (for DNA-damage-binding protein 1) and Cul4 (for Cullin4), causes reactivation defects. CRL4 likely promotes neuroblast reactivation by its conventional ubiquitin ligase activity because the ubiquitin-ligase-impaired form of Cul4, Cul4A, fails to rescue reactivation defects of cul4" mutants. We also demonstrate that the CRL4 complex functions downstream of Insulin receptor in promoting neuroblast reactivation. The identification of the relevant substrate receptors and substrates of CRL4 ligases in neuroblast reactivation is in progress. The highly-conserved nature of CRL4 complex suggests the findings from this study could be relevant to quiescence-reactivation regulation in other organisms, including humans, and be generally relevant to stem cell homeostasis.
460 Eyeless uncouples neuroblast proliferation from dietary nutrients in *Drosophila*.  
Conor Sipe, Sarah Siegrist  Department of Biology, University of Virginia, Charlottesville, VA.

All neurons in the *Drosophila* central brain are generated from asymmetric cell divisions of neural stem cells, known as neuroblasts (NBs). Most NBs enter quiescence at the end of embryogenesis coincident with declining maternal nutrient stores; upon larval feeding, these nutrient-sensitive NBs reenter the cell cycle and begin a second round of proliferation that continues until early pupal stages. In contrast, a small subset of central brain NBs, the mushroom body (MB) NBs, never enter quiescence and divide continuously throughout development regardless of nutrient intake. Both NB subtypes reside in close proximity to one another and share a common macroenvironment, suggesting that quiescence versus proliferation decisions are regulated in a cell-intrinsic manner. We have demonstrated that the transcription factor Eyeless (Ey), a conserved Pax-6 ortholog predominantly expressed in MB NBs, is required for nutrient-independent NB proliferation. When Ey is knocked down, MB NBs exit the cell cycle in response to dietary nutrient withdrawal; conversely, when Ey is ectopically expressed in all NBs, some non-MB NBs continue to divide independent of dietary nutrient conditions. Therefore, Ey is a cell-intrinsic factor that is both necessary and sufficient to uncouple NB proliferation from dietary nutrient intake. We are currently investigating if conventional cell growth pathways are required for Ey to exert this function. We find that the transcription factor Myc is also required for nutrient-independent proliferation of MB NBs, whereas PI3-kinase signaling is not. Myc expression in MB NBs with reduced Ey can rescue their ability to proliferate during dietary nutrient withdrawal. However, since Myc protein and transcript levels are not altered in Ey RNAi MB NBs, we propose that Ey and Myc act in parallel pathways to control MB NB nutrient-independent proliferation. Our work highlights an important role for lineage-specific factors in regulating proliferation decisions in response to nutrient availability in a cell-autonomous manner.

461 Groucho controls proliferation and differentiation of *Drosophila* intestinal stem cells by co-operating with E(spl) factors.  
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*Groucho* is an evolutionarily conserved transcriptional co-repressor and has been implicated in regulating cell proliferation and cell fate decisions in many developmental processes. But whether it has a role in intestinal stem cell lineages is unknown. From an RNAi genetic screen, we identified *groucho* as a critical regulator of cell proliferation and differentiation of intestinal stem cells in *Drosophila* midgut. We find that depleting *groucho* in ISCs leads to rapid accumulation of ISC-like cells and failure of enteroendocrine cell differentiation. It is known that Notch signaling activation is essential for ISC differentiation, but loss of *groucho* does not compromise Notch pathway activation. Furthermore, ectopic expression of NICD or the Notch target *e(spl)* genes fails to induce differentiation of *groucho*-depleted ISCs. Together with the analysis on its role in ISC proliferation, we propose that Groucho acts as a co-repressor of E(spl) factors to regulate ISC differentiation and proliferation.

462 Coordinated regulation of adult stem cell proliferation and differentiation by Sox21a and Sox100B in the *Drosophila* intestine.  
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The precise control of stem cell proliferation and differentiation in a coordinated manner is essential to maintain tissue homeostasis, allowing both normal tissue turnover at homeostatic condition and tissue repair in response to various stresses. In great contrast to the fact that the majority signaling pathways involved in regulating proliferation and differentiation are known, the transcriptional networks downstream of these pathways remain largely unclear. We previously found that the Sox family transcription factor Sox21a is specifically expressed in the progenitor cells in the *Drosophila* intestine, including intestinal stem cells (ISCs) and enteroblast cells (EBs) which are committed to differentiation into polyploidy enterocytes (ECs). We showed that the ISC-specific Sox21a expression is essential for their proliferation both at homeostasis and stress conditions. Interestingly, we demonstrated that in response to stress, Sox21a expression level is induced downstream of EGFR/Ras and JNK signaling pathways to accelerate stem cell proliferation. Confirming the findings by other groups, we also found that Sox21a expression in EBs is essential for their differentiation into ECs, further highlighting the role of Sox21a in coordinating ISC proliferation and EB differentiation to maintain intestinal homeostasis. We now show that another Sox family transcription factor Sox100B is expressed in ISCs and EBs, and is required for optimal Sox21a mRNA and protein expression. We identify a putative Sox21a enhancer which is regulated by Sox100B, strongly suggesting that Sox21a is a direct target gene of Sox100B. We find that while Sox100B is not required for ISC proliferation, Sox100B is required in the EB differentiation process. Currently, we are characterizing the mechanism by which Sox100B controls Sox21a expression, as well as the mechanisms by which Sox100B-Sox21a transcriptional network precisely regulates EB differentiation.

463 The POU/Oct transcription factor Nubbin controls the balance of intestinal stem cell maintenance and differentiation by isoform-specific regulation.  
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*Drosophila* adult midgut epithelium is composed of two major differentiated cell types termed enterocytes (ECs) and enteroendocrine (EE) cells. The differentiation of these two cell types is tightly and precisely controlled by the multipotent
intestinal stem cells (ISCs), which are non-homogeneously distributed along the basement membrane of the midgut. During normal gut regeneration, ISCs divide asymmetrically into a transient enteroblast (EB) that subsequently differentiates into a mature and absorptive EC, while another daughter cell accounts for the secretory EE cell specification. The well-tuned balance between ISC proliferation and differentiation contributes to the construction of normal gut epithelium and in turn, sustains epithelium homeostasis. Likewise, disrupted ISC activity can promote hyperplastic growth and tumor formation or to regeneration failure and tissue loss.

*Drosophila* POU/Oct transcription factors have been shown to be involved in specification of embryonic stem cell fate, while little is known about their roles in regulation of adult stem cell activity. Here, we show that Nubbin (Nub)/Pdm1, homologous to mammalian Oct1/POU2F1 and related to Oct4/POUSF1, is not only expressed in adult midgut differentiated ECs but also in progenitor cells (ISC+EB). The *nub* gene encodes two proteins, Nub-PB and Nub-PD, with a common C-terminal part comprising the POU DNA binding domains. We demonstrate that these two isoforms play opposite roles in regulation of ISC proliferation. Depletion of Nub-PB in progenitor cells increased ISC proliferation by derepression of *escargot* expression. In contrast, loss of Nub-PD reduced ISC proliferation both in basal conditions and upon infection, suggesting a role of Nub-PD in maintaining stemness and multipotency, analogous to mammalian Oct4/POUSF1 functions. Furthermore, we demonstrate that Nub-PB is specifically required in EBs to promote differentiation and that it acts as a strong tumor suppressor of Notch RNAi-driven hyperplasia. We suggest that the relative abundance of Nub-PD/Nub-PB in progenitor cells is important for maintaining gut epithelium homeostasis.

**464 Mitochondrial pyruvate metabolism suppresses stem cell proliferation both cell autonomously and non-autonomously.** D. Roonalkia. Wisidagama, Carl S. Thummel Human Genetics, University of Utah, Salt Lake city, UT.

The Mitochondrial Pyruvate Carrier (MPC) is necessary and sufficient for mitochondrial pyruvate import in yeast, *Drosophila* and mammals, linking cytoplasmic glycolysis with mitochondrial oxidative phosphorylation. This central role in metabolism positions the MPC at a critical point in determining the rate of cellular energy production. Previously we showed that high rates of mitochondrial pyruvate metabolism mediated by the MPC are required for efficient glucose-stimulated insulin secretion in *Drosophila* and mice. Here we show that the MPC is necessary and sufficient for suppressing excess stem cell proliferation. Using mutant clonal analysis and cell-specific RNAi we show that a loss of MPC function increases stem cell proliferation in the *Drosophila* intestine in a cell autonomous manner. Conversely, overexpression of the MPC in wild-type intestinal stem cells (ISCs) reduces proliferation. Genetic studies of enzymes upstream and downstream from the MPC support these results, demonstrating that mitochondrial pyruvate metabolism plays a direct role in controlling the rate of stem cell proliferation. In addition, we discovered that a loss of the MPC in differentiated enterocytes remotely controls stem cell proliferation. Loss of MPC in enterocytes results in increased acidosis and Unpaired 3 (Upd3) expression. This inflammatory signaling, in turn, leads to increased JAK/STAT activity in ISCs, stimulating proliferation. Additionally, both Upd3 and Lactate Dehydrogenase (LDH) are necessary in enterocytes for increased ISC proliferation in the absence of the MPC. Our current studies are investigating the cell autonomy of MPC function in enterocytes and the mechanisms by which a shift in cellular pyruvate metabolism leads to increased JNK and/or Hippo signaling and downstream upd3 expression. Taken together, our results show that metabolism does not only provide a permissive environment for cellular function but can play an active and direct role in cell fate and tissue homeostasis.

**465 A positive feedback regulatory circuit mediated by Phyllopod promotes enteroendocrine cell commitment from *Drosophila* intestinal stem cells.** C. Yin1,2, RW. Xi1,2,3 1) National Institute of Biological Sciences Beijing, BEIJING, Beijing, China; 2) Graduate School of Peking Union Medical College, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; 3) Institute for Regenerative Medicine, Shanghai East Hospital, School of Life Sciences and Technology, Tongji University, Shanghai, China.

The intestinal epithelium in the *Drosophila* midgut is maintained by intestinal stem cells (ISCs), which are capable of generating both enterocytes and enteroendocrine cells (EEs) via alternative cell fate specification. Activation of Delta-Notch signaling directs ISCs for enterocyte generation, but how EEs are generated from ISCs remains poorly understood. Here we identified Phyllopod (Phyl) as a key regulator that drives EE generation from ISCs. Phyl, which is normally suppressed by Notch, functions as an adaptor protein that bridges Tramtrack 69 (Ttk69) and E3 ubiquitin ligase Sina for degradation. Degradation of Ttk69 allows the activation of the Achaete-Scute Complex (AS-C)- Pros regulatory axis that promotes EE specification. Interestingly, expression of AS-C genes in turn further induces Phyl expression, thereby establishing a positive feedback loop for continuous EE fate specification and commitment. This positive-feedback-circuit-driven regulatory mechanism could represent a common strategy for the reliable and irreversible cell fate determination from progenitor cells.

**466 Transcriptional targets in the neuronal regulation of resident hematopoietic sites.** Glenda Li1,4, Leire Herboso2,4, Katelyn Kukar2,4, Debra Ouyang2,4, Corinna Wong2,4, Katja Brückner1,2,3,4 1) Broad Center of Regeneration Medicine and Stem Cell Research; 2) Dept. Cell and Tissue Biology; 3) Cardiovascular Research Institute; 4) University of California San Francisco, CA.
One of the outstanding questions in animal development and tissue homeostasis is how extrinsic stimuli regulate cell signaling and development that allow adaptation to the environment. Our goal is to understand, at the molecular level, how the peripheral nervous system (PNS) and its activity regulate organs and target tissues during development and homeostasis, using a *Drosophila* model of sensory neuron-dependent blood cell production. The model shows parallels with vertebrate hematopoiesis in the bone marrow niche, and self-renewing tissue macrophages such as the Langerhans cells of the skin (Gold and Brückner 2014; Gold and Brückner 2015). In the *Drosophila* model, blood cells (hemocytes) reside in contact with sensory neurons in specialized microenvironments known as hematopoietic pockets (Makhijani et al. 2011; Makhijani et al. 2012). Sensory neurons are crucial for hemocyte proliferation, survival and localization (Makhijani et al. 2011; Makhijani et al. 2017). We have identified PNS neuron-produced Activin-β (Actβ), a TGF-β family ligand, as one of the signals that translates neuronal activity into blood cell proliferation (Makhijani et al. 2017).

To identify transcriptional targets downstream of sensory neuron activation and Actβ/dSmed2 signaling, we initiated RNAseq transcriptome analysis of hemocytes, compared to control tissue. Specifically, we examined transcriptional changes in hemocytes following PNS neuronal stimulation (+/− carbachol, a pan-Acetylcholine Receptor agonist), and transient sensory neuron silencing (+/− UAS-Kir2.1 under control of tub-GAL80ts). To identify which aspects of the transcriptional response are promoted by Actβ/dSmed2 signaling, we silenced components of the Actβ/dSmed2 pathway in hemocytes (+/− RNAi silencing of dSmed2, or + RNAi of the Actβ type II receptor put, respectively). Our analysis suggests a shift in the translational and metabolic status of hemocytes following neuronal activation. It further identifies expression of previously unrecognized signaling molecules that are conserved with vertebrate self-renewing macrophages. Candidate genes are being tested functionally for their roles in hemocyte proliferation, survival, and differentiation.

### 467 Role(s) of bric-à-brac and engrailed in Germline Stem Cell Niche (GSC) formation, in the *Drosophila melanogaster* ovary.

* L. Miscopin Saler1, M. Bartoletti4, L. Varoquaux1, M. Madoni1, H. Tardivel1, P. Dumas1, F. Chalvet1, S. Netter1,2

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Stem cell recruitment and maintenance depends on the environment provided by a specific niche. The Germline Stem Cell (GSCs) niche of the *Drosophila melanogaster* ovary is an excellent model to study niche formation: first, the signaling pathways involved in GSC maintenance are very well characterized, and second, markers have been identified for the different somatic cell types composing the niche. In the adult ovary, there are around twenty niches, each composed of a stack of flattened cells forming the Terminal Filament (TF), associated Cap Cells (CCs) and Escort Cells (ECs). Each niche allow the maintenance of a small pool of GSCs and, thus, the continuous production of oocytes throughout the lifetime of the adult female. In contrast to what is known about the maintenance of GSCs in the adult niches, little is known concerning the molecular networks involved in the formation of functional niches in the prepupal ovary. Niche formation is a morphogenetic process during which TF cells (TFCs) progressively flatten, intercalate to form stacks and recruit CCs, GSCs and ECs. TFCs are characterized by the specific expression of three transcription factors: Bric-à-brac 1 (Bab1), Bric-à-brac 2 (Bab2) and Engrailed. Our results indicate that Bab1 and Bab2 have redundant functions in TFCs for their flattening and stacking to form TFCs, for recruitment of GSCs, as well as for engrailed expression. To determine whether the implication of Bab1 and Bab2 genes in the formation of functional niches is linked to the regulation of engrailed expression, we conducted engrailed knock-down experiments and show that (1) *engrailed IS NOT necessary for GSCs recruitment in prepupal stages*, whereas it has been shown to be necessary for GSCs maintenance in the adult; and (2) with a gain of function approach, we show that ectopic expression of bab1, bab2 and engrailed is sufficient, under certain conditions, to recruit ectopic GSCs in the prepupal ovary. Taken together, our results indicate that niche establishment in the larval ovary and niche maintenance in the adult are controlled by distinct mechanisms.

### 468 BMP Signaling in the CySCs of the *Drosophila* Testis Stem Cell Niche.

* N. Mues, J. Major, J Koretko, B Johnson, M Ahmed, J Leatherman School of Biological Sciences, University of Northern Colorado, Greeley, CO.

Adult stem cells live in different tissues, and they support and regenerate the tissue they reside in and themselves. The stemness behavior is tightly regulated by the niche. The *Drosophila* testis is a valuable model niche that has been used to study how stem cells are maintained in the niche. In this niche, there are two populations of stem cells; germline stem cells (GSCs) and somatic cyst stem cells (CySCs), which provide the cells to maintain the production of sperm in *Drosophila* males throughout their adult life. These stem cells co-mingle around a group of non-dividing somatic cells known as the hub, which is the niche that provides molecular signals to instruct the behavior of the surrounding stem cells. The fate of GSCs and differentiating germ cells is dependent on CySCs and their descendants, cyst cells, because if we block these cells, GSCs are unable to be maintained normally as stem cells or differentiate properly.

The main self-renewal regulator in GSCs is the BMP signaling pathway, and inhibition of signaling in germline cells has been shown to eliminate the population of GSCs. Recent work on merlin from Inaba et al. (2017) revealed for the first time that BMP signaling may be mitogenic for the cyst stem lineage, as constitutive tkv receptor caused cyst cell tumors. We also found that
activated tkv caused tumors of cyst lineage cells in the testis. We have also been examining the requirement for BMP signaling in the CySCs. When we ablated tkv in the cyst lineage cells by RNA interference, we observed a partial loss of CySCs and loss of differentiated cyst cells. In the absence of cyst cells, the germline cells also failed to differentiate properly. Mutant clone analysis of BMP pathway components revealed that CySCs deficient for the ability to transduce BMP signaling were not maintained as stem cells as well as control cells. In the future, we will determine how BMP pathway inhibition affects the cycling rate of the CySCs.

469 Development of ovarian Follicle Stem Cells during pupal stages. A. Reilein, H.V. Kogan, K.S. Park, D. Kalderon Biological Sciences, Columbia University, New York, NY.

As we learn how adult stem cell communities and their supporting cells are arranged, it becomes possible to ask how that organization arises during development. We are interested in how patterned extracellular signals guide the initial specification and behavior of Follicle Stem Cells (FSCs) and associated niche cells in the ovary. In the pupal ovary, germline cells are mixed with somatic progenitors. The somatic progenitors differentiate into 14-16 FSCs in the adult ovary and 25-30 post-mitotic Escort Cells (ECs). We are using lineage analysis and direct examination of pupal ovaries to test the hypothesis that the adult organization of ECs and FSCs derives from a Wnt gradient of anterior origin that imposes EC potential, followed by the later development of an inverse JAK-STAT pathway gradient that promotes FSC behavior and institutes the graded proliferation pattern seen in adults. We conducted a lineage analysis in which wild-type MARCM clones were induced on different days prior to adult eclosion and scored in 2d-old adults. Most FSC-producing lineages initiated prior to -3d before eclosion also included ECs, implying a common precursor. The number of ECs per clone declined significantly when precursors were marked at -3d or later, indicating a large reduction in dividing precursors during the second half of pupation. We derived an estimate for the number of precursors of each type (those that give rise to ECs, FSCs, or both) at each time point, calculated from the observed proportion of clone types and their average yield of each cell type in the adult.

470 The Role of Centromere Components in Germline Stem Cell Identity and Asymmetric Division. B. Carty, A. A. Dattoli, E. M. Dunleavy Centre for Chromosome Biology, National University of Ireland, Galway, Galway, IE.

Stem cells are unspecialised cells that are essential to the generation of all multicellular organisms during embryogenesis and throughout adult-life. Unlike normal cell division which produces two identical daughter cells, stem cells have a distinctive ability to divide asymmetrically to produce: 1) a daughter cell that maintains itself as a stem cell (exact copy of the parent cell), 2) a differentiating daughter cell, capable of becoming a specialised cell type. The asymmetric division of Germline Stem Cells (GSCs) in the Drosophila germline produces gametes (sperm/oocyte). Thus, disruption to the balance of this process gives rise to devastating diseases of tissue homeostasis and aneuploidy, such as tumour development and infertility. A key question, therefore, is to elucidate the molecular control of asymmetric stem cell maintenance versus differentiation.

Epigenetic mechanisms (heritable changes in DNA that do not alter the primary genomic sequence) have been shown to play a role in maintaining stem cell identity during stem cell division. Centromeres are key chromosomal loci that coordinate chromosome segregation at cell division and are epigenetically defined by the histone H3 variant CENP-A. In addition, CENP-C constitutes the primary inner kinetochore component, which binds to and is required for CENP-A assembly and maintenance. In human cells, fluorescent recovery after photo-bleaching (FRAP) analysis has indicated that CENP-A is loaded to the centromere in early G1-phase, with CENP-C only being stably bound in mid-late S-phase. Moreover, it has been previously shown that CENP-C is assembled with CENP-A at early anaphase in Drosophila embryos. Here, we show that CENP-C is loaded post-replication in G2-phase (concurrently with CENP-A) in female Drosophila GSCs. Furthermore, we show that when we knock down CENP-C in GSCs, we see a tumour-like phenotype with disrupted asymmetric cell division, along with a 50% reduction in CENP-A. This evidence suggests that the centromere components, CENP-A and CENP-C, might stabilise the epigenetic maintenance of stem cell identity and proper asymmetric division.

471 Brain Tumor promotes axon growth across the midline through interactions with the microtubule stabilizing protein Apc2. E. Arbeille, G.J. Bashaw University of Pennsylvania, Department of Neuroscience, Philadelphia, PA.

Commissural axons must cross the midline to establish reciprocal connections between the two sides of the body. This process is highly conserved between invertebrates and vertebrates and depends on guidance cues and their receptors to instruct axon trajectories. The DCC family receptor Frazzled (Fra) signals chemoattraction and promotes midline crossing in response to its ligand Netrin. However, in Netrin or fra mutants, the loss of crossing is incomplete, suggesting the existence of additional pathways. Here, we identify Brain Tumor (Brat), a tripartite motif protein, as a new regulator of midline crossing in the Drosophila CNS. Genetic analysis indicates that Brat acts independently of the Netrin/Fra pathway. In addition, we show that through its B-Box domains, Brat acts cell autonomously to regulate the expression and localization of Adenomatous polyposis coli-2 (Apc2), a key component of the Wnt canonical signaling pathway, to promote axon growth across the midline. Genetic evidence indicates that the role of Brat and Apc2 to promote axon growth across the midline is independent Wnt and Beta-catenin-mediated transcriptional regulation. Instead, we propose that Brat promotes midline crossing through directing
the localization or stability of Apc2 at the plus ends of microtubules in navigating commissural axons. These findings define a new mechanism in the coordination of axon growth and guidance at the midline.

472  **d-HURP (Mars) cooperates with the Frazzled receptor to promote axon growth across the midline in the Drosophila embryonic CNS.**  *Katherine M. Blocklove, Samantha A. Russell, Greg J. Bashaw*  
Neuroscience, University of Pennsylvania, Philadelphia, PA.

The reliable navigation of axons to their correct targets is crucial for the development of functional neural pathways. In the *D. melanogaster* ventral nerve cord, commissural axons cross the midline to innervate targets on the contralateral side of the body to form circuits that are important for coordinated behavior. Commissural axons cross the midline only once, in either the anterior or posterior commissure of each segment, before projecting ipsilaterally towards their targets. Highly conserved ligand-receptor families signal to guide axons through modifications of the growth cone, a specialized structure at the tip of extending axons. Frazzled (Fra), a DCC-family receptor, promotes midline crossing through two distinct pathways: interaction with the secreted ligand Netrin signals outgrowth, and transcriptional regulation of *commissureless* down-regulates Slit-mediated repulsion in pre-crossing axons. Efforts to determine additional effectors of Fra-mediated axon guidance have identified several candidate genes, including *d-HURP (mars)*, which was isolated independently in two screens. In vivo, neuronal expression of the dominant-negative truncated Fra receptor (FraΔC) results in a midline crossing phenotype that is sensitive to reduced function of other factors that promote midline crossing. A genetic screen for enhancers of the FraΔC midline crossing phenotype identified mars in vivo, and a concurrent yeast two-hybrid screen detected Mars as an interactor with the intracellular domain of Fra in vitro. The Mars protein regulates the cell cycle through stabilization of the mitotic spindles, but recent works suggest that its functions may include the regulation of cell migration, adhesion, and synaptogenesis. Homozygous removal of Mars further enhances the midline crossing defects observed through FraΔC mis-expression, and mars homozygous-null embryos present mild midline crossing defects in the eagle subset of commissural neurons. Preliminary results indicate that exogenous expression of Mars can rescue the mutant phenotype, but overexpression does not induce ectopic crossing of ipsilateral axons. Genetic experiments to determine how Mars contributes to midline attraction, and biochemical assays to define the interaction of Mars and Frazzled are ongoing. Although the role of Mars in axon guidance requires further investigation, these results suggest that our studies will elaborate on the current model of axon guidance at the embryonic midline.

473  **Minimal structural elements required for midline repulsive signaling and regulation of the Drosophila axon guidance receptor Robo1.**  *H. Brown, T. Evans*  
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The repellant ligand Slit and its Roundabout (Robo) family receptors regulate many aspects of axon guidance in bilaterians, including midline crossing of axons during development of the embryonic CNS. Slit proteins are produced by midline cells and signal through Robo receptors expressed on the surface of axonal growth cones to repel axons from the midline. Disruption of Slit-Robo signaling causes ectopic midline crossing phenotypes in the CNS of a broad range of animals, including insects and vertebrates.

*Drosophila* Robo1 has a conserved ectodomain structure of five immunoglobulin-like (Ig) domains plus three fibronectin (FN) repeats. We have previously shown that the Ig1 domain is the only ectodomain element essential for Robo1's midline repulsive activity in the *Drosophila* embryonic CNS. Here, we test how much of the receptor is required for Robo1's midline repulsive function, by using a genomic rescue construct based on endogenous *robo1* regulatory regions to restore expression of Robo1D1g2-5, Robo1DFN1-3, and Robo1D1g2-FN3 in embryonic neurons of *robo1* mutants.

We find that making combinatorial deletions of either the Ig domains (D1g2-5) or the FN repeats (DFN1-3) does not disrupt Slit binding or midline repulsion. But, when these two deletions are combined (D1g2-FN3), so that only the Ig1 domain remains, midline repulsion is not completely restored to that of wild-type embryos. Interestingly, Robo1D1g2-FN3 is still able to bind Slit, indicating that Ig1 alone is both necessary and sufficient for Slit binding by Robo1, but not sufficient on its own for Robo1's *in vivo* function. Furthermore, we find that while the D1g2-5 variant is sensitive to downregulation in vivo, the DFN1-3 and D1g2-FN3 variants are insensitive to the Robo1 antagonists *Commissureless* (Comm) and Robo2, revealing a novel regulatory role for Robo1's FN repeats.

While our previous studies demonstrated that only Robo1's Ig1 domain is individually required for the receptor's midline repulsive function *in vivo*, we now report that the Ig1 domain by itself is insufficient to rescue midline repulsion despite proper Slit binding and trafficking to axons. This partial rescue phenotype of Robo1D1g2-Fn3 suggests that additional ectodomain elements of Robo1 apart from Ig1 may play a permissive role in repulsive signaling, and that a minimal number of domains, rather than a specific set, may be necessary for Slit-dependent signaling by Robo1.

474  **The Scar/Wave complex as a direct downstream effector of axon guidance receptors.**  *K. Chaudhari, G. Bashaw*  
Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.
Proper function of the nervous system relies on the formation of correct neural circuits, which in turn depends on the precise guidance of axons to their appropriate targets. The Roundabout (Robo) guidance receptor family induces repulsion in axons in response to its ligand, Slit, while the Frazzled (Fra) guidance receptor induces attraction in response to its ligand, Netrin. Robo and Fra function in guidance by inducing local cytoskeletal changes in axons. Although some downstream effectors of these guidance receptors have been identified, the exact links to the cytoskeleton and the nature of these cytoskeletal changes are still unclear. Further, as many effectors have been implicated downstream of both attractive as well as repulsive guidance receptors, the signaling differences that discriminate between attraction and repulsion remain elusive. Recent studies have implicated the heteropentameric Scar/Wave Complex (SWC), a complex involved in actin polymerization, in axon guidance. We will investigate the possibility that attractive and repulsive receptors may modulate the activity of the complex in different ways. Drosophila mutants of the complex show phenotypes similar to robo1 mutants and the cytoplasmic domains of both Robo and Fra have the conserved binding sequence (the WIRS motif) for the Scar/Wave Complex. This suggests that the complex might function as an effector of Robo and Fra, and serve as a link to the cytoskeleton. Preliminary results show that mutants of members of the Scar/Wave complex enhance crossing defects seen in the ventral nerve cords of Drosophila embryos with reduced Robo or Fra function. We will determine whether mutating the WIRS binding site for the Scar/Wave complex can disrupt the function of these guidance receptors using rescue assays and gain-of-function assays in the Drosophila embryo. Further, we will investigate whether the complex is required for the repulsive response of axons to Slit using cultured vertebrate neurons. Identifying downstream effectors of guidance receptors that can modulate the cytoskeleton will provide insight into how these receptors function in neural circuit formation and wiring specificity as well as aid in generating improved treatment strategies for nerve injury.

475 A novel role for Plexin A in photoreceptor axon targeting. J. Douthit1, S. Astigarraga2, G. Lee3, J. Treisman4  1) Cell Biology, Skirball Institute, New York University School of Medicine, New York, NY; 2) Cell Biology, Skirball Institute, New York University School of Medicine, New York, NY; 3) Cell Biology, Skirball Institute, New York University School of Medicine, New York, NY; 4) Cell Biology, Skirball Institute, New York University School of Medicine, New York, NY.

Normal nervous system functioning is dependent upon very defined and precise network formation during development. Aberrant synaptic connectivity caused by mutations in axon guidance molecules and cell adhesion proteins has been associated with neurodevelopmental and psychiatric disorders such as intelligence disability, epilepsy, Autism Spectrum Disorders, and schizophrenia. The Drosophila visual system is an excellent model system for studying the basic mechanisms of axon pathfinding and neural circuit formation. The terminals of R7 and R8 photoreceptors, responsible for color vision, are segregated into distinct target layers of the medulla, a central region of visual processing in the brain. We have found that null mutations in plexA cause R7 photoreceptors to prematurely terminate in the R8 layer of the medulla and fail to expand their axon terminals. Labeling of presynaptic sites in R7 axons shows fewer synapses when plexA is knocked down in neurons using RNAi. Mosaic analysis and plexA RNAi experiments indicate that PlexA is required in the brain and not in the eye. PlexA is strongly expressed in medulla tangential neurons, which occupy the layer just beyond where R7 axons terminate and arise from the tips of the outer proliferation center (OPC). Deleting plexA from the progeny of the OPC using somatic CRISPR/Cas9 causes R7 mistargeting, supporting a function for PlexA in medulla tangential neurons. Additionally, we find that loss of plexA affects the organization of medulla tangential neurons and R7 target cells, Dm8 neurons. Misexpression of PlexA in photoreceptor axons results in their hyperfasciculation and premature termination, consistent with PlexA acting to promote attraction or adhesion of R7 axons. We are using genome editing to delete the cytoplasmic domain of PlexA in order to determine whether it acts as a receptor or a ligand in this context. R7 mistargeting is not observed in mutants for Semaphorin-1a or Semaphorin-1b, the known PlexA binding partners. Lastly, we are investigating whether a novel receptor may mediate this function of PlexA. In RNAi and mosaic analysis experiments, we have found that this novel receptor is required in the eye for proper R7 axon targeting. Investigation of the role of this novel receptor may have implications for how Plexin family members control axon pathfinding in higher organisms.

476 Slit-independent guidance of longitudinal axons by Drosophila Robo3. A. Carranza, H. Brown, T. Evans  Biological Sciences, University of Arkansas, Fayetteville, AR.

Axon guidance receptors of the Roundabout (Robo) family regulate a number of axon guidance outcomes in bilaterian animals in addition to their canonical role in Slit-dependent midline repulsion. In Drosophila, three Robo family members (Robo1, Robo2, and Robo3) each have specialized roles in regulating midline crossing and the formation of longitudinal axon pathways in the embryonic ventral nerve cord. All three fly Robos can act as receptors for a common Slit ligand, but we do not have a good understanding of which Robo-dependent axon guidance decisions depend on Slit, and which do not. Drosophila Robo3 does not regulate midline crossing, but is required for the formation of longitudinal axon pathways in specific medial-lateral positions in the ventral nerve cord. In the absence of robo3, axons that normally form pathways in the intermediate region instead join medial pathways closer to the midline. Although Robo3 has been presumed to act as a canonical Slit receptor to position longitudinal axons in response to a midline-derived Slit gradient, this model has not been
directly tested.

To determine whether Robo3’s role in longitudinal pathway formation depends on its ability to bind Slit, we used CRISPR/Cas9-based gene modification to replace the robo3 gene with a version of Robo3 that is unable to bind Slit. Using a cell culture-based Slit binding assay, we show that deletion of the Robo3 Ig1 domain prevents Slit binding. Using a series of modified robo3 alleles in which Ig1 has been deleted or otherwise modified, we show that Robo3 does not need to bind Slit to properly guide longitudinal axons. We further show that Robo3 Ig1 has a Slit-independent role in promoting the proper localization of Robo3 protein on neuronal axons in vivo. Together, our results indicate that Drosophila Robo3 guides embryonic axons independently of Slit, and contradict the established model of Robo3 acting to position longitudinal axons in response to midline-derived Slit.

477 Identifying Natural Variation in Midline Axon Guidance Using the Drosophila melanogaster Genetic Reference Panel.  M.L. Gosztuya, M.A. Seeger Department of Molecular Genetics, The Ohio State University, Columbus, OH.

The central nervous system (CNS) midline is an important choice point for many pathfinding axons during neural development. Previous studies have searched for novel regulators using mutagenesis experiments involving a few inbred laboratory strains of Drosophila melanogaster. An alternative strategy is to utilize the polymorphic variation that exists in natural populations to study embryonic axon guidance at the CNS midline. This approach was recently enhanced by the creation of the D. melanogaster Genetic Reference Panel (DGRP), which consists of more than 200 isogenic, sequenced strains derived from an outbred population. In the present study, embryos from 141 DGRP strains were stained using one of two antibodies to visualize different aspects of the embryonic CNS: BP102, which labels all axon pathways, or 1D4, which labels a subset of longitudinal axons that normally do not cross the CNS midline. We then selected a minimum of n = 5 embryos per strain for each antibody and scored for the presence of missing commissures or ectopic midline crossovers. We identified 39 strains where at least one embryo showed one or more defects in axon guidance. Of these, 18 showed only missing commissures, 16 showed only ectopic crossovers, and 5 showed both types of defects. Between these 39 strains, we observed considerable variation in the penetrance of the observed phenotypes, ranging from 5% to 65% of embryos showing a defect within a strain. Furthermore, in these defective embryos the proportion of defective segments varied from 8% to 33%. These observations demonstrate that natural variation exists among genes influencing midline axon guidance in D. melanogaster. We are now repeating these experiments using the remaining DGRP strains and further analyzing the strains exhibiting defects. In addition, we are testing the DGRP in several sensitized genetic backgrounds to potentially reveal additional genetic variation that influences embryonic axon guidance. In the long-term, this research may provide insight into the complex and redundant network of ligands, receptors, and signaling molecules that regulate axon guidance.


Slit is a large secreted molecule that normally repels growing nerves in the central nervous system. Slit is cleaved into two fragments in vivo, but the function of this cleavage is unknown. Our analysis suggests that Slit cleavage provides a means for nerve growth via alternative signaling pathways involving the formation of a complex where the N terminus fragment of Slit binds to Robo1 and Dscam1 receptors. Recently, we have identified the protease responsible for cleaving Slit in flies. Both the fly and vertebrate proteases are capable of cleaving fly Slit in cell culture. We are currently testing human Slit2 and the related protease. Using a series of in vivo experiments confirming that Slit is not cleaved in the protease null mutant, and that these mutants display defects consistent with a role in longitudinal axon guidance. Using CRISPR, we have generated a new uncleavable slit allele. Preliminary data suggests that longitudinal axon guidance is disrupted but midline repulsion is unaffected. Our results will provide insight into how nerves normally grow. Spatial and temporal application of our construct has implications for treatment of spinal cord injury and neurodegeneration. Moreover, understanding the functionality of Slit fragments can help us better understand how they impact metastasis, immune responses, and regulation of body temperature.

479 The functional and structural analysis of Drosophila Robo2.  L. Howard, T. Evans Biological Sciences, University of Arkansas, Fayetteville, AR.

No matter the complexity of bilateral animals, axons are posed with the central problem of whether or not they should cross the midline. Roundabout (Robo) family proteins regulate many axon guidance decisions in the Drosophila embryonic central nervous system. Robo1 and Robo2 facilitate midline repulsion in response to Slit, while Robo2 and Robo3 define the lateral position of longitudinal axon pathways. In addition to these shared roles, Robo2 can also promote midline crossing of axons, an activity that is not shared by the other Drosophila Robos. My project will give me insight on molecular mechanisms of Robo2’s functional diversity as a transmembrane receptor and therefore also may apply to vertebrate species.

Drosophila Robo2 plays at least three distinct roles in axon guidance in the fly embryo (midline repulsion, pro-midline crossing, and lateral positioning). Previous gain of function and genetic rescue studies suggest that the different roles of Robo2 are specified by individual immunoglobulin-like (Ig) domains within the receptor. Ig2 is required for Robo2’s pro-
crossing function, while Ig1 and Ig3 are thought to regulate lateral positioning. It has been assumed (but not directly demonstrated) that Robo2 acts as a canonical cell-autonomous Slit receptor to signal midline repulsion; if so, this activity would likely require the Slit-binding Ig1 domain of Robo2.

We are using a CRISPR/Cas9-based gene replacement approach to investigate which domains of Robo2 (Ig & Fn) are required for each of its axon guidance activities. By replacing the robo2 coding region with epitope-tagged cDNAs, in which individual domains have been deleted, we are examining the contributions of each domain to receptor localization, regulation, and Robo2-dependent axon guidance outcomes. We observed a mislocalization of protein when looking at Robo2 without its Ig1 and Ig3 domains. We are also currently examining the roles of the cytoplasmic domains of Robo2 with a similar approach. Our results promise to increase our understanding of how individual receptors can contribute to multiple axon guidance outcomes during developmental wiring of the nervous system.

480 Regulating the Slit-Robo system in flies and mice.  R. Kellermeyer1, L. Heydman1, B. Bjorke2, T. Gillis1, R. Allen1, G. Mastick1, T. Kidd1 1) Biology, University of Nevada, Reno, Reno, NV; 2) Neuroscience, Carleton College, Northfield, MN.

The secreted protein Slit is proteolytically cleaved, with fragments that function independent of Slit-Robo axon repulsion in axon growth and branching, metastasis, and metabolic functions. Of the cleaved fragments, Slit-N can form a complex with Dscam1 and Robo1 receptors to promote longitudinal outgrowth. We have identified the Slit protease in Drosophila, supported by data in vivo and in cell culture (see abstract by Heydman et al.). Slit protease mutants are unable to cleave Slit in vivo and have severely disrupted longitudinal axon tracts, as expected from our previous work. Epistasis experiments reveal that slit and the protease genetically interact in vivo, and protease mutants have defects in the growth rates of longitudinal axons. Expression patterns of the Slit protease in a mouse model supports evolutionary conservation.

Additionally, we identify PRRG3 as the functional vertebrate orthologue of Commissureless (Comm) in the embryonic mouse. Both PRRG3 and PRRG4 are expressed in the cortex and corpus callosum, the largest brain commissure, and have function in sequestering Robo from the axon growth cone. Protein expression of PRRG3 in vivo shows expression at the right time and place to allow commissure formation, and PRRG3 accumulates in the midline spinal cord axons, similar to Comm. PRRG3 and PRRG4 both have Gla domains, extracellular regions that are gamma-carboxylated, and Comm has a degenerate Gla domain. Ongoing experiments testing the role of gamma-carboxylation of Comm will be reported as this would provide further evidence that PRRG3/4 are true orthologs of Comm. We report on the identification of the Slit protease and evidence for the functional vertebrate homologue of Comm.

481 Evolutionary conservation of axon guidance: midline repulsive signaling by Robo family receptors in flies and mice.  A. Loy, T. Daiber, T. Evans  Biological Sciences, University of Arkansas, Fayetteville, AR.

As the nervous system develops in animal embryos, neuronal axons are guided to their synaptic targets by extra cellular cues that signal through axon guidance receptors expressed on the surface of the axon. In animals with bilateral symmetry, one of the important decisions made by nearly every axon in the embryonic nervous system is whether to stay on its own side of the body, or to cross the midline and connect to cells on the opposite side. The Roundabout (Robo) family is an evolutionarily conserved group of axon guidance receptors that regulate midline crossing in a wide range of animal groups, by signaling midline repulsion in response to their ligand Slit. Despite their strong evolutionary conservation, it is unknown if the mechanisms of Robo signaling are conserved across different species.

Can Robo receptors from mice regulate axon guidance decisions in Drosophila embryos, or do species-specific difference exist in the cellular signaling mechanisms by which Slit and Robos regulate midline crossing? To investigate the evolutionary conservation of Robo signaling mechanisms, we are using the GAL4/UAS system in Drosophila to express Robo receptors from mice in fly neurons during embryonic development. We find that mammalian Robo receptors can repel axons from the midline in Drosophila embryos, which suggests that the mechanisms by which they signal midline repulsion are conserved in insects and mammals. However, a further study involving a GAL4/UAS rescue of mutated Drosophila Robo receptors with mouse Robo receptors indicates that mammalian Robo genes cannot successfully effect midline repulsion in fly embryos on their own. Therefore it is still uncertain how clearly the mechanisms of Robo signaling are conserved from insects to mammals.

In addition, we are creating chimeric receptor genomes combining one Drosophila Robo domain with all other domains of mouse Robo. The effectiveness of the chimeric receptor will be tested in another GAL4/UAS rescue of mutated Drosophila Robo receptors and will again demonstrate whether or not the mechanisms of Robo signaling are evolutionarily conserved.

482 Conservation of the Netrin receptor Frazzled in insects.  B. Wadsworth, L. Terry, T. Evans  Biological Sciences, University of Arkansas, Fayetteville, AR.

Axons in the developing embryo receive and react to signals that direct their growth to reach target tissues at specified
locations. The signal pathways that direct midline crossing of axons during embryonic development have been comprehensively examined using the Drosophila melanogaster ventral nerve cord or the vertebrate spinal cord as a model. A number of these signaling mechanisms are conserved; however, disparities have been found between species either in the general strategy or the molecular signals controlling axonal response to guidance cues. The Netrin-Frazzled/DCC pathway has been shown to aid in midline crossing of axons in the embryonic ventral nerve cord of Drosophila. However, it is uncertain if this function of Frazzled is conserved in other insects. The goals of this research are to gain insight into the evolutionary conservation of axon guidance by the Netrin receptor Frazzled (Fra) and to expand our understanding of how Frazzled affects midline crossing in the flour beetle Tribolium castaneum.

Our lab has successfully cloned the frazzled ortholog from Tribolium and employed it in gain of function studies. In these studies ectopic expression of TcFra induces midline crossing in Drosophila embryos. We will apply a CRISPR approach to replace the Dmfra gene with Tcfra in Drosophila embryos to assess whether TcFra is sufficient to rescue the loss of endogenous Fra. We will also examine Tcfra in Tribolium embryos to elucidate the function of TcFra in vivo. We expect the Frazzled ortholog in Tribolium (Tcfra) to be sufficient for replacing loss of function in Drosophila Fra. Additionally, we expect to see a similar function of Frazzled in beetles to those observed in Drosophila. These studies will expand our knowledge of axon guidance of midline crossing in a species that does not share some of Drosophila’s derived guidance characters, ultimately allowing us to see a more ancestral guidance scheme. Therefore, understanding the fundamental mechanisms of midline crossing for differing evolutionary trajectories will provide a clearer understanding of axon guidance in the developing embryo across organisms.

483 Formin3 regulates dendritic architecture via microtubule stabilization and is required for peripheral sensitivity to noxious stimuli. J.M. Letcher1, R. Das1, J.M. Harris1, I. Foldi, S. Nanda2, B.D. Grantier1, J. Mihaly2, G.A. Ascoli1, D.N. Cox1 1) Neuroscience Institute, Georgia State University, Atlanta, GA; 2) Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; 3) Krasnow Institute for Advanced Study, George Mason University, Fairfax, VA.

Specialized neural morphologies are required for detection and transduction of sensory stimuli and emerge via complex growth mechanisms modulated by intrinsic and extrinsic signaling. These signaling mechanisms ultimately converge on the cytoskeleton, however the regulatory factors that drive the formation of cell-type specific dendritic architectures remain incompletely understood. In a neurogenomic-driven screen of cytoskeletal regulators, we identified several members of the Formin gene family as putative regulators of class IV (CIV) nociceptive sensory neurons. Among these, we demonstrate that Formin3 (Form3) functions cell-autonomously in CIV neurons to stabilize distal higher order branching. Live confocal imaging of multi-fluor cytoskeletal reporters and IHC analyses reveal the form3 mutants exhibit specific collapse of the dendritic microtubule (MT) cytoskeleton. The functional consequences of MT destabilization include defective trafficking of mitochondria and satellite Golgi. Biochemical studies demonstrate that Form3 directly interacts with MTs via FH1-FH2 domains and promotes MT stabilization via acetylation. Mutations in human INF2 (form3 ortholog) are causally linked to Charcot-Marie-Tooth (CMT) sensory neuropathies that lead to impaired peripheral sensitivity to stimuli such as heat, cold, and pain. Moreover, CMT is a progressive neurological disorder, and developmental analyses of form3 mutants reveal a progressive dendritic arbor and MT collapse throughout larval development. In CIV nociceptive neurons, form3 disruption severely impairs noxious heat-evoked behaviors. Behaviorally, form3 is not required for general neuronal excitability suggesting that impaired peripheral sensitivity may be altered at the sensory transduction stage. TRP channels including Painless have been implicated in mediating noxious heat-evoked nocifensive behavior and intriguingly, we observed defects in dendritic trafficking of Painless in form3 mutant CIV neurons as well as altered calcium dynamics, both of which may contribute to impaired peripheral sensitivity. Finally, form3 mutant defects in MT stabilization and nocifensive behaviors can be rescued by INF2 FH1-FH2 expression revealing conserved functions and thereby providing novel mechanistic insights into potential etiological bases of CMT sensory neuropathies.

484 Mediating activity-dependent plasticity of Drosophila central synapses by tuning nicotinic receptor clustering. J.S. Rosenthal1, J. Yin, C. Long, Q Yuan National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD.

During nervous system development, it is critical to balance the intrinsic and stereotyped developmental program with modifications generated by changing external inputs. Using Drosophila melanogaster as a model, we built a formidable understanding of the mechanisms of synapse formation as well as its modulation in response to neural activity. However, previous studies mainly focused on the glutamatergic synapses at the Neuromuscular Junction (NMJ). Consequently, less is known about the formation and regulation of cholinergic synapses, which are the main excitatory synapses in the Drosophila central nervous system (CNS). In this study, we analyze activity-dependent modification of cholinergic synapses in ventral Lateral Neurons (LNv) of the Drosophila larval visual circuit, which receive cholinergic inputs from presynaptic photoreceptors.

LNvs respond to variations in visual input and exhibit homeostatic structural plasticity via robust changes in dendrite volume during development. Specifically, chronically elevated activity reduces the dendritic size of LNvs, which are also less
responsive physiologically as demonstrated by calcium imaging. Using LNv-specific transcriptome analysis followed by in vivo transgenic RNAi screens, we isolated candidate molecules that potentially contribute to LNv plasticity. Among them were several genes encoding postsynaptic cholinergic receptors. Cell-specific knockdown of two genes, nAchRα1 and nAchRα6, resulted in similar LNv dendrite sizes regardless of the level of chronic presynaptic input, indicating these receptor subunits may be involved in the LNv plasticity mechanism. The conclusions from these preliminary experiments are currently being validated using null mutants and rescue constructs. Additionally, GFP-tagged transgenes for these receptors are being generated and will be used to observe subunit trafficking and receptor clustering during development. Future experiments will be performed to determine which transcription factors up- and/or downregulate these subunit genes, as well as to identify additional, auxiliary proteins which are localized on or near the postsynaptic density and contribute to synaptic formation and plasticity.

485 Uncovering the role of a Drosophila tRNA methyltransferase in neurons. Caley Hogan1, Xueyang He1, Joseph Bruckner2, Scott Gratz1, Kate O’Connor-Giles1 1) University of Wisconsin-Madison, Madison, WI; 2) University of Oregon, Eugene, OR.

tRNAs are ubiquitous adaptor molecules that decode mRNAs through codon-anticodon base pairing and insertion of the appropriate amino acid into growing polypeptide chains. tRNAs undergo extensive posttranscriptional modifications that affect stability, efficiency, and fidelity. Disruptions to the posttranscriptional processing of tRNAs have recently been linked to neurological disease. Although once thought to function as simple adaptor molecules, it is becoming apparent that tRNAs can function as signaling molecules in the dynamical regulation of translation.

In a genetic screen, we identified a neuronal tRNA methyltransferase as a novel regulator of synaptic growth. This tRNA methyltransferase is one of two metazoan paralogs of a yeast enzyme, TRM9, that methylates uridines found in the wobble position of the anticodon loop to increase translational efficiency, and has been named TRM9L in humans. Drosophila TRM9L was also identified in a recent RNAi screen for mutations that alter larval responses to pain, and named fire dancer (fid). Interestingly, TRM9L/Fid is expressed predominantly in neurons, whereas the second TRM9 paralog, CG17807, is broadly expressed. This suggests the exciting possibility that TRM9L/Fid plays a specific role in the dynamic regulation of translation in neurons. Here, we present our findings demonstrating the diverse neuronal functions for TRM9L/Fid in synaptic development, neurotransmission, and response to stress.

To determine the function of TRM9L/Fid in the nervous system, we generated a deletion allele using CRISPR-Cas9. Consistent with our previous RNAi results, TRM9L/fid mutants exhibit significant overgrowth at the neuromuscular junction. Despite ectopic synapse formation, synaptic transmission is significantly decreased in TRM9L/fid mutants, demonstrating that TRM9L/Fid is necessary for proper synaptic function as well as growth. Finally, because yeast TRM9 mutants are sensitive to a variety of stressors, we examined the role of TRM9L/Fid in stress response and found that loss of TRM9L/Fid increases sensitivity to paraquat, a toxin that induces oxidative stress. This suggests that translational regulation of stress response genes may be a conserved function of TRM9-family enzymes.

486 Synaptic development within the optic glomeruli of Drosophila melanogaster. B. Walter. McFarland1, L.J. Solomon1, D.P. Gallagher1, A. Nern2, T. Godenschwege3, C.R. von Reyn1,4 1) School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA; 2) Janelia Research Campus, HHMI, Ashburn, VA; 3) Department of Biological Sciences, Florida Atlantic University, Boca Raton, FL; 4) Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA.

Development of the brain is a dynamic process whereby axons extend and navigate to specific brain regions, recognize specific synaptic partners, and form stable connections. While research is beginning to describe molecular proteins involved in circuit development, we still know little about how these molecules communicate in a concerted effort to control precise partner recognition and direct circuit development. For example, each type of lobula columnar neuron (LCN) in the optic lobes of Drosophila melanogaster projects axons out of the optic lobe to terminate in their appropriate central brain region (optic glomeruli). Descending interneurons in the central brain project dendrites into a subset of optic glomeruli to receive visual feature information that guides behavioral responses. The optic glomeruli therefore serve as an ideal model to study the molecular mechanisms involved in precise neuronal pairing, synapse development, and sensorimotor integration. To date, neither the temporal dynamics of synapse formation nor the cues responsible for selective synaptic pairing in the optic glomeruli have been established. Here, we investigate how one type of descending neuron that drives escape behavior (giant fiber, GF) establishes synaptic connections with appropriate LCNs that encode features of a predator’s approach. Using the GAL4/LexA systems, we labeled GF dendrites and LC axons within the same fly, allowing us to track synapse formation across larval and pupal stages of synaptic development. Using immunofluorescence, we investigated the accumulation of presynaptic active zones (T-bars) during formation of the optic glomeruli, by labeling for Bruchpilot (Brp, a nc82) a protein integral for active zone structural integrity and synaptic function. Furthermore, we utilized GFP reconstitution across synaptic
partners (GRASP) to track the proximity of GF dendrites and LC axons through subsequent stages in development. Together, our data shows GF initiates contact with a subset of LCNs.

487  **Tao is required in neurons to restrict neuromuscular junction development.**  P.J. Vanderzalm, S.F. Politano, R.R. Salemme, T.A. Bakula, K.A. Puhalla  
Biology, John Carroll University, University Heights, OH.

Tao is a conserved Sterile20-family kinase with multiple known roles in the cell. In Drosophila, it inhibits growth of epithelial tissues and neuroblasts through its function in the Hippo pathway, where it phosphorylates and activates the Hippo kinase. In addition to this growth-restricting role in development, in both mammals and in Drosophila, Tao also promotes neurite outgrowth, regulates links between actin and microtubules, and signals upstream of p38 MAPK in activity-dependent dendrite formation.

We have found that Tao also plays a role during synaptic development in Drosophila. Loss of Tao function in motoneurons leads to overgrowth of neuromuscular junctions (NMJs) in 3rd instar larvae, while postsynaptic loss of Tao function has no effect on NMJ formation. However, presynaptic loss of other Hippo pathway components does not lead to similar overgrowth phenotypes, and loss of *yorkie* function does not affect the NMJ overgrowth due to loss of Tao. Together, this strongly suggests that Tao is not acting through the Hippo pathway to restrict NMJ development. We will present preliminary evidence regarding alternative signaling pathways in which Tao may be involved during synaptic development.

488  **Effect of thrombospondin in synaptogenesis at the Drosophila larval NMJ.**  Eve Lowenstein, Norma Velazquez Ulloa  
Biology, Lewis and Clark College, Portland, OR.

Thrombospondin (TSP) is an extracellular matrix glycoprotein that has been shown to have a role in synaptogenesis in the mammalian brain. In mammals, TSP is released by astrocytes at glutamatergic synapses. The TSP family of glycoproteins is composed of 5 members. There is a single homologous protein in *Drosophila melanogaster* (D-TSP) and there is conservation between proteins. In *Drosophila*, D-TSP has mostly been studied in the myotendinous junction at embryonic stages. Not much is known about the role of D-TSP at larval stages or whether D-TSP plays a role in synaptogenesis. Hence, we set out to determine if D-TSP functions at glutamatergic synapses in the larval neuromuscular junction (NMJ). We hypothesized that D-TSP would be necessary for normal NMJ formation. To test this hypothesis, we used immunohistochemistry to visualize larval NMJ structure at the 6/7 muscles in segments A3 and A4. We quantified established NMJ markers (muscles with phalloidin, presynaptic axons with HRP, and post-synaptic density with DLG) in flies with normal expression of D-TSP and flies with decreased expression of D-TSP. Decreased expression was achieved by RNA interference. Our initial data shows an effect in larva with pan-neuronal D-TSP knockdown using an elav-GAL4 driver. Our results show that the attachment size of muscle 7 was significantly reduced and we observed large gaps between muscles and detachments in the knockdown not present in control. We detected a significant decrease in DLG area in the knockdown compared to control and a compact branching pattern in the knockdown. No significant differences were found for HRP area, but we noticed that the HRP axons of the knockdown appeared thinner overall and did not have distinct boutons. Our results suggest that D-TSP plays a role in synaptogenesis at the larval NMJ. We are currently testing whether knockdown of D-TSP in other tissues (i.e. muscle, glia, specific neurons) produces the same effects we found. We are also in the process of validating the knockdown.

489  **A conserved BMP-responsive cis-regulatory activation element mediates widespread target-dependent gene activation in Drosophila neurons.**  Robin Vuilleumier¹, Tianshun Lian¹, Stephane Filbotte², Zaynah Khan¹, Alisa Fuchs², George Pyrowolakis², Douglas Allan¹  
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In *Drosophila*, efferent neurons gain access to a target-derived BMP signal in the periphery that is required for differentiation and/or synaptic growth and neurotransmission. This requires transcriptional activity of the BMP-activated Smad transcription factors, but Smad-bound enhancers and the BMP-regulated genes they control remain largely unidentified. Here, we test whether Smads act at archetypal Smad-binding motifs to directly co-regulate batteries of BMP-responsive genes. To target these motifs for analysis, we employed a bioinformatics approach to identify candidate motifs conforming to the consensus 15bp BMP-Activation Element (BMP-AE). After filtering for high conservation and proximity to neurally-expressed genes, we prioritized 62 BMP-AEs for analysis. Testing the *in vivo* enhancer activity of 58 genomic fragments containing these BMP-AEs in transgenic reporters, we show that 61% are functional BMP enhancers in diverse motoneuron and neuromodulatory neuronal populations, and that the BMP-AE motif itself is required for the fragments BMP-dependent enhancer activity. Moreover, 60% of these functional BMP-AEs were located within 20kb of at least one BMP activated gene, as identified by RNAseq analysis. Finally, we show that the BMP-AE motif plays a conserved activator function in the vertebrate nervous system, by demonstrating the BMP-dependent activity of a reporter driven from a BMP-AE concatamer electroporated into the developing chick neural tube. Our results provide evidence that BMP signaling controls neuronal development and function by directly coordinating networks of genes through widespread deployment of conserved, consensus Smad-binding motifs in the enhancers of a battery of BMP-regulated genes.

In *Drosophila* neurons, target-derived BMP signaling induces a diversity of subtype-specific genes. Towards understanding how a common BMP signal generates this diversity, we identified a 39bp BMP-Response Element (BMP-RE) required for selective BMP-dependent FMRFa neuropeptide gene expression in Tv4 neurons. Here, we examine how specificity is generated from this cis-element. Genetic analyses confirmed activator roles for Mad and Medea, with no involvement of common co-regulators brinker and schnurri. Reporter analysis and in vitro Smad binding assays defined an essential Smad-binding motif of sequence GGC/CC(N)5GTAT. This matches consensus BMP-REs except for a C>A switch in the functionally crucial penultimate nucleotide, which we show results in reduced Mad binding. Conversion of the suboptimal FMRFa BMP-RE to an optimal consensus BMP-RE sequence resulted in ectopic BMP-dependent reporter activity in other VNC neurons. Thus, we find that BMP-RE suboptimization contributes to restriction of BMP-dependent genes to specific neuronal subtypes in the *Drosophila* nervous system.

**491** The function of intrinsic and extrinsic factors on temporal identity transitions in mushroom body progenitors. A. M. Rossi, C. Desplan Department of Biology, New York University, New York, NY.

The nervous system is comprised of diverse neuron types organized into complex networks. Neural stem cells, called neuroblasts in *Drosophila*, sequentially produce different types of neurons as they age. The time dependent birth of different neuron types is an evolutionarily conserved mechanism called temporal patterning. We use the developing *Drosophila* mushroom bodies as a model to study the molecular mechanisms regulating temporal patterning. Four identical mushroom body neuroblasts per hemisphere produce only three main neuron classes in a sequential order: γ, then αβ' and finally αβ. Importantly, transitions between temporal windows occur at stereotyped, developmental time points, suggesting a role for extrinsic signaling. To identify genes that regulate the temporal birth of the three mushroom body neuron types we performed an immunohistofluorescence screen against 150 transcription factors within each temporal window and identified 13 genes that appear to be differentially expressed. To test the temporal function of each candidate gene, we used Mosaic Analysis with a Repressible Cell Marker (MARC) to induce mutant neuroblast clones and then characterized the adult axonal projections of mutant neurons compared to the remaining, non-mutant mushroom body neurons. We identified 2 genes (Rz-f1 and Groucho) that when removed from mushroom body neuroblasts resulted in the loss of the second and third born neurons (αβ’ and αβ) in the adult clone, demonstrating that Rz-f1 and Groucho are necessary for the first temporal transition. We then took a candidate approach to test extrinsic signals that might regulate temporal transitions. Since the first transition from γ to αβ’ occurs at mid-L3, a time point when TGFβ signaling is active in the mushroom body, we made mutant neuroblast clones for the activin signaling receptor, baboon. Mutant neurons did not project into the adult αβ’ lobe, suggesting that activin signaling is necessary for the first transition. We are further testing how and if Ftz-f1 and Groucho interact with each other and how they may interact with additional, extrinsic signaling cues to regulate temporal identity.

**492** Shep regulates neuronal remodeling by controlling expression of its chromatin target genes. D. Chen, R. Dale, E. Lei National Institutes of Health, NIH, Bethesda, MD.

Nervous systems are actively remodeled during the developmental transition from juvenile to adult in a wide range of organisms. This process often involves pruning of existing connections and regrowth of adult-specific connections. Dysregulation of neuronal remodeling leads to improper connections that are associated with neurological diseases such as autism. However, our understanding of molecular mechanisms of neuronal remodeling remains far from complete. We have previously shown that the RNA-binding protein Shep promotes neuronal outgrowth during metamorphic remodeling. Loss of shep leads to locomotor deficits, abnormal sexual behaviors, and morphological defects of synapses and neurons specifically at the adult stage. Shep physically interacts with gypsy chromatin insulator proteins and inhibits their activities specifically in the nervous system. In this study, we sought to explore molecular mechanisms underlying shep regulation of neuronal remodeling.

In order to determine neuronal transcriptome profiles before and during metamorphosis, we performed RNA-seq analysis on FACS-sorted larval and pupal neurons. Consistent with stage-specific phenotypes, we observed strong effects on the transcriptome specifically in pupal neurons of shep-depleted flies. Our results demonstrate that shep regulates synaptic signaling factors and gene expression regulators that control neuronal remodeling during metamorphosis. Bioinformatic analysis further determined that a significant proportion of these affected genes are chromatin targets of Shep as well as gypsy insulator proteins. By isolating and quantifying nascent RNA, we find that Shep inhibits expression of its chromatin binding target genes by repressing their transcription. Chromatin conformation capture assays further revealed that shep inhibits promoter-enhancer interactions in chromatin of shep-regulated genes, such as brat and Myc. Finally, we employed behavioral and cellular assays and found that knock down of brat rescues shep-dependent wing expansion defects and remodeling of burricon neurons in abdominal ganglion that promote wing expansion after eclosion. Taken together, our findings provide new insights into gene expression profiles during normal neuronal remodeling as well as the role of Shep in facilitating the re-wiring of the nervous system toward full maturation and function.
silm is required for proper lch5 chordotonal neuron morphology and migration in the embryonic PNS. Madison Gonsior, Afshan Ismat  Department of Biology, University of St. Thomas, St. Paul, MN.

Cells migrate along pathways to their target during embryogenesis, responding to different repulsive and attractive cues in their environment. The slt-robo signaling pathway is a repulsive signaling pathway that repels axons away from the midline of the CNS via Robo, a transmembrane protein, and Slit, a secreted protein in the extracellular matrix (ECM). In the peripheral nervous system (PNS), the lateral chordotonal neurons (lch5) are a group of five neurons that repel away from the midline of the CNS while migrating laterally. These neurons display a slightly overlapped, teardrop shape with each dendrite facing the same direction. The absence of slt showed a change in the lch5 migration pattern. The lch5 neurons migrated more dorsally, and were not aligned properly. Moreover, the morphology of the lch5 neurons was also altered in the absence of slt. Specifically, the lch5 neurons did not display a teardrop shape, did not interact with each other properly, and their dendrites were pointing in various directions. These results suggest an important role for slt in sensory neuron migration and morphology. It is possible that the secondary cells, such as scolopale cells, ligament cells, cap cells, and attachment cells, interact with the lch5 neurons and influence these migration and morphological defects that are present in slt mutants. Current work is being done in order to elucidate the exact mechanism of lch5 chordotonal neuron migration, especially the role of the secondary cells, in lch5 neuron migration and morphology.

Nopo is an E3 ubiquitin ligase linking DNA damage response and centrosomes in mushroom body development. R.S. O’Neill, B.J. Galletta, C.J. Fagerstrom, N.M. Rusan  Cell Biology and Physiology Center, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Microcephalic primordial dwarfism (MPD) is a class of rare genetic disorders characterized by a reduction in growth that is especially pronounced in the brain. Most genes mutated in MPD have functions relating to the DNA damage response (DDR) or centrosomes. While functional connections between centrosomes and DDR have been established, the striking similarities in “DDR” and “centrosome” MPD phenotypes suggest a deeper and more direct link. As such, MPD genes are prime candidates for uncovering cross-talk between DDR and centrosome functions, and for understanding how these seemingly distinct gene classes function together to affect neurogenesis. As part of a screen for MPD-related structural abnormalities in the Drosophila brain, we focused on nopo, the ortholog of human MPD gene TRAIP, which encodes an E3 ubiquitin ligase with known roles in regulating DDR and apoptosis. Interestingly, nopo (No Poles) was named for its mutant phenotype of acentrosomal spindles, suggesting that this DDR gene might have additional functions at the centrosomes. Our screen revealed that nopo mutants have a mushroom body (MB)-specific defect: nopo mutant MB lobes are fused at the midline, are extremely thin or missing, and often contain axons with aberrant pathfinding. This defect begins to arise in the late 3rd instar, as MB cell subpopulation analysis shows that α′/β′ and α/β, but not γ, MB lobes are affected. We targeted 3rd instar neuroblasts for live imaging, finding that GFP-nopo localizes to nuclei during interphase as expected for a DDR protein; however, at the onset of mitosis, GFP-nopo streams along mitotic spindles towards centrosomes and transitions to the midbody during cytokinesis. Yeast two-hybrid analysis reveals extensive interactions between Nopo and several core centrosome proteins, including Sas4, Ana2 and Plk4. To further understand the function of Nopo in the developing MB, a follow-up screen targeting E2 conjugating enzyme-encoding genes has revealed strikingly similar phenotypes, thus identifying candidate E2s that function with Nopo. Current work also focuses on identifying the ubiquitination substrates of Nopo required for MB development, including Nopo direct binding partners at the centrosome. Together, our discoveries in nopo uncover a novel link between DDR and centrosomes, further emphasizing the deep level of functional coordination at these distinct yet intertwined cellular locations.

Motor Neurons and the Path to Synaptic Specificity. L. Venkatasubramanian1, Z. Guo1, J. Enriquez2, S. Xu3, L. Tan4, Q. Xiao5, S. L. Zipursky4, R. S. Mann3  1) Department of Biological Sciences, Columbia University, New York, NY; 2) Institut de Génomique Fonctionnelle de Lyon, Lyon, France; 3) Department of Biochemistry and Molecular Biophysics, Mortimer B Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY; 4) Department of Biological Chemistry, UCLA, Los Angeles, CA.

The ability of animals to perform coordinated movements depends on the precise organization of neural circuits controlling motor function. Motor neurons (MNs), which are a key component of these circuits, execute coordinated movements by forming distinct connections between the central nervous system and muscles in the periphery. In adult Drosophila melanogaster, ~50 morphologically unique MNs innervate 14 muscles in each leg, each projecting their axon to a specific muscle in a highly stereotyped manner (Baek and Mann, 2009) thereby enabling us to precisely study the development of their neuromuscular architecture. Born shortly after embryogenesis, leg MNs undergo axon defasciculation and terminal branching through the course of metamorphosis, coordinating with overall leg development to reach their synaptic targets. We have previously demonstrated the contribution of combinatorial codes of morphological transcription factors (mTFs) for the proper targeting of MN axon projections to specific muscle targets (Enriquez et al., 2015; unpublished). mTFs are likely involved in several aspects of leg MN development by activating a range of downstream effectors that enable MN projections to interact with their environment and form connections with their synaptic partners. With this in mind we have studied the spatial and temporal expression patterns of a novel group of hetero-binding transmembrane proteins – the DIPs and Dprs...
(Ozkan et al., 2013). The expression of these molecules in components of the leg neuromusculature could serve as a mechanism for establishing distinct MN morphologies. Indeed, we find that the interacting pair Dipa and Dpr10 are expressed in subsets of leg MNs and muscles respectively, and cause terminal branching defects when mutated. Additionally, the dynamic expression patterns of the Dips and Dprs in several distinct units of the leg neuromusculature suggest roles in mediating interactions between developing leg MNs not only with muscles in the periphery but also with themselves and/or leg sensory neurons. The combinations of such interactions throughout development may ultimately lead to synaptic specificity between distinct leg MNs and muscles.

**496 The role of molecular heterogeneity at individual active zones in establishing diverse neurotransmitter release properties.** Scott Gratz1, Karam Khateeb1, Joseph Bruckner1, Roberto Hernandez4, Gregory MacLeod4, Kate O'Connor-Giles1,2 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 3) Institute of Neuroscience, University of Oregon, Eugene, OR; 4) Department of Biological Science and Wilkes Honors College, Florida Atlantic University, Jupiter, FL.

Complex behaviors are established by neural circuits, whose function is defined by their component neurons and synaptic communication between them. The strength of individual synaptic connections is a key determinant of neural circuit function and can be modified in response to experience. In the signal sending, or presynaptic, neuron, strength can be defined as the probability a neurotransmitter-filled synaptic vesicle is released in response to an action potential. Evidence in multiple systems indicates that synaptic vesicle release properties can vary significantly even between neighboring synapses of the same neuron, suggesting that these properties are determined locally at individual AZs and raising the question of how this complex regulation is achieved. At the Drosophila neuromuscular junction, single motorneurons form hundreds of glutamatergic synapses with a single postsynaptic muscle. These synapses exhibit great diversity in neurotransmitter release probabilities, providing an excellent model for understanding the local determination of release parameters. Using a combination of endogenous tagging of synaptic proteins and functional imaging, we are investigating the role of key synaptic proteins in establishing and modulating local release properties. We have endogenously tagged the voltage-gated calcium channel, Cacophony, and observe a heterogeneous, non-normal distribution of Cacophony levels at individual AZs of single neurons with small numbers exhibiting very high levels. Functional imaging revealed a strong correlation between the level of Cacophony and neurotransmitter release probability at individual AZs. We hypothesize that functional heterogeneity enables neurons to adapt to a broad variety of changing inputs. We will present our progress in elucidating the molecular mechanisms through which synaptic strength is established and modified at individual AZs to support circuit function.

**497 Glial-expressed BMP ligand impacts motor neuron development.** T. Knight, M. Bartoletti, A. Held, K. Wharton  Brown University, Providence, RI.

The nervous system of vertebrates is a complex circuitry that depends on dynamic interactions between multiple cell types. In vertebrates, non-neuronal cells, such as glia play important roles in coordinating brain circuitry development, including axonal growth, neuronal progenitor proliferation, neuronal differentiation, as well as in processes later in life that guarantee neuronal maintenance and function. They accomplish these tasks through intimate interactions with neurons. Consequently, the communication between different types of glial cells with neurons is crucial for establishment and maintenance of a functional neural circuit. As in vertebrates, the Drosophila glia are a major component of the nervous system and with many fewer neurons and glial cells overall, Drosophila is a powerful system to study the communication between different glial subtypes and neurons. We have found that Bone Morphogenetic Protein (BMP) 5/6/7 ligand orthologue, Glass bottom boat (Gbb), contributes in distinct ways to glial-neuron interactions. gbb is expressed in discrete sets of cells in the Drosophila brain. Using RNAi to knock down gene expression, we found that glial cell expression of gbb is necessary for a normal synaptic growth at the neuromuscular junction (NMJ), suggesting that Gbb acts from the glia to regulate motor neuron development. Electrophysiological properties, however, do not show a significant change in synaptic activity when gbb is knocked down in glia, which suggests that the glial pool of Gbb is involved in the development of the synapse but not in its function. Interestingly this reduced growth of the NMJ is also accompanied by a strong reduction in the active form of the BMP effector, phosphorylated Mother against dpp (pMad). This suggests that glial cells and motor neurons communicate through a canonical Smad-mediated pathway to allow proper synapse development. We are currently investigating the role of gbb in different glial populations, as well as other cell types in order to: 1) better understand the role of glial gbb for synapse development, 2) to specify which cell types, glia or otherwise, are important for robust neurotransmission.

**498 Maintaining Neuronal Function: The Role of the Transcription Factor Gooseberry in Synaptic Growth and Stability.** M. Perez1,2, C. Dominicci-Cotto1, B. Marie1 1) Institute of Neurobiology, Anatomy and Neurobiology Department, University of Puerto Rico, Medical School; 2) Department of Biology, University of Puerto Rico, Rio Piedras Campus.

Within the nervous system, transcription factors control gene expression to regulate early neuroblast fate, neuronal differentiation, axonal guidance and target recognition. Once a neuron has reached its fully developed state, it must function for an entire lifespan. Are developmental transcription factors also important for the maintenance of neuronal function? Our lab is interested in Gooseberry (Gsb), a paired homeodomain transcription factor homologous to the vertebrate pax3/7,
present in mature motoneurons (MNs) and required for the maintenance of homeostatic compensation at the neuromuscular junction (NMJ). Nonetheless, little else is known about the role of gsb in the developed nervous system.

To assess the role of gsb in the mature nervous system we manipulated its expression at different stages of MNs development and asked whether the growth and stability of the NMJ was affected. Perturbing gsb expression at both early (pan-neuronal; embryo) and late (after initial synaptic growth; larval stage 2) stages of synapse development affected synaptic growth, suggesting that gsb is not only an early fate determinant but can also control synaptic growth at different stages of MNs development. Furthermore, early or late gsb overexpression and loss of function led to cytoskeletal alterations at the NMJ, a hallmark of synaptic instability. We therefore quantified the frequency of synaptic retractions in synapses where gsb expression was altered. We found that increasing or decreasing gsb expression led to a significant increase in synaptic retraction frequency, suggesting that gsb is critical to maintain synaptic integrity. We argue that gsb controls mechanisms involved in both synaptic stability and elimination, and that its expression level is critical in directing these processes. We also found that gsb and wingless (wg) maintain an antagonistic relationship in synaptic growth and stability, and we propose that gsb affects a molecule downstream of wg. Our data suggest that Gsb, a transcription factor involved in neuroblast differentiation and synaptic homeostasis, controls, in fully developed MNs, an array of synaptic processes essential to the maintenance of neuronal function.

499 The class I bHLH protein Daughterless interacts with the class V HLH protein Extramacrochaetae in postmitotic neurons. E.A. Waddell1, M. D'Rozario2, D.R. Marenda1,3 1) Department of Biology, Drexel University, Philadelphia PA ; 2) Department of Development Biology, Washington University School of Medicine, St. Louis MO ; 3) Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia PA.

Class I basic Helix Loop Helix (bHLH) proneural proteins are highly conserved transcription factors. Class I bHLH proteins are broadly expressed in multiple tissues and have critical roles in many developmental processes such as neurogenesis. However, little is known about how class I bHLH proteins function in mature, differentiated neurons. Class I bHLH proteins function during development by forming heterodimers with class II bHLH proteins to activate transcription or by forming homodimers to both activate or restrict transcription of target genes. Class I bHLH proteins can also heterodimerize with class V HLH proteins, preventing gene expression. Our laboratory has shown that the class I bHLH transcription factor Daughterless (Da) is present in neurons and is required for neuroplasticity. Da functions to restrict axonal branching and bouton number through binding to cis regulatory regions of neurexin (a cell adhesion molecule required for synaptic formation, stability, and maintenance) in neurons. Furthermore, our laboratory has recently shown that the class V HLH protein Extramacrochaetae (Emc) functions in neurons to promote axonal branching and bouton number through a potential interaction with Da. Neuromuscular junction dissections were performed on third instar larvae and mature motor neuron synapses were imaged from this tissue. Additionally, Emc has been shown to interact with Da during development where it functions to restrict Da DNA binding. Proximity Ligation Assay (PLA) was performed to determine if Emc and Da interact and it appears that this interaction is taking place primarily in the cytoplasm of neurons, which has not been previously described in the literature. Furthermore, through direct immunohistochemistry on third instar larval ventral nerve cords, alterations in Emc expression affect Da subcellular location. It will be necessary to understand the mechanisms behind this interaction in order to determine whether Emc inhibits the ability of Da to transcriptionally repress neurexin in neurons.

500 Screening the Candidacy of Monosodium Glutamate (MSG) as an Addictive Substance in Drosophila melanogaster. Curteisha L Jacobs, Sandra M Leal Math and Science, Harris- Stowe State University, Saint Louis, MO.

Monosodium glutamate (MSG) is a common salt additive used to enhance the flavor of food. We hypothesize that consuming food containing MSG on a frequent basis develops into an addictive behavior. Thus, consuming MSG may either directly or indirectly increase neurotransmitter (NT) levels in the reward center of the brain. Before determining whether MSG consumption affects systemic NT levels, we first used Drosophila larvae as a simple model system to validate whether MSG, like the NT glutamate, affects simple behaviors mediated by the peripheral and central nervous systems (PNS and CNS). Oregon-R (OR) larvae developing throughout the first-, second-, and third-instar stages were fed either regular yeast paste or yeast paste containing increasing doses of sodium chloride (NaCl) or MSG (0, 1, 10, 50, and 100 mg/ml). Four-day old third-instar larva were then subjected to behavioral assays to measure the effects of NaCl and MSG on either rhythmic behaviors (body wall and mouth hook contractions) or non-rhythmic behaviors (righting reflex and mechanosensory assays). Preliminary results show that low doses of MSG (1mg/ml) significantly inhibit locomotor body wall contractions, but not other behaviors assayed. Currently, we are immunolabeling third-instar larval nervous and metabolic tissues dissected from wild-type OR and drug-treated animals for a variety of NT enzymes and specific NTs including dopamine (DA) and serotonin. Future studies will measure transcript levels for tyrosine hydroxylase, the precursor for DA. Addictive assays will be further developed for adult flies. Potentially addictive food additives, such as MSG, must be examined as contributing factors towards the development of unhealthy eating habits that lead to obesity and other diseases in the general human population.

501 Epigenetic reprogramming of courtship behaviors with social experience. Bryson Deahardt1, Songhui Zhao1, Sachin Sethi2, Jing Wang2, Pelin Volkan1 1) Neurobiology, Duke University, Durham, NC; 2) Biological Science, University of
California at San Diego, San Diego, CA.

Epigenetic regulation of gene expression is associated with long-lasting behavioral changes in animal models and humans. In response to environmental cues, epigenetic programs regulate gene expression in matured neurons via chromatin modifications and DNA methylation resulting in enduring neurophysiological changes. However, establishing a causal link between an epigenetic mechanism and a behavioral change has been elusive, mainly due to the difficulty in identifying the behaviorally relevant neural circuit that is targeted by epigenetic regulation in the nervous system. Using *Drosophila* as a model, we identified an epigenetic mechanism by which sensory experience-dependent changes in gene expression modulate neurophysiology and behavior. We found that social experiences enhance the sensitivity of a pheromone-sensitive olfactory receptor neurons (ORNs) and courtship behavior of male flies. Specifically, we found that group housing increases male courtship behavior and olfactory response of Or47b ORNs, which respond to the courtship-promoting fly odor palmitoleic acid (PA). PA can mimic group housing effect on enhancing Or47b response in socially isolated males. Social context-dependent change in the sensitivity of Or47b ORNs are due to olfactory experience dependent changes in the expression of the transcriptional factor fru. Fru is a sex determining gene that is required for all aspects of male courtship behavior and expressed in approximately 2,000 interconnected neurons in the male courtship circuit. Or47b mediated calcium signaling, and the histone acetyl transferase p300 are both required for expression of fru in Or47b ORNs. In addition, social isolation decreases fru expression in Or47b ORNs, which can be rescued by PA activation. The effects of social isolation on fru transcription in Or47b ORNs are due to a decreased accumulation of p300/CREB, RNA Polymerase II, and open chromatin marks at fru promoter. Our results suggest that epigenetic changes in fru expression with social experience can modulate downstream genes regulated by Fru, that affect ORN physiology and courtship behavioral function.

502 Gut microbiota influences the severity of neurological phenotypes in *Drosophila* voltage-gated sodium channel mutants. Patrick Lansdon, Junko Kasuya, Toshihiro Kitamoto 1,2 1) Dept Anesthesia, Univ Iowa Col Med, Iowa City, IA; 2) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA.

It is widely acknowledged that mutations in genes encoding voltage-gated sodium (Na+) channels contribute to the etiology underlying various seizure disorders. *Shudderer (Shu)* is a dominant mutant allele of the *Drosophila* Na+ channel gene, *paralytic (para)*. *para<sup>M</sup>* exhibits neuronal hyperexcitability and severe seizure-like behavioral defects, including spontaneous jerking and heat-induced convulsion. Our microarray analyses indicated that genes involved in the innate immune response were significantly upregulated in *para*<sup>M</sup> mutants relative to wild-type flies. In accordance with the fact that the host immune system and commensal gut bacteria interact bidirectionally, we observed significant differences between gut microbiota of wild-type flies and *para*<sup>M</sup> mutants. Encouraged by these results, we investigated a potential role for the gut microbiota in *para*<sup>M</sup> phenotypes. Intriguingly, removal of microbiota by antibiotic administration significantly suppressed *para*<sup>M</sup> behavioral phenotypes while having no observable effect on wild-type behavior. Since the microbiota can influence the production of reactive oxygen species (ROS), we next examined the activity of CncC, a master regulator of antioxidant gene transcription in *Drosophila* and the homolog to mammalian Nrf2. Using a GFP reporter, we found that CncC activity was significantly increased in the gut following antibiotic treatment. Overall, our findings suggest that commensal bacteria modulate seizure severity in *Drosophila* Na+, channel mutants by affecting oxidative stress conditions.

503 Wide spread identification of an Axon Initial segment like region in the neurons of *Drosophila* Melanogaster using para-GFP. T.A. Ravenscroft, P.T. Lee, H.J. Bellen 1,2 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 5) Howard Hughes Medical Institute, Houston, TX.

The Axon Initial Segment (AIS) is a key component of neurons, allowing the establishment of neuronal polarity and the initiation of action potentials (AP). The AIS comprises predominantly of scaffold proteins (Ankyrin G, BIV spectrin) and ion channels (Nav1.x, Kv1). Prior belief was that the AIS was a vertebrate specific structure, adapted for rapid and precise signaling. However, recent identification of a neuronal diffusion barrier and ankyrin patterning in the invertebrate model, *Drosophila Melanogaster*, indicates the AIS is conserved in invertebrates. An eGFP containing Minos Mediated Integration Cassettes (MIMIC) inserted into the endogenous locus of another AIS component, voltage gated sodium channels (para) using Recombinase Mediated Cassette Exchange (RMCE), confirms the presence of AIS-like structures throughout the neurons of the *Drosophila* central nervous system (CNS). The expression pattern of Para revealed the majority of neurons have restricted sodium channel expression at an AIS-like region that develops in the larval stage of fly development. The size and distribution of the AIS is not uniform amongst the neuronal population, displaying variation in the propensity for signaling capacity and polarity enforcement across neurons. The identification of an AIS-like structure across *drosophila* neurons provides an exciting new platform to study and model the AIS and its role in the invertebrate system.

504 Interactions between photoreceptors and wrapping glia to control neuronal and glial morphogenesis in developing visual system. Y. Chang 1,2 1) Department of Life sciences and Institute of Genome sciences, National Yang-
dietary nutrients are withdrawn, cortex glia fail to elaborate membrane and ensheath NBs, and tracheae fail to infiltrate the well as trachea require food consumption and locally or systemically, for growth. Like NBs, we find that the cortex glia, which ensheath NBs and their newborn progeny, as well as trachea require food consumption and p1 kinase growth signaling pathway in NBs, leading to their reactivation. While NBs rely on Dilps to reactivate, it remains unclear whether other cell types within the early larval brain also require Dilp signals, either locally or systemically, for growth. Like NBs, we find that the cortex glia, which ensheath NBs and their newborn progeny, as well as trachea require food consumption and p1 kinase activation for growth. When p1 kinase activity is reduced or when dietary nutrients are withdrawn, cortex glia fail to elaborate membrane and ensheath NBs, and tracheae fail to infiltrate the brain and ventral nerve cord, and crawling assays reveal that larvae exhibit reduced locomotion, demonstrating defects in neuron function. Examination of the number of glia along peripheral nerves reveals a reduction in glial number upon raw knockdown. The reduced number of glia along peripheral nerves occurs as a result of decreased glial proliferation and increased cell death. As Raw has been shown to negatively regulate Jun Kinase (JNK) signaling in other developmental concepts, we examined the expression of the downstream Jun-related antigen (Jra) target, matrix metalloprotease 1 (mmp1), and find that raw knockdown results in an increase in mmp1 levels. These results are consistent with previous studies showing increased Mmp levels lead to nerve cord defects similar to those observed upon raw knockdown. In addition, knockdown of puckered, a negative feedback regulator of JNK signaling, also causes a decrease in glial number. Thus, our studies have resulted in the identification of Raw as a novel regulator of glial development. Reduced levels of Raw result in increased JNK signaling, which negatively impacts glial development. Experiments are ongoing to understand how Raw functions at the molecular level to negatively regulate JNK signaling.

505 Identification of a Novel Regulator of Glial Development. J.C. Jenc, D. Luong, L. Perez, M. Davis Dept. of Biology, Loyola University Chicago, Chicago, IL.

Glia cells perform numerous roles to support neuron development and function, including axon wrapping, formation of the blood brain barrier, and enhancement of synaptic transmission. We have identified a novel gene, raw, which functions in the glia of the central and peripheral nervous systems in Drosophila. Reducing raw levels in glia by RNAi results in morphological defects in the brain and ventral nerve cord, and crawling assays reveal that larvae exhibit reduced locomotion, demonstrating defects in neuron function. Examination of the number of glia along peripheral nerves reveals a reduction in glial number upon raw knockdown. The reduced number of glia along peripheral nerves occurs as a result of decreased glial proliferation and increased cell death. As Raw has been shown to negatively regulate Jun Kinase (JNK) signaling in other developmental concepts, we examined the expression of the downstream Jun-related antigen (Jra) target, matrix metalloprotease 1 (mmp1), and find that raw knockdown results in an increase in mmp1 levels. These results are consistent with previous studies showing increased Mmp levels lead to nerve cord defects similar to those observed upon raw knockdown. In addition, knockdown of puckered, a negative feedback regulator of JNK signaling, also causes a decrease in glial number. Thus, our studies have resulted in the identification of Raw as a novel regulator of glial development. Reduced levels of Raw result in increased JNK signaling, which negatively impacts glial development. Experiments are ongoing to understand how Raw functions at the molecular level to negatively regulate JNK signaling.

506 Regulation of retinal basal glia differentiation in Drosophila eye disc. Chia-Kang Tsao1,2, Yu Fen Huang1,2, Y. Henry Sun1,2 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, Republic of China; 2) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan, Republic of Chin.

The retinal basal glia (RBG) is a group of glia that migrates from the optic stalk into the third instar larval eye disc while the photoreceptor cells (PR) are differentiating. There are three major classes of RBG, namely surface glia (SG), wrapping glia (WG) and carpet glia (CG), based on molecular and morphological characteristics. The SGs migrate and divide. The WGs are postmitotic and wraps around PR axons. There are two CGs per eye disc and they have giant nucleus and extensive membrane extension that each covers half of the eye disc. It has been proposed that the SGs migrate under the CG membrane, which prevented their contact with the PR axons lying above CG membrane. Upon passing the front of the CG membrane, which lags slightly behind the morphogenetic furrow marking the front of PR differentiation, the migratory SG can contact the nascent PR axon and be induced to differentiate into WG. We have developed an ex vivo culture system to follow the migration, division and differentiation of RBGs by live imaging. We found that the increase of RGB cell number is primarily (92%) due to SG divisions and only 8% contributed by migration from optic stalk. The WGs showed no cell divisions and appeared de novo in the anterior region, presumably differentiating from the migratory SG. However, we found that SGs are migrating both above and below the CG membrane, thus the CG membrane cannot be a physical barrier to prevent contact between SG and PR axon. We will present new findings suggesting a novel mechanism for the regulation of the SG-to-WG transition by CG.

507 Nutrient-dependent Development of the Gial Niche Coordinates Neuroblast Exit from Quiescence. X. Yuan, S. Siegrist Biology, University of Virginia, Charlottesville, VA.

Neuroblasts (NBs), the Drosophila neural stem cells, enter quiescence at the end of embryogenesis, and reactivate after freshly hatched larvae consume their first complete meal. In response to food consumption, insulin-like peptides (Dilps) are produced and secreted locally from glia and systemically from insulin producing cells in the brain. Local Dilp signaling activates the highly conserved PI3-kinase growth signaling pathway in NBs, leading to their reactivation. While NBs rely on Dilps to reactivate, it remains unclear whether other cell types within the early larval brain also require Dilp signals, either locally or systemically, for growth. Like NBs, we find that the cortex glia, which ensheath NBs and their newborn progeny, as well as trachea require food consumption and PI3-kinase activation for growth. When PI3-kinase activity is reduced or when dietary nutrients are withdrawn, cortex glia fail to elaborate membrane and ensheath NBs, and tracheae fail to infiltrate the brain and ventral nerve cord, and crawling assays reveal that larvae exhibit reduced locomotion, demonstrating defects in neuron function. Examination of the number of glia along peripheral nerves reveals a reduction in glial number upon raw knockdown. The reduced number of glia along peripheral nerves occurs as a result of decreased glial proliferation and increased cell death. As Raw has been shown to negatively regulate Jun Kinase (JNK) signaling in other developmental concepts, we examined the expression of the downstream Jun-related antigen (Jra) target, matrix metalloprotease 1 (mmp1), and find that raw knockdown results in an increase in mmp1 levels. These results are consistent with previous studies showing increased Mmp levels lead to nerve cord defects similar to those observed upon raw knockdown. In addition, knockdown of puckered, a negative feedback regulator of JNK signaling, also causes a decrease in glial number. Thus, our studies have resulted in the identification of Raw as a novel regulator of glial development. Reduced levels of Raw result in increased JNK signaling, which negatively impacts glial development. Experiments are ongoing to understand how Raw functions at the molecular level to negatively regulate JNK signaling.
brain hemispheres. Growth and elaboration of both cortex glial membranes and tracheae coincide with NB reactivation from quiescence, but only cortex glial growth is required for NB reactivation. When PI3-kinase is reduced in cortex glia, NBs fail to reactivate in response to food consumption. NBs also fail to reaggregate in response to larval feeding when cortex glia are genetically ablated. We conclude that the development of the NB glial microenvironment is nutrient-regulated and is required for NB reactivation. Furthermore, we are investigating dynamic changes within the brain tissue architecture that occur in response to animal feeding, and how tissue architecture impacts NB proliferation decisions.

### 508 Identification of a novel neuronal circuit controlling Dilps secretion and systemic growth according to nutrition.

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Body growth is tightly regulated by nutrient availability. Upon nutritional shortage, animals harmoniously reduce their body size by modulating the activity of insulin/IGF signaling (IIS). Drosophila has a conserved IIS with 8 insulin-like peptides (Dilps), a unique insulin receptor and a conserved downstream signaling cascade. Several Dilps are produced by specialized neurons, the Insulin-Producing-Cells (IPCs), functionally related to vertebrate beta cells. Dilps are secreted in the hemolymph under normal nutrient condition, but reducing amino acid in the diet induces a strong blockage of this secretion. Several cross-talks between the fat body and the brain have been recently identified that control this secretion. One particular set of EGF-like peptides produced by the fat body in response to amino acids, called GBP1 and 2, acts on the IPCs to induce insulin release, but the neuronal circuitries at play are unknown.

Using the GRASP technique (GFP Reconstitution Across Synaptic Partners), we identified a pair of neurons harboring synaptic connections with the IPCs (IPC-connecting neurons, ICNs). We next determined that these ICNs become active upon amino acids starvation and induce a blockade of Dilp secretion by the IPCs. Moreover, in rich nutrient conditions, EGFR signaling prevents activation of the ICNs, allowing Dilp release from the IPCs. Finally, using ex-vivo brain culture, we show that the presence of the fat body-derived signal GBP1 in the hemolymph activates EGFR signaling in the ICNs and alleviates their inhibitory input on the IPCs, allowing Dilp release.

In conclusion, we identify a novel neuronal circuitry responding to fat-derived EGF-like GBP1s, allowing coupling dietary amino acids to the release of insulin-like peptides and systemic growth.

### 509 A genetic approach for the understanding of the brain micro-environment that regulates the plasticity of neural stem cells.

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It has long been reported that adult mammalian central nervous system (CNS) possess significant plasticity. It has also been reported that some patho/physiological stimuli can activate the proliferation of slow-growing or quiescent NSCs in many experimental models. Interestingly, the plasticity of NSCs is also observed in lower organisms such as invertebrates. For example, most of the Drosophila neural stem cells, neuroblasts (NBs), become quiescent during late embryonic stage; however, their proliferation is reactivated at around twelve hours after larvae hatched. Thus, the principle molecular mechanism would be conserved.

We identified that an evolutionarily conserved metabolic enzyme, whose function is required for the lipid metabolism, is required for the proper reactivation of NBs. The loss of function mutant animals for this gene showed a cleaved caspase 3-positive signal in the developing NBs, presumably indicating cell death. We also found that this phenotype appeared at around 12hrs after larvae have hatched, suggesting a developmental timing-dependent machinery. Finally, a series of genetic rescue experiments suggested that this phenotype was regulated by surrounding neurons. Taken together, these results suggest that lipid homeostasis in neurons regulate the reactivation of NBs non-cell autonomously.

### 510 Combinatorial action of Grainyhead, Extradenticle and Notch in regulating Hox mediated apoptosis in Drosophila larval CNS.

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Neuroblasts (NBs) are mother cells of all neurons in a fully functional Drosophila brain. Each NB attains its specific daughter neuron numbers through regulation of apoptosis, proliferation and quiescence which are also prime tools for sculpting the Drosophila central nervous system (CNS) during anterior-posterior (A-P) patterning by Hox genes. Hox mediated Nb apoptosis is prevalent way to pattern developing Drosophila CNS. The mechanism for Hox mediated specific Nb apoptosis is not well understood both in embryonic and larval stages. In our present study, for the first time, we investigated in detail molecular mechanism of Hox gene Abdominal-A (AbdA) mediated larval Nb (pNB) apoptosis in abdominal segments. We extend our investigation to Dfd expressing region of larval sub-esophageal ganglia and find that common players are involved in NB apoptosis in abdominal and Dfd-SEG regions.

We have supported our conclusions with both in vitro and in vivo data. The in vivo study includes MARCM (Mosaic analysis with a repressible cell marker), RNA interference and reporter analysis. The in vitro investigation was carried out with
the help of Electrophoretic Mobility Shift Assay (EMSA) to study protein-DNA interactions while protein-protein interactions were showed using pull downs.

Our study shows that AbdA, its cofactor Extradenticle (Exd) along with a bHLH transcription factor Grainyhead (Grh) collaborate with Notch signaling on 717bp subfragment of NBRRR (neuroblasts regulatory region) to transcriptionally activate (RHG family) death genes and cause pNb apoptosis. We also find that, Notch has a direct role in this apoptosis and Hox cofactor Exd function independent of known partner Homothorax (hth). Our in vitro binding studies show that these factors bind on multiple binding sites and mutating them in the 717bp subfragment causes the enhancer to lose its maintenance activity in vivo leaving its initiation activity intact. Further, we also report that, Hox, Exd, Grh, and Notch also play a role in causing the death of pNbs in Dfd expressing region of sub-esophageal ganglia (Dfd-SEG), through a yet to be isolated SEG specific enhancer. We propose a mechanism where common players like Exd-Grh-Notch work with different Hox genes through region specific enhancers to pattern respective segments of larval CNS.

511 Drosophila globin 1 is required for the development of nervous system. N. Kumari University of Delhi South Campus, New Delhi, India.

(Hemo-) globins (Hbs) are evolutionarily conserved heme containing metallo-proteins which could be characterized by the presence of distinct "globin fold". Although, globin genes have been classically associated with the oxygen transport function; however, recent studies have linked globins to various other biological and physiological processes. Drosophila genome harbours three globin genes, namely, glob1, glob2 and glob3. Our lab has successfully established the role of glob1 in various aspects of development such as tracheal liquid clearance, regulation of cellular level of ROS during oxidative stress and maintenance of cytoskeleton integrity during oogenesis. The present study was undertaken to investigate the cellular expression profile and functional relevance of glob1 in central nervous system development in both embryonic as well as larval tissues. Our study reveals a robust expression of Glob1 in the neuronal tissues, specifically around physiologically active and dividing cells such as outer proliferation centre (OPC) of the optic lobes, in the optic stalk, in the region of eye imaginal discs below the morphogenetic furrow. In addition a robust expression of Glob1 has been observed in various cells of embryonic ventral nerve cord, and A1-A8 (abdominal) neuromeres of the larval brain ganglia. Ubiquitous or neuronal cell specific reduced expression of glob1 causes abnormal development of nervous system and also results in significant lethality during embryonic and larval development. Our study, therefore, suggests a novel and critical role of glob1 in the development of nervous system in Drosophila.

512 E93 activates autophagy to terminate mushroom body neuroblast proliferation. Matthew C. Pohl, Susan E. Doyle, Sarah E. Siegrist Biology, University of Virginia, Charlottesville, VA.

A remarkable number of morphologically and functionally distinct neuron types are generated within the CNS during development from the asymmetric cell divisions of a defined number of neural progenitors, known as neuroblasts (NBs). As development nears completion, NB cell divisions terminate in a spatially and temporally defined manner and no new neurons are generated during adulthood. Mushroom body neuroblasts (MB NBs), a small subset of central brain neuroblasts, that generate neurons important for memory and learning continue dividing well into late pupal stages, much longer than all other NB lineages. Shortly before adulthood, MB NBs undergo a period of reduced growth and proliferation, which primes them for elimination by programmed cell death. To identify genes required for termination of the MB NB proliferation, we performed a directed RNAi screeen of candidate regulators. One of the candidate genes that we identified is E93, an edcsynone-induced transcription factor that is required for elimination of the salivary glands and other tissues via autophagy. When E93 is knocked down in NBs, we find that MB NBs ectopically persist into adulthood, whereas when E93 is overexpressed, MB NBs terminate prematurely. Using an autophagy reporter, we find an absence of autophagosome formation in E93 RNAi MB NBs compared to controls. Somewhat surprising, we find that MB NBs initiate E93 expression during early to mid pupal stages, likely in response to the last pupal pulse of edcsynone, and maintain E93 expression until their elimination two days later. Other non-MB NBs also express E93, yet in these lineages E93 is expressed during larval stages and is not required for termination of non-MB NB divisions. We are investigating how E93 is regulated in MB NBs and propose that E93 functions as a late temporal factor, providing MB NBs competence to undergo autophagy and programmed cell death.

513 JAK/STAT guarantees robust differentiation of neural stem cells by shutting off biological noises in the developing fly brain. M. Sato1, T. Yasugi1, Y. Tanaka2, M. Nagayama3, S Ei2 1) Institute for Frontier Science Initiative, Kanazawa University, Kanazawa, Ishikawa, JP; 2) Department of Mathematics, Faculty of Science, Hokkaido University, Sapporo, Hokkaido, JP; 3) Research Institute for Electronic Science, Hokkaido University, Sapporo, Hokkaido, JP.

The development of live organisms is precisely regulated by a sequence of gene functions even in the presence of biological noises. However, it is difficult to evaluate the effect of the noises in vivo and the mechanisms how the noise is filtered during development is largely unknown. To unveil the noise canceling mechanism, we use the fly visual system, where the timing of differentiation of neural stem cells is spatio-temporally ordered. Our mathematical model predicts that JAK/STAT signaling plays a role in noise canceling to guarantee the robust progression of the differentiation wave in silico. We further
demonstrate that the suppression of JAK/STAT signaling causes stochastic and ectopic neural stem cell differentiation in vivo, suggesting an evolutionarily conserved function of JAK/STAT to regulate the robustness of stem cell differentiation.

514 Regulation of the Drosophila ID protein Extramacrochaetae by proneural dimerization partners.  
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Functions of proneural bHLH transcription factors depend on the availability of the E-protein Daughterless (Da) in the face of competition from the ID-protein Extramacrochaetae (Emc). Emc expression is reduced where neurogenesis occurs, which is proposed to define the prepattern for neural differentiation independently of proneural bHLH genes. Here we investigate directly how levels of Da and Emc are regulated. Unexpectedly, we find Emc levels are actually determined by other bHLH proteins. Outside proneural regions, Emc levels are quantitatively matched to Da levels, apparently by stabilization in heterodimers. Emc levels preclude Da activity in heterodimers, which is necessary for proliferating cells to survive (since high Da activity is toxic outside proneural regions). In proneural regions, it is proneural genes such as Ato or AS-C that reduce Emc levels. This is opposite to the expectation that low Emc levels define a neural prepattern. We provide evidence that Emc protein is destabilized by proneural bHLH proteins via local competition for heterodimer formation, and not through the transcriptional activity of proneural proteins. Emphasizing the post-transcriptional regulation of Emc, and the fact that its spatial patterning is downstream of other proneural regulators, uniform emc transcription is sufficient for almost normal patterns of protein expression and almost normal neural patterning in multiple tissues. Dynamic exchanges between dimers that change protein stability may be a general feature of bHLH-mediated developmental events.

515 Characterization of epithelial machinery required for ensheathment of somatosensory neurons.  
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In vertebrates and invertebrates alike, peripheral arbors of some subtypes of somatosensory neurons become physically embedded inside of epithelial cells. Structurally, this epithelial ensheathment of sensory neurons resembles schwann cell ensheathment of sensory neurons, though functional roles for epithelial sensory dendrite ensheathment have not been defined. And while prior studies have shown that epithelial dendrite ensheathment constrains somatosensory dendrite growth and structural plasticity, the cellular machinery that regulates epithelial dendrite ensheathment has not been defined. Here, we examined the development and progression of neuron ensheathment by epithelial cells throughout Drosophila larval development. To identify the molecules present at sites of epithelial ensheathment, we screened a large collection of fluorescently-tagged proteins expressed in the epithelium of live animals for enrichment along sensory dendrite branches. From this screen, we identified markers that define specialized plasma membrane domains in epithelial cells that ensheathe somatosensory neurons. Using these markers in combination with high resolution imaging approaches including expansion microscopy (ExM) and serial block-face scanning electron microscopy (SBFSEM), we have characterized the stepwise assembly of these structures. Dendrite-derived signals initiate clustering of epithelial membrane phosphoinositides in a modality-specific manner, with class IV da neurons inducing the most extensive phosphoinositide clustering. Concomitant with phosphoinositide clustering, the endocytic adaptor protein dArf6 clusters at epithelial sites of dendrite ensheathment, followed by regulators of the actin cytoskeleton and finally by components of septate and adherens junctions. By using a variety of approaches to perturb phosphoinositide signaling we find that PIP2 clustering is required for sheath formation and hence proper dendrite growth, and that the protein composition of the sheaths change as they mature. Among somatosensory neurons, nociceptive neurons exhibit the largest extent of ensheathment, and we have found that this ensheathment is critical for behavioral responses to nociceptive stimuli. Given that similar structures are present in organisms ranging from nematodes to humans, we speculate that epithelial ensheathment of somatosensory neurons may define a conserved mechanism for nociceptive signaling.

516 Gal80ts analysis defines threshold for WND-driven axonal sprouts in R7 photoreceptor cells.  
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Our laboratory has used the GAL4-UAS system to express the dual leucine kinase WND specifically in photoreceptors. WND expressed by this system promotes the growth of axonal sprouts in synaptic regions of R7 photoreceptors. In the study reported here, GAL80ts was introduced to limit GAL4 activity at lower temperatures, thereby providing an experimental method to limit WND expression in a temperature dependent manner. Flies carrying Rh3-GAL4 express GAL4 specifically in the R7 photoreceptors. Experimental flies also carried tubGAL80ts, to express GAL80ts in all tissues, and UAS-WND, to express WND only in R7 photoreceptors. They were reared at 25°C until 3rd instar larvae, then shifted to 18°C until 3 days post-eclosion, to provide repression of WND activity during retinal development. At 3 days post-eclosion, flies were shifted to temperatures of 20°C, 22°C, 24°C, and 25°C and reared for 5 additional days prior to histological examination. R7 axon outgrowths were observed in animals shifted to the higher temperatures of 24°C and 25°C, but not in those shifted to 20°C
and 22°C. At higher temperature, inactivation of GAL80ts allows sufficient WND expression to drive the cellular signaling pathways required for axonal growth. The results indicate that temperature control of GAL80ts is an useful experimental means to maintain WND activity near the critical threshold of cellular signaling required for production of ectopic axonal sprouts, likely a key initial step in the axon regeneration process.

517 Roles of N-cadherin in columnar unit organization in the medulla. O.I. Trush, C. Liu, M. Sato Developmental Neurobiology, Grad. School of Med. Sciences, Kanazawa Univ., Kanazawa, JP.

Columnar organization of neurons is an elementary anatomical and functional unit for information processing and is found in brains of a wide variety of organisms. However, the developmental mechanisms of columnar unit formation remain largely unclear. The medulla in the fly optic lobe, which receives visual input from the eye, shares structural characteristics with the mammalian cerebral cortex, such as columnar and layered structures. In the mammalian brain, each columnar unit consists of more than 10,000 neurons. In contrast, a columnar unit in the fly medulla contains only about 100 neurons. In addition, powerful techniques of fly genetics are available. Thus, the medulla is an excellent model system to investigate mechanisms of columnar unit formation.

To understand molecular mechanisms of medulla column formation, we screened a series of markers that can visualize the columns. We identified that N-cadherin (Ncad) shows a characteristic donut-like distribution pattern in each medulla column, while the others are distributed in the central or peripheral regions of the donut shape of Ncad. Combining these molecular markers and Gal4/UAS system, we examined how the donut-like structures are formed during development. We found that one of the medulla neurons, Mi1, acts as primary neuron during column formation. Mi1 axons project to the medulla neuropil earlier than other neurons and gradually form a donut-like projection pattern that overlaps with Ncad. In the absence of Mi1 neurons, the column formation is severely affected.

Next, we investigated the function of Ncad in medulla column formation. Manipulation of Ncad level in Mi1 showed autonomous mistargeting within the column as well as non-autonomous disorganization of column boundaries and fusion of the columns. Additionally, Ncad knock-down in neurons that usually project to the central part of each column caused the expansion of their neurites towards the peripheral part. These results suggest that Ncad expression in Mi1 is essential for proper column formation and that column formation can be explained by the Differential Adhesion Model in three-dimensional space.

518 Logic of an aminergic/glutamatergic circuit in egg-laying. Sonali Deshpande1, James Asuncion1,2, Daniel Suto1, Pei-Tseng Lee3, Hugo Bellen4,5,6,7, David Krantz1 1) Department of Psychiatry, University of California, Los Angeles, Los Angeles, CA, United States; 2) Medical Scientist Training Program, University of California, Los Angeles, Los Angeles, CA, United States; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States; 4) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX, United States; 5) Program in Developmental Biology, Baylor College of Medicine, Houston, TX, United States; 6) Department of Neuroscience, Baylor College of Medicine, Houston, TX, United States; 7) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, United States.

Biogenic amines such as dopamine, serotonin and octopamine regulate multiple behaviors in Drosophila; however, the underlying mechanisms and potential interactions with other signaling pathways remain unclear. To address these questions, we are studying the neural circuitry involved in regulating egg-laying. Previous studies in larger insects as well as Drosophila suggest a relatively simple regulatory mechanism in which glutamate and proctolin cause contractions whereas octopamine promotes oviduct relaxation. However, most previous studies have assessed the function of oviducts as whole rather than at specific anatomical regions. In addition, the localization and function of specific octopamine and glutamate receptors have remained unclear. To overcome these limitations we have used calcium imaging to visualize muscle activity in specific regions of the reproductive tract including the anatomically distinct lateral and common oviducts. In addition, we developed molecular markers based on MIMIC insertions to determine the localization of specific receptors. These MIMICS also allowed us to tag the genes encoding the receptors with SA-T2A-GAL4-polyA and perform precise RNAi knockdowns of these specific receptors. Our data indicate that glutamate and octopamine have different effects in the lateral versus common oviducts. In addition, at both sites, interactions between glutamatergic and octopaminergic signaling pathways can reverse the sign of their respective effects. The distribution of specific octopamine receptors in the oviducts may explain differences in the regulation of the common versus lateral regions. In addition, expression of other octopamine receptors in local interneurons expressing the marker pickpocket suggest that some interactions may be mediated via previously unknown pathways. Our data provide fundamental information on the logic underlying aminergic neuromodulation of circuit function that may be applicable to more complex pathways in the fly and other systems.

519 Genome-wide association analysis of amphetamine sensitivity. Coline Karam1,2, Brenna Williams1, Elizabeth Neuriter1,2, Rebecca Mirhashem1, Diana Ortiz1, Jonathan Javitch1,2 1) Division of Molecular Therapeutics, Research Foundation for Mental Hygiene, New York, NY, USA; 2) Department of Psychiatry, College of Physicians and Surgeons, Columbia University, New York, NY, USA.

Abuse of psychostimulants is a major public health problem with profound psychiatric, medical and psychosocial
complications. Genetic factors contribute substantially to an individual's susceptibility to developing addiction; however, the search for risk alleles has yielded limited success. The initial sensitivity to psychostimulants varies significantly, and has been associated with continued use and abuse. This trait can be studied in animal models, which have emerged as powerful tools to investigate the behavioral response to drugs in a controlled and systematic manner. We are currently screening the sequenced, inbred lines of the D. melanogaster Genetic Reference Panel (DGRP) for amphetamine-induced behaviors, with the aim of performing genome wide association studies (GWAS) to identify new genes and gene variants associated with the sensitivity to amphetamine. The DGRP consists of 203 lines that were created by collecting mated females from a natural, outbred population followed by 20 generations of full-sibling inbreeding. The lines harbor most common variants and a representative sample of rare variants, with ~2.5 million SNPs available for analysis. We are quantifying amphetamine-induced increases in locomotor activity, changes in circadian activity, as well as amphetamine-induced mortality, at two distinct doses, using the Trikinetics Drosophila Activity Monitors (DAMs). Our analyses have identified substantial variation in the response to amphetamine for all 3 phenotypes. Upon completion of the screen, we will perform GWAS analyses to identify genes and gene networks associated with the psychostimulant response, with the aim of identifying novel mechanisms that modulate the neurobiology of amphetamine action and influence both the initial as well as the prolonged behavioral sensitivity to the drug.

520  Nitric oxide signaling in Drosophila ethanol sedation.  

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Nitric oxide (NO) is a key gaseous neurotransmitter in both the vertebrate and invertebrate nervous systems. Nitric oxide synthase (NOS) produces NO during the conversion of L-arginine to L-citrulline. NO produced from this reaction regulates the function of downstream proteins via S-nitrosylation (the addition of NO to the metal moieties of metal-containing proteins) and S-nitrosothiolation (the conversion of thiol groups to S-nitrosothiol). Although studies in rodents implicate NO signaling in multiple ethanol-related behaviors, the molecular mechanisms underlying these effects have not been identified. We are using flies to investigate these mechanisms. We have backcrossed and characterized three transposon insertions and a null allele in the sole NOS-encoding orthologue in flies, Nos. Flies homozygous for each of the transposon insertions had decreased levels of total Nos mRNA expression, indicating that the insertions cause partial loss of function in the gene. Additionally, flies homozygous for any of the three insertions had increased ethanol sedation sensitivity compared to isogenic controls, but had no change in ethanol uptake/metabolism or locomotor behavior. Nos null flies have a premature stop codon N-terminal to the reductase domain in NOS, rendering the protein nonfunctional. Consistent with our transposon insertion studies, Nos null flies had increased ethanol sensitivity without a change in ethanol uptake/metabolism or locomotor behavior. To understand the mechanism underlying the role of Nos and NO signaling in ethanol sedation, we are investigating multiple candidate downstream effectors. One of these candidates, UNF, is a heme-binding transcription factor that is an S-nitrosylation target of NO. Pan-neuronal expression of RNAi against UNF increased ethanol sedation sensitivity. In contrast, expression of RNAi against other candidate effectors including two members of the soluble guanylate cyclase family did not impact ethanol sedation. Our results to date indicate that ethanol sedation in flies is influenced by NO signaling, possibly via the transcription factor UNF.

521  Effect of point mutations in the vesicular acetylcholine transporter on acetylcholine-linked behavioral paradigms in Drosophila.  

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Cholinergic neurotransmission is essential for key organismal functions such as movement, vision, and learning and memory. As a consequence of this role, deficits in acetylcholine (ACh) are an important cause of locomotion and cognitive deficits. However, the manner through which changes in ACh regulation lead to these deficits remains poorly understood. Another important unanswered question is the precise contribution of central ACh release to the regulation of cognitive and locomotion performance. Here we are using point mutations in the vesicular acetylcholine transporter (Vacht) which regulates the packaging of ACh into synaptic vesicles for release at the plasma membrane. Our working hypothesis is that point mutations in Vacht will lead to deficits in cholinergic-mediate behaviors such as locomotion and courtship learning in a manner that varies with the severity of the mutations. Indeed, we report just such a differential effect of Vacht on locomotion behavior using different locomotion paradigms. Vacht the most severe allele shows a strong phenotype in all three matrices of locomotion tested. By contrast, Vacht our least severe mutation allele shows no discernible effect on average speed relative to wildtype but displays a phenotype when more subtle measures of locomotion were assessed. Furthermore, we report the effect of Vacht mutations on courtship learning behavior. Together, these data provide evidence for a role for central cholinergic release in the mediation of key neuronal functions and sets the stage for a more detailed behavioral analysis as well as functional studies in cholinergic neurons.

522  Central Neuropeptide Neurons that Relay Sex Peptide Signaling and Regulate Female Mating Receptivity in Drosophila melanogaster.  

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Upon mating, fruit fly females become refractory to further mating for several days. An ejaculate protein called Sex Peptide
(SP) acts on uterine sensory neurons to trigger this behavioural switch, but it is still unclear how the SP signal modifies the mating decision. Here, we describe two groups of female-specific local interneurons that are important for this process—the *ventral abdominal lateral* (vAL) and *ventral abdominal medial* (vAM) interneurons. Both vAL and vAM express myoinhibitory peptide (Mip)-GAL4. vAL is positive for Mip neuropeptides and the sex determining transcriptional factor *doublesex*. Silencing the Mip neurons in females induces active rejection of male courtship attempts, whereas activation of the Mip neurons makes even mated females receptive to re-mating. vAL and vAM are located in the abdominal ganglion (AG) where they relay the SP signal to other AG neurons that project to the brain. Genetic analyses with a Mip-null mutant suggest that the Mip neuropeptide produced in vAL appear to promote mating receptivity both in virgins and mated females, although it is dispensable for normal mating in virgin females.

### 523 Calcium activated chloride channels are required for distinguishing between noxious and innocuous stimuli in multimodal sensory neurons.

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Multimodality is a common functional characteristic of somatosensory neurons that confers these neurons with the ability to sense and respond to more than one sensory modality. Multimodal and monomodal molecules and neural circuits have been identified across a number of species, however the mechanisms by which multimodal sensory neurons can integrate and/or distinguish between sensory modalities—thereby driving stimulus-appropriate behaviors—are poorly understood. Elucidating generalizable mechanisms of multimodality is particularly important for understanding neuropathic pain, wherein otherwise innocuous stimuli can erroneously engage nociceptive circuitry, thereby causing pain. In *Drosophila melanogaster*, class III (CIII) multidendritic sensory neurons mediate both noxious cold nociception and gentle touch mechanosensation. CIII neurons are thought to operate under a high-low threshold mechanism, whereby high levels of activation drive noxious cold-evoked nocifensive behaviors, and low levels of activation drive gentle touch-evoked behaviors. Neurogenomic analyses of CIII neurons revealed expression of a suite of calcium-activated channels. Genetic analyses coupled with established behavioral assays revealed that disruption of calcium-activated chloride channel (CaCC) expression inhibits the ability of larvae to initiate and sustain noxious cold-evoked behaviors, whereas gentle touch sensitivity was not perturbed. Furthermore, genetic manipulations of secondary active transporters responsible for regulating chloride homeostasis indicate that high intracellular chloride concentrations are necessary for CaCC-mediated behavioral regulation—a feature also typical of vertebrate dorsal root ganglion (DRG) neurons. Collectively, these data suggest that cold-evoked, calcium-activated, excitatory chloride currents contribute to the high levels of activation required for driving cold-mediated nocifensive behaviors. These findings demonstrate that CIII neurons provide a model for elucidating molecular and neural mechanisms underlying modality-specific sensory integration, as well as suggest potentially conserved neural mechanisms between *Drosophila* nociceptive neurons and vertebrate DRG neurons.

### 524 A forward genetic screen to identify modifiers of neurological phenotypes in *Drosophila* voltage-gated sodium channel mutants.

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Voltage-gated sodium (Na⁺) channels are essential for the generation and propagation of action potentials in neurons. *Shudderer* (Shu) is a dominant mutant allele of the *Drosophila* Na⁺ channel gene, *paralytic* (para). *para⁰⁰⁰* exhibits neuronal hyperexcitability and severe seizure-like behavioral defects, including spontaneous jerking and heat-induced convulsion. We carried out an unbiased forward-genetic screen to identify genes that functionally interact with *para⁰⁰⁰* to influence the severity of its neurological phenotypes. Our working hypothesis was that the mutant phenotypes would be significantly altered when the activity of a gene functionally interacting with *para⁰⁰⁰* was reduced by 50%. We systematically deleted particular genomic regions by crossing para⁰⁰⁰* to a panel of flies carrying molecularly defined chromosomal deficiencies. Of the 176 chromosome deficiencies we have thus far examined, at least six had a robust modifying effect on *para⁰⁰⁰*, and greatly reduced the severity of their neurological phenotypes. One candidate deficiency that covers the second chromosome genomic region 3D6-3F11 was selected for further analysis. We found that an overlapping smaller deficiency, *Df(2R)BSC433 (5F3F4-5F3F8)* covering only 6 genes, phenocopied the original large deficiency. We next used RNAi-mediated knockdown of these 6 genes and among them identified *Glutathione S-transferase S1* (GstS1) as a modifier of *para⁰⁰⁰*. Finally, we introduced a GstS1 null allele (*GstS1⁰⁰⁰*) into *para⁰⁰⁰* and demonstrated that 50% reduction of GstS1 function in this system also causes significant suppression of *para⁰⁰⁰* neurological phenotypes. We are now studying the underlying mechanisms by which the rescue effects of GstS1-downregulation upon *para⁰⁰⁰* phenotypes by utilizing the UAS/GAL4 binary expression system in combination with a *UAS-GstS1-RNAi* transgene. We expect to determine the spatiotemporal requirements for GstS1-dependent modification of *para⁰⁰⁰* phenotypes, and gain fundamental insights into its specific mechanisms of action in the fly nervous system.

### 525 Unveiling Mechanisms that Regulate Glial Signaling to Neurons.

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Despite increased efforts on glia studies, we still know very little about how glia communicate to neurons. Recent studies
have shown that overexpressing TRPA1, a heat-sensitive cation channel, in glia causes neuronal hyper-excitability and leads to rapid paralysis upon exposure to restrictive temperatures. Taking advantage of this behavioral phenotype, we employed an un-biased modifier screen to knockdown genes or overexpress genes in glia while simultaneously overexpressing TRPA1 in glia to determine which genes influence the kinetics of TRPA1-induced paralysis. Genes that increased the paralysis duration are enhancers, and genes that decreased or abolished the paralysis duration are suppressors. We screened various category of glia genes including those encoding calcium channels and pumps, gap junctions, transporters, vesicle trafficking, and others. After identifying some suppressors and enhancers, we then verified neuronal responses by recording synaptic transmission at the larval NMJ. One of the enhancers is the SNARE protein Syntaxin, and overexpression of the constitutively active Syntaxin and TRPA1 in glia resulted in a dramatic increase in paralysis duration and slow recovery. One of the suppressors we identified is EAAT1. Knockdown of EAAT1 enhanced temperature-induced paralysis and slows the kinetics of recovery whereas overexpression of EAAT1 fully abolished the paralysis behavior.

We then wanted to know if any particular subset of glia are better suited for influencing neuronal behavior. By expressing TRPA1 in various subset glial GAL4 drivers, we found that astrocyte glia account for the majority of the paralysis behavior and duration. We then knocked down candidate genes from our screen in these various subset glia while also expressing TRPA1 in those subsets. In general, suppressors continued to suppress in the various subsets, and enhancers enhanced in the various subset glia. We conclude that glial transmission is critical for triggering neuronal hyperexcitability and that uptake of synaptic glutamate by glia helps tune down such hyperexcitability. We are now focusing on identifying glia transmitters and or transporters that alter neuronal excitability.

526 Transgenerational inheritance of behavior in Drosophila. J.E. Bozler, B.Z. Kacsoh, G. Bosco  Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Changing environmental conditions is one of the greatest challenges to an organism's long-term survival. Considerable research has explored behavioral changes as a form of adaptation; however, considerably less is known about how environmental circumstances and adaptive behavior effect subsequent generations. Multigenerational effects have been observed in many species including C. elegans, mice, and humans. Therefore, understanding mechanisms behind these epigenetic programs may provide insight into basic principles of biology and inform a more comprehensive understanding of genetics as it relates to our interactions with the environment.

To explore these topics we employ a predator-prey system, utilizing Drosophila melanogaster and predatory parasitic wasps. Larval stages of the fruit fly are susceptible to several species of endoparasitoid wasps: The presence of these wasps triggers an ethanol seeking behavior in adult flies as a protective measure for their offspring. This behavior is governed by complex neural circuitry, linking inputs from the visual system to the learning center of the brain as well as the germline. We found that this ethanol seeking behavior is heritable through five generations and activation of this epigenetic program is linked to some of the same neurocircuits governing the ethanol seeking behavior itself. Inheritance of ethanol seeking behavior is not dependent on ethanol itself, and instead requires neuropeptide signaling, protease activity within the brain and activated caspases Dcp-1 and drice in the female germline. We also find important transcriptional changes and signal peptides in the activation and maintenance of the ethanol seeking behavior across generations.

527 Gut microbial diversity is important for the maintenance of learning behavior in Drosophila melanogaster. M. DeNieu, K. Mounts, M. Manier  The George Washington University, Washington, DC.

The microbiota-gut-brain axis describes the complex, two-way communication system through which microbes in the gut influence brain function and behavior. How communication between the gut microbiota and the central nervous system occurs is still poorly understood, so we are using Drosophila as a model to explore the link between microbes in the gut and learning. We first investigated the adaptive characteristics of individual microbes to learning behavior. To do this we isolated two strains of bacteria, one Acetobacter and one Lactobacillus, from our melanogaster stock. We then used these bacteria to create a panel of 5 microbiota treatments by exposing sterile, dechorionated eggs to bacteria for the following treatments: Acetobacter only, Lactobacillus only, and combined Lactobacillus and Acetobacter, in addition to axenic (germ free) and conventional (full microbiota) controls. These treatments were then tested for learning using the Aversive Phototaxis Suppression Assay in which a light source is paired with an aversive quinine stimulus in order to test the ability of flies to associate the positive light stimulus with the quinine. We performed additional taste assays to determine if disruption of the microbiota had any effect on the ability of the flies to sense the quinine. Next, we wanted to determine whether exposure to the bacteria during development was necessary for production of the behavioral patterns that we observed or if adult exposure was sufficient. To test this, we conventionalized adult flies reared in axenic conditions, and treated conventionally raised adult flies with antibiotics. We then retested learning behavior as described above along with axenic and conventional controls. None of the treatments differed in their ability to taste or their propensity to avoid quinine. However, we found that the axenic and both mono-association treatments showed learning deficits as compared to conventional flies, but that the combined treatment was sufficient to recapitulate normal learning behavior. We also found that bacterial exposure after larval development was not sufficient to recover learning behavior in axenically reared flies, but that continued exposure to bacteria in conventionally
reared flies was necessary to maintain learning. These results suggest that there is indeed a link between learning and the gut microbiota, and that not only is the community composition important but the timing of exposure is important as well.

528 Drosophila species learn dialects through communal living. B.Z. Kacsoh, J. Bozler, G. Bosco Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

In nature, many species are able to share information about their environment by communicating by means of auditory, visual, and olfactory cues. Perception of and communication of environmental threats can have immediate survival benefits and long-term evolutionary implications within and between species. Although a species may have evolved alarms specific to its own predators, other species may also benefit from alarms pertaining to a common predator. In Drosophila melanogaster, exposure to parasitoid wasps leads to a decline in oviposition (egg laying). Following wasp removal, wasp-exposed females communicate the threat of wasps to naive flies, which also depress egg laying through caspase activation. Using this fly-fly social communication paradigm we asked (1) whether social communication is conserved among other Drosophila species, (2) if Drosophilids engage in interspecies communication, and (3) which environmental and genetic factors are required for interspecies communication. We find that species across the genus Drosophila respond to wasps by oviposition reduction, activate cleaved caspase in oocytes, and communicate the presence of wasps to naive individuals. Communication within a species and between closely related species is efficient, while more distantly related species exhibit the ability to only partially communicate. When two species are only able to partially communicate, they can learn each other’s dialect after a period of cohabitation, yielding inter-species communication enhanced to levels normally observed among conspecifics. This cohabitation period requires exchange of visual and olfactory signals dependent on visual cues provided by wing movement, olfactory and ionotropic receptor presence, in addition to full spectrum light. This interspecies “dialect learning” requires neuronal cAMP signaling in the mushroom body, suggesting neuronal plasticity facilitates dialect learning and memory. We suggest that this study points to previously unappreciated functions of the Drosophila mushroom body in integrating information from multiple olfactory and visual inputs. Such cognitive plasticity that allows for dialect learning from many different species hints that adult behaviors could only emerge in a manner that is dependent on previous social experiences where relevant ecological pressures are ever present and multiple species co-exist in nature.

529 Walking down the Line: Fruit Flies’ Decision-making Behavior inside a Heat-Box. R. Sun1,2, E. Sereno1,2,6, J. Delly1,2, S. Wong1,2, Y. He1,2, R. Greenspan1,2,3 1) Division of Biological Sciences, UC San Diego, La Jolla, CA; 2) Kavli Institute for Brain and Mind, UC San Diego, La Jolla, CA; 3) Center for Brain Activity Mapping, UC San Diego, La Jolla, CA; 4) Genomic Analysis Laboratory, The Salk Institute for Biological Studies, La Jolla, CA; 5) Bioinformatics and Systems Biology Program, UC, San Diego, La Jolla, CA; 6) School of Speech, Language, and Hearing Sciences, San Diego State University, San Diego, CA.

Decision-making, behaviorally, is a process of choosing between at least two options. This process is fundamental to all multicellular organisms. Decision making can be modulated by many different factors and how these factors influenced one’s decision is still an open question. In humans, abnormal modulation of decision making is part of the etiology in neurological diseases such as depression, anxiety and Parkinson’s disease. Addressing this process holistically in humans, however, is still not possible, due to the extreme complexity of the human brain.

Fruit flies resemble human in many biological aspects. It offers an amenable test system for the mission of identifying genes, neural circuitry, and molecules capable of influencing behaviors such as learning, memory, and decision making. For example, the circadian rhythms and its molecular mechanisms discovered in fruit flies are found to be similar to that of humans (a seminal discovery warranted the 2017 Nobel Prize in Physiology and Medicine). Like humans, fruit flies make life and death decisions. The fly’s binary decision between walking and stopping presents an ideal accessible neuro-behavioral model for decision making.

Using a novel behavioral setup recently developed in our lab, Bob-Dylan box, (named after the Bob-Dylan’s Walking down the line song), we found that wild type fruit flies showed decreased tendency to initiate walking after being consistently heat-punished for walking. Such a decrease was not found in dopamine 1-like receptor 1 (DopR1) – deficient flies, while a greater decrease was seen in dopamine 1-like receptor 2 (DopR2) – deficient flies. This result indicated that dopamine modulates decision making patterns in a yin/yang fashion. Using a variety of genetics, molecular, behavioral and imaging techniques, we further identified that such initiation of walking behaviors involved both central complex and mushroom bodies substructures.

530 Neuronal fruitless and doublesex Expression Clusters in Brains across Drosophila Species. S. Ali, Sergey Nuzhdin Molecular & Computational Biology, University of Southern California, Los Angeles, CA.

The link between sex-specific expression patterns that lead to dimorphic phenotypes within and between species remains unclear. How do genes, regulatory networks, cells and tissues integrate the information necessary to generate various developmental outcomes? Drosophila melanogaster and Drosophila simulans provide an ideal system for understanding these large-scale processes on multiple biological levels. The sex-determination hierarchy is involved in several dimorphic phenotypes via the sex-specific alternative splicing of fruitless and doublesex. The sex-specific expression of these two transcription factors leads to the formation of sexually dimorphic interconnected neural circuits. The neurons that form
these fruitless and doublesex expression clusters show interspecies differences despite the conservation of the sex-determination hierarchy. This, along with evidence from other studies, suggests an alternative pathway controlling dimorphic neural patterning via upstream fruitless and doublesex regulators, independent of the sex-determination hierarchy pathway. By using the hybridization chain reaction, we will compare fruitless and doublesex-expressing neuron clusters between species and potential upstream regulators. These cell-specific, quantitative microscopy methods will broaden our understanding of the complex spatial and expression patterns that can lead to developmental differences.


Sperm competition occurs when sperm from multiple males compete for an opportunity to fertilize an egg. Male genotype, female genotype, and male x female interactions can all influence the sperm competition outcome. However, the nature of female contributions to the process is not well understood. A previous GWAS study of sperm competition outcomes across the DGRP lines identified 33 top-associated candidate female genes. We are functionally testing these candidates for their roles in sperm competition, including in different female tissues using UAS/GAL4-based RNAi knockdown. These experiments identified 10 genes that affected sperm competition outcomes when knocked down in females ubiquitously. Eight of the genes are expressed in the nervous system, and their pan-neuronal knockdown (and, in some cases their knockdown in sensory ppk neurons), altered sperm competition phenotypes. Furthermore, females having a knockdown of one of the genes in octopaminergic tdc2 neurons exhibited severe egg laying defects. These results suggest that the expression of these 8 genes in the female’s nervous system is important for her control of sperm competition dynamics. Future work is directed at determining how these genes function in the female’s nervous system to determine sperm competition outcome.

532 A neural mechanism that biases sperm storage according to the nutritional status of both sperm donor and acceptor in Drosophila melanogaster.  K-M. Lee, M. Yun, Y-J. Kim  Gwangju institute Science and Technology, Gwangju, KR.

To maximize reproductive fitness, some polyandrous females use preferentially sperm from the male that offers food items. It remains unknown how females evaluate the nuptial gift and bias sperm usage. Here, we show Drosophila melanogaster female in nutritional debt stores more sperm when her mating partner is well nourished than otherwise. The brain pars intercerebralis neurons that produce a neuropeptide diuretic hormone 44 (Dh44-PI) are essential for sperm storage. Dh44-PI also functions as a brain sugar sensor that regulates the post-ingestive feeding behavior. The secretory activity of Dh44-PI increases upon feeding or mating. The female with Dh44-PI lacking a trehalose transporter fails to alter sperm storage according to the nutritional status of her mating partner. In addition, the body sugar content in female increases significantly upon mating. Thus, we propose that the ejaculate-born sugars enter the female hemocoel and act on Dh44-PI to facilitate sperm storage. This study offers a mechanism by which females evaluate male ejaculate quality and bias sperm storage accordingly.


Female preference usually determines whether or not mating occurs within a species, and can serve as a barrier between species. We used a novel genetic mapping approach to identify three genes that affect female preferences underlying behavioral isolation between two species of Drosophila. For two of these genes, we have performed neural expression assays to identify which regions of the brain these genes are acting through to influence female preference. Lastly, we assessed which components of the male courtship ritual are being assessed through these pathways.

534 Elucidating the contribution of central brain histamine-mediated signaling in courtship behavior in Drosophila melanogaster.  A. Van Velsen*, T. Van Velsen*, M. G. Burg1,2 1) Biomedical Sciences, Grand Valley State University, Allendale, MI; 2) Cell & Molecular Biology, Grand Valley Sate University, Allendale, MI.

Histamine is a biogenic amine that has been shown to be necessary for a number of functions including vision, grooming, temperature preference, and sleep. Mutations in the Hdc gene, which disrupts histamine synthesis, have in the past been used to identify the effects of histamine deficiency on these types of behaviors. Histamine has been localized to peripheral sensory receptor cells (photoreceptor and mechanosensory receptor cells) and a small number of central brain neurons. A deletion in the 5’ noncoding region of the P[ghdc;w+] transgene was made (P[ghdcΔ32;w+]) that has been shown to disrupt Hdc expression in a subset of adult central brain neurons when placed in a HdcD0510 mutant background, determined through confocal microscopy. We have used this ghdcΔ32 transgene deletion mutation to determine whether histamine deficiency in the central brain could disrupt a complex behavior, such as courtship. Results indicate that a lack of histamine, caused by the HdcD0510 mutation, has a profound effect on the ability of flies to exhibit a normal courtship behavioral repertoire. Additionally, flies bearing the HdcΔ32 and HdcΔ278 mutant alleles were found to consistently exhibit a disrupted courtship behavior in
homotypic courtship experiments, indicating that the lack of histamine was the cause of the behavioral disruption. Homotypic courtship experiments with mutants in the HcIA receptor gene (ort\textsuperscript{PAS}) also revealed a similar disrupted courtship behavior. Results from these homotypic as well as heterotypic courtship assays (mixing sex genotypes) indicate that both male and females with only a CNS histamine deficiency appear to be disrupted in separate aspects of courtship. Thus, the disruption of histamine levels in certain CNS neurons appears to affect specific components of courtship, indicating that these histaminergic neurons are likely involved in regulating specific aspects of this behavior in both males and female flies. *contributed equally to work presented

535  **Discerning the sex peptide sperm-binding pathway.**  A. Vogel\textsuperscript{1,2}, A. Singh\textsuperscript{1}, G. Findlay\textsuperscript{2}, M. Wolfner\textsuperscript{1}  1) Dept. of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Dept. of Biology, College of the Holy Cross, Worcester, MA.

Post-mating behavioral responses in Drosophila melanogaster females can last for several days. Previous research showed that this response is mediated by the seminal fluid protein sex-peptide (SP), which is retained in females through its binding to sperm. Several seminal fluid proteins (Sfps) in the pathway that binds SP to sperm are known, but the pathway is, as yet, incomplete. Additional candidates have been selected through mass spectrometry and evolutionary rate covariance. Fifteen of these candidates are currently being tested through RNA-interference using UAS-Gal4 knockdowns to determine which are needed for extended egg laying and receptivity changes in females through mediating SP binding. As additional members of the network are identified, we will use Sfps known to be in the SP pathway to place the newly discovered proteins in order, and will determine their persistence in the female.

536  **Network and environmental regulation of circadian behavioral phase.**  S. Haase, X. Lu, A. Iyenger, B.C. Lear  University of Iowa, Iowa City, IA.

Endogenous circadian pacemakers allow animals to align daily behaviors to the external environment. Drosophila exhibit prominent peaks of locomotor activity at dawn and dusk, and these behaviors are known to be regulated by pacemaker neurons in the fly brain. A subset of Drosophila pacemaker neurons, the posterior dorsal neurons (DN1p), have been shown to promote activity during the morning hours, but have also recently been implicated as sleep-promoting neurons. We have assessed the effects of disrupting DN1p activity through RNAi knockdown of the narrow abdomen (na) ion channel or its regulators. Surprisingly, we find that such manipulations have relatively little effect on locomotor activity levels or profiles during light:dark entrainment conditions (LD). However, we observe major changes in behavioral phase during constant darkness (DD), including a prominent shift in the distribution of locomotor activity from evening to middle/early daytime hours. As wild-type DN1p neurons are thought to exhibit little activity during the evening hours, we have sought to determine how these neurons impact evening behavior. We find that tissue-specific rescue of NA channel expression in the DN1p group has only a modest effect on evening behavior in DD conditions. However, na rescue in the LNd circadian neurons, which have previously been implicated in the regulation of evening behavior, can promote either a discrete or broad evening peak in DD. Moreover, combining na rescue in the DN1p and LNd groups strongly sharpens and enhances DD evening peak. We propose that inhibition of the LNd by the DN1p during subjective daytime hours regulates the onset of evening activity, independent of any effects on the molecular circadian clock. We are currently using neuronal activity imaging methods to determine how LNd and DN1p activity patterns correspond to the behavioral profiles observed in na rescue flies. We are also further evaluating the impact of light on evening activity regulation. Our data thus far indicate that light input pathways have complex effects on behavioral phase.

537  **A sleep state in Drosophila larvae required for neural stem cell proliferation.**  M. Szuperak\textsuperscript{1}, M. Churgin\textsuperscript{2}, A. Borja\textsuperscript{1}, D. Raizen\textsuperscript{3}, C. Fang-Yen\textsuperscript{1,4}, M. Kayser\textsuperscript{1,4}  1) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 2) Department of Bioengineering, University of Pennsylvania, Philadelphia, PA; 3) Department of Neurology, University of Pennsylvania, Philadelphia, PA; 4) Department of Neuroscience, University of Pennsylvania, Philadelphia, PA.

Sleep during development is involved in refining brain circuitry, but a role for sleep in the earliest periods of nervous system elaboration, when neurons are first being born, has not been explored. Here we identify a sleep state in Drosophila larvae that coincides with a major wave of neurogenesis. Mechanisms controlling larval sleep are partially distinct from adult sleep: octopamine, the Drosophila analog of mammalian norepinephrine, is the major arousal neuromodulator in larvae, but dopamine is not required. Using real-time behavioral monitoring in a closed-loop sleep deprivation system, we find that sleep loss in larvae impairs cell division of neural progenitors. This work establishes a system uniquely suited for studying sleep during nascent periods, and demonstrates that sleep in early life regulates neural stem cell proliferation.

538  **Translational profiling of the head fat body yields insights into the function of the Drosophila adipose tissue clock.**  A.M. Yu\textsuperscript{1,2}, Y. Huang\textsuperscript{2}, F.R. Jackson\textsuperscript{2}  1) Department of Biology, University of Wisconsin - La Crosse, La Crosse, WI; 2) Department of Neuroscience, Tufts University School of Medicine, Boston, MA.

Circadian clocks govern the rhythmicity of a wide variety of physiological processes. The best characterized clocks are composed of transcription-translation negative feedback loops, and are thought to exert their effects by controlling daily
rhythms in gene expression. Clocks are expressed in a wide variety of tissues. The functions of neural clocks are well characterized, but the clocks in other cell types less so. It is reasonable to assume that clocks in specific cell types control tissue-specific programs of gene expression, and studies using dissected tissue support this notion. However, genome-level circadian profiling of cell type-specific gene expression has been hampered by the difficulty of isolating mRNA from specific cell types.

To circumvent this difficulty, our lab has adapted translating ribosome affinity purification (TRAP) to selectively isolate ribosome-associated mRNAs from cell types of interest. In TRAP, a GFP-tagged ribosome is selectively expressed in the target cell type, allowing immunoprecipitation of associated mRNA.

In this study, we used TRAP to profile circadian gene expression in cells of the fly head fat body. The fat body is a complex organ that shares functions with mammalian adipose tissue and liver and synthesizes many secreted proteins. While eye-specific transcripts were prominent in total head RNA, this enrichment was absent in fat body TRAP RNA, demonstrating specificity of the protocol. We identified 329 genes that cycle in head fat body TRAP RNA. A number of these genes have not been previously shown to cycle. Of these, expression of 78 was significantly enriched relative to total head RNA, indicating that these are fat body-specific cycling genes. Contrary to previous TRAP results profiling all clock cells of the head, cycling fat body TRAP RNAs were expressed in a continuum of phases. Genes of similar function, however, tended to be translated at similar times of the day, and potentially secreted proteins were translated at two distinct times of day. Studies are currently underway to confirm that these cycling genes are clock-controlled and to determine whether their cycling depends on the local fat body clock.

539  **Sleep deprivation suppresses aggression via the dorsal fan-shaped body.**  B. Mainwaring, M.S. Kayser  University of Pennsylvania, Philadelphia, PA.

Sleep deprivation impairs a wide range of essential processes such as cognition, alertness, and metabolism. Insufficient sleep has also been shown to influence emotional processing and aggression, though mechanisms linking sleep loss to changes in affective state are largely unknown. We previously found that acute sleep deprivation profoundly suppresses aggression in *Drosophila*, opening the possibility of using the fruit fly to study the neural basis coupling sleep and aggression. Here, we provide evidence that a sleep homeostat in the fly brain, the dorsal fan-shaped body (dFB), resides upstream of aggression centers: sleep rebound is attenuated with acute inhibition of dFB neurons following sleep deprivation, and the suppression of aggression is relieved. In addition, recent work indicates that activation of different classes of wake-promoting neurons does not necessarily induce subsequent recovery sleep, suggesting that the sleep homeostat is not always engaged following enforced wake. We have shown that suppression of aggression after mechanical sleep deprivation resolves following recovery sleep, and predicted that sleep loss without subsequent recovery sleep would lead to a long-lasting reduction in aggression. Consistent with this idea, activation of octopamine neurons to promote wake, which is not followed by recovery sleep, leads to a sustained impairment of aggression. Finally, we find that following sleep deprivation, acute activation of P1 neurons but not tachykinin neurons restores aggression to normal levels despite sleep loss. These results suggest that P1 is located downstream of sleep centers in the fly brain, and positioned to receive input from multiple modulators of behavioral state. Together, our findings further elucidate the mechanisms governing the interaction between two phylogenetically conserved behaviors, sleep and aggression, granting mechanistic insight into how sleep loss can impact behavior.

540  **Developmental gating of aggressive behaviors in Drosophila.**  E.H. Moscato, B. Mainwaring, M.S. Kayser  Department of Psychiatry, University of Pennsylvania, Philadelphia, PA.

A fundamental challenge in neuroscience is determining how complex behaviors arise from rapidly changing circuitry in the brain. Early in life, animals initiate a broad repertoire of innate behaviors essential for survival, such as feeding, sleeping, and aggression. Often considered 'hard-wired,' these behaviors can arise from immature underlying neural processes, show dramatic ontogenetic change, and be altered by experience, indicating that these circuits are highly dynamic and modifiable. Aberrant emergence of social behaviors such as aggression in early life is a hallmark of human neurodevelopmental disorders, emphasizing the importance in gaining a mechanistic understanding of behavioral ontogeny. We are using the model system *Drosophila* to dissect the neural, molecular, and genetic logic underpinning the gating of aggression in early life. We have found that newly eclosed male flies do not exhibit aggressive behaviors for the first 24 hours of adult life; this finding does not reflect a generalized inability to enact complex behaviors, as male flies begin courting females as early as 4 hours post eclosion. As flies mature, aggression becomes increasingly robust. Activation of some neurons known to promote aggression in mature adults, such as P1, likewise drives aggression in juvenile flies; others, such as tachykinin neurons, induce aggression in mature adults but not young flies. To discover novel regulators of aggression ontogeny, we performed a thermogenetic neural activation screen to identify neurons and circuits that can drive aggression in juvenile flies. We identified a population of neurons, YF1, that when activated strongly promote aggressive behaviors on the first day of adult life, when flies are normally non-aggressive. YF1-Gal4 is expressed in multiple neuronal populations; on-going work aims to identify the specific subset of neurons required for inducing juvenile aggression, and determine how these neurons interact
with previously characterized regulators of mature aggression. These studies will provide fundamental insights into the neurobiological mechanisms that regulate the maturation of an essential behavior.

541 The Molecular and Cellular Basis of Food Texture Sensation. Y. Zhang, J. Mack Monell Chemical Senses Center, Philadelphia, PA.

Food texture, the physical properties of food such as hardness and softness, plays a critical role in controlling food preference. Although food texture has enormous effects on feeding behavior, the molecular and cellular identities of mechanosensory receptors responsible for food texture sensation were unknown. Akin to mammals, we found that fruit fly, Drosophila melanogaster prefers food with a specific viscoelasticity. In Drosophila, the transmembrane channel-like (TMC) ortholog was required to discriminate food stiffness. tmc defined a previously unknown class of multidendritic neurons (md-L) in the primary taste organ, the labellum, which extended elaborate dendritic arbors to innervate the base of taste hairs. Deflecting taste hairs triggered prominent neural responses in the md-L neuron. The md-L neurons exhibited selectivity in response to mechanical forces applied to taste hairs in distinct directions. However, genetic ablation of tmc selectively abolished the mechanical responses in md-L neurons. We demonstrate that the single Drosophila transmembrane channel-like (TMC) is expressed in md-L neurons, where it is required for sensing food hardness and viscosity. We propose that md-L neurons are long-sought-after mechanoreceptor cells through which food mechanics are perceived and encoded by a taste organ, and this sensation depends on TMC.

542 Carbon dioxide inhibits Drosophila startle responses. G.E. Merrill1, J.M. VandenBrooks2, M.C. Quinlan3, G.B. Calo1 1) Department of Biomedical Sciences, College of Health Sciences, Midwestern University, Glendale, AZ; 2) Department of Pharmacology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ; 3) Department of Physiology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ.

Drosophila rely on their Giant Fiber System (GFS), a pair of large diameter neurons originating in the brain and synapsing on leg and wing muscles, for rapid signal transmission to escape threatening situations. The GFS startle response begins with a jump followed immediately by uncoordinated flight. Data from our laboratory shows a blunted startle response after exposure to CO₂. To measure this, we have developed a novel startle response assay comprised of a multibeam activity monitor that continuously records the position of individual flies in a vial. This monitor is mounted on top of a vortex to provide a two-second vortex event, which we hypothesize to trigger a startle response in the flies causing them to jump from their position and fall to the bottom of the vial. The multibeam monitor can record 16 individual flies simultaneously, allowing for a high-throughput startle response method. Prior to this assay, the flies are exposed to 100% CO₂ or air for 10 minutes. The air-exposed, Ore-R control flies show a very consistent response to the vortex event with 96% of all flies startling. However, when Ore-R flies are exposed to 100% CO₂ for 10 minutes and allowed to recover for one hour before the assay, only 43% of the flies startle, which is different from the control flies (P₂ exposure. When recording flight initiated by a puff of air in a standard startle response assay, we see similar patterns of behavior. Indeed, the CO₂-exposed flies, actively hold onto the surface and do not show a startle response.

OreR flies exposed to 100% N₂ for 10 minutes do not have a reduced startle response compared to air-exposed controls (73% vs. 96%, P=0.07). This indicates that CO₂ is disrupting the startle response through a CO₂-specific mechanism and not anoxia. We exposed Gr63Al mutants, which have a non-functional CO₂ receptor, to 100% CO₂ and found that they also do not have a reduced startle response (77% vs. 96%, P=0.29). This higher percentage of startle behavior in the CO₂ receptor mutants was significantly different from the 100% CO₂-exposed OreR flies (P₂-specific effect on the startle response is acting via the CO₂ receptor pathway.

This study provides more evidence against the use of CO₂ anesthesia prior to performing Drosophila behavior assays as it shows a detrimental effect on the involuntary reflex pathway that is the startle response.

543 The role of ovipositor bristles in tasting in Drosophila melanogaster. Julianne Pelaez, Kristin Scott, Noah Whiteman University of California, Berkeley, Berkeley, CA.

For insects with larvae that have limited mobility, female oviposition site selection has important ecological and evolutionary consequences because it determines resource availability to her offspring. While the role of chemoreceptors in the antennae, mouthparts, and legs in determining oviposition site preference has been well-studied in Drosophila melanogaster, it has been repeatedly speculated throughout the literature, that the female ovipositor may also be chemosensory and aid in egg-laying site selection. Here, we present evidence that D. melanogaster ovipositor bristles are chemosensory, and present candidate chemoreceptors that may be implicated in sensing chemical cues from the substrate. Using scanning electron microscopy (SEM), we discovered gustatory pores at the tips of the ovipositor bristles, through which chemical compounds can enter the bristle to reach the chemosensory neurons. Furthermore, we performed RNA sequencing on three pooled replicates of 300 ovipositors, and identified several specific chemoreceptors from the ovipositor tissue that may be involved in detecting key chemical cues to subsequently stimulate egg-laying. Finally, using chemoreceptor specific GAL4 driver lines, we confirmed the presence of these chemoreceptors in bristles lining the ovipositor.
Insects primarily use olfaction to target hosts, yet the functional contributions of individual and grouped components within the circuit remain poorly understood. Due to lack of this knowledge, contemporary odor coding models fail to reliably predict insect behavior. Recent evidence from our lab demonstrated that 5 of the 21 Olfactory Receptor Neurons (ORNs) of the *Drosophila melanogaster* larva contribute differently to its navigational behavior. We sought to determine the contributions of all 21 larval ORNs to behavior and ask whether the larval ORNs could be classified into distinct groups based on function. Our overall strategy was to measure the impact of individual ORNs on larval behavior by activating each of the 21 ORNs. We used chemical and optogenetic approaches to stimulate individual ORNs and a novel tracking assay to measure the corresponding larval behavior in the absence or presence of directional cues. We then used a number of clustering analyses to group ORNs based on their functional impact. ORNs were found to cluster into 4 distinct groups. Classification of ORNs into a small number of groups is significant in that it provides new insights into the functional relationship between olfaction and behavior. Optogenetic techniques allowed us to control for spatial as well as temporal aspects of ORN stimulation. We found that larval behavior was impacted not only by which ORN was stimulated but also by the temporal pattern of stimulation. Further, some ORNs impacted behavior in response to increase in light stimuli while some impacted behavior in response to decrease in light stimuli. Overall, this research provides direct experimental measures of neuronal diversity among an entire repertoire of an animal's primary olfactory epithelium, as well as provides new insights for incorporation of neuronal diversity into computational models. By doing so, we expect to significantly improve the predictive power of future odor coding models.

**545 Novel olfactory coding mechanisms in response to repellent odors.** *J.T. Clark*, Jadrian Ejercito, Ryan Arvidson, Anandasankar Ray Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA.

Odor-based repellents offer a powerful approach for preventing the spread of insect-borne diseases such as malaria, Zika, and dengue. Despite extensive research, the molecular and cellular mechanisms through which repellents such as DEET elicit aversion in insects are not fully understood and are controversial. We have now used sensitive electrophysiological and imaging analyses to study the responses to such repellents in the *Drosophila* olfactory system and confirmed in the mosquito. To do so, we first developed novel odor-delivery methods that better replicate stimuli that cause aversion. We uncover an unusual phenomenon not reported previously: DEET causes widespread activation across numerous olfactory neuron classes, X of Y tested so far. Imaging of calcium levels in cells indicates that calcium is mobilized from internal stores in response to the repellent. We also find a second class of repellents, having amine groups, responding with a short burst of action potentials followed by a prolonged silencing of neural activity. Experimentation with structural derivatives allowed us to identify physicochemical factors that correlate with the inhibition of neurons. The inhibition can be blocked in a dose-dependent manner by the co-application of odorants with physicochemical factors that counter it. The effects of the second class of repellents are consistent across multiple chemosensory neuron types, including ORNs and the GR-expressing CO2 neuron, suggesting a common physiological mechanism that is also conserved in other Diptera such as mosquitoes. Our results uncover novel mechanisms for aversion to common, naturally occurring compounds, and a new potential avenue for repellent discovery.

**546 The evolution of olfactory receptors coupled with transition to herbivory in Drosophilidae.** *T. Matsunaga*, M. Karageorgi, H. Suzuki, B. Goldman-Huertas, N. Whiteman 1) Integrative Biology, University of California Berkeley, Albany, CA; 2) Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ.

Insects detect odorants primarily using odorant receptors (Ors) housed in the dendritic membrane of olfactory sensory neurons (OSNs). Some Ors in insects are activated by several ligands, while the others are activated by only ecologically relevant unique odorants. This suggests that both broadly and narrowly tuned ORs work together. But how newly generated Ors remodel tuning and expression patterns in evolutionary time are largely unexplored. In the herbivorous drosophilid *Scaptomyza flava*, Or67b paralogs are triplicated and show signatures of positive selection, while in the other microbe-feeding *Scaptomyza*, only one copy of Or67b homolog lies in the genome. Homologs of Or67b in *Drosophila melanogaster* respond to several ligands, including the green-leaf volatile, (2)-3-hexenol, which suggests Or67b paralogs in *S. flava* are involved in detection of green leaf volatiles. We hypothesized that Or67b in S. flava remodeled its expression pattern in the antennae and tuning curve with the transition to herbivore. To analyze change of the expression pattern of Or67b paralogs in the antennae, we performed in situ hybridization both in leaf-feeding *S. flava* and in microbe-feeding species of *Scaptomyza*: *S. pallida* and *S. apicata*. Then, to test if *S. flava* shows stronger chemotaxis to green leaf volatiles more than *S. pallida* and *S. apicata*, we performed four-arm olfactometer assays. Additionally we explored candidate odorants that attract *S. flava*. We discuss the evolutionary and behavioral implications of the Or67b expansion in *S. flava* and the evolution of herbivory in general.
A major question in neuroscience is the circuit mechanisms underlying action selection. While many action selection tasks involve complex circuit interactions, relatively simple circuits are capable of producing meaningful action selection variations. By studying these simpler sensorimotor systems, we gain insight into how this class of decision making circuits function, while illuminating principles of decision making circuits generally. Escape behavior in the fruit fly, Drosophila.

Drosophila Gr64e mediates fatty acids sensing via phospholipase C pathway. Hyeon Kim¹, Haein Kim², Jae Young Kwon², Seok Jun Moon¹ 1) Department of Oral Biology, Yonsei University College of Dentistry, Seoul, KR; 2) Department of Biological Sciences, Sungkyunkwan University, Suwon, KR.

Animals use taste to sample and ingest essential nutrients for survival. Free fatty acids (FAs) are energy-rich nutrients that contribute to various cellular functions. Recent evidence suggests FAs are detected through the gustatory system to promote feeding. In Drosophila, phospholipase C (PLC) signaling in sweet-sensing cells is required for FA detection but other signaling molecules are unknown. Here, we show Gr64e is required for the behavioral and electrophysiological responses to FAs. GR64e and TRPA1 are interchangeable when they act downstream of PLC. TRPA1 can substitute for GR64e in FA but not glycerol sensing, and GR64e can substitute for TRPA1 in aristolochic acid but not N-methylmaleimide sensing. In contrast to its role in FA sensing, GR64e functions as a ligand-gated ion channel for glycerol detection. Our results identify a novel FA transduction molecule and reveal that Drosophila Grs can act via distinct molecular mechanisms depending on context.

m. melanogaster, provides an excellent model for examining action selection circuitry as D. melanogaster select between easily quantifiable motor programs in response to predation attempts and provide tractable neural complexity and a robust genetic toolkit that enable stimulation, silencing, and imaging of precisely targeted neural populations. In response to a single looming visual stimulus presentation, freely behaving flies respond with either a short or long duration takeoff escape sequence (‘short’ and ‘long’). Using a novel tethered assay, we examine the response of flies to repeated stimulus presentation, gaining insight into the changes in frequency and distribution of action selection over time as well as the individual behavioral bias of flies in contrast to their population distribution.

In this assay, a looming stimulus (r/v = 40ms) projected on a cylindrical screen was presented to the central visual field of tethered flies every 15 seconds for 20 trials. Behavioral responses were recorded at 1300 fps and behaviors were manually classified. We validated that the escape responses of tethered flies recapitulate the responses observed for freely behaving flies to identical stimuli in terms of absolute rate of takeoff escapes to initial stimulus presentations and the distribution between short and long duration responses. We also confirmed similar behavioral alteration when silencing key circuit elements required for takeoff escapes in freely behaving flies.

For repetitive stimulus presentations, fly takeoff rates decreased by 20 percentage points between the first 5 and final 5 trials (χ² test of homogeneity, n=572 trials, p < 1e-3). L1/L2 silencing abolished escape responses, while Giant Fiber silencing significantly reduced short mode escapes (χ² test of homogeneity, n=572 trials, p < 1e-3). Flies showed significant bias in escape type selection (Fischer’s exact test, p << 1e-7). Future work will utilize this assay for neurogenetic screens, where the tethered fly can be recovered for dissection. The presentation mechanism can also be easily adapted for more complex stimuli. In conclusion, our tethered, visually-evoked escape behavior assay provides a robust means of investigating action selection in a controlled and flexible visual environment.

551 The Role of the IgSF Protein Dpr11 in the Development of Neural Circuits for Nociception. M.R. Chin¹,²,³, W.D. Tracey¹,²,³ 1) Department of Biology, Indiana University Bloomington, Bloomington, IN; 2) Program in Neuroscience, Indiana University Bloomington, Bloomington, IN; 3) Gill Center for Biomolecular Sciences, Indiana University Bloomington, Bloomington, IN.

Nociception is the act of sensing and responding to noxious environmental stimuli. This ability is vital to escape injury and for general organismal survival. To better understand this phenomenon, we use Drosophila larvae as a model system to identify and characterize genes that have a role in nociceptive responses. Nociceptive responses are facilitated by neural circuits, which provide efficient pathways for first transmitting information from the environment to the organism, and then generating an appropriate response. Genes that have a role in building these circuits are good candidates for genes that specify behaviors. One candidate gene, defective in proboscis 11 (dpr11), shows enriched expression in class IV multidendritic arborization (cIVda) nociceptive neurons. dpr11 is part of a large immunoglobulin superfamily (IgSF) gene family that is widely expressed throughout the Drosophila nervous system. Dpr11 is known to have a role in synapse development in the developing Drosophila eye and larval neuromuscular junction, and this function is thought to be based upon a direct protein interaction across synapses with another IgSF gene, dpr-interacting protein-y (DIP-y). RNAi knockdown of dpr11 in cIVda neurons causes larvae to exhibit a severely impaired nociception phenotype. The goal of this project is to further characterize the role of dpr11 in cIVda neurons, with the hypothesis that Dpr11 is required for nociceptive behavior through its role in specifying synapse formation during neuronal development.

552 Investigation of the role of smoke alarm in sensory dendrite morphogenesis. K.H. Fisher, S Jeffirs, E Kumar, S.E. Mauthner, W.D. Tracey Gill Center for Biomolecular Sciences and Department of Biology, Indiana University, Bloomington, IN.

The detection and processing of sensory input is dependent on proper functioning of many sensory neurons in the peripheral nervous system. Drosophila dendritic arborization (da) sensory neurons extensively cover the larval body wall and are grouped into four distinct classes based on morphology. Class IV da neurons are the pain-sensing neurons, called nociceptors, which encode and process noxious stimuli. When presented with a noxious stimulus, larvae exhibit a highly stereotyped behavioral response, termed nocifensive escape locomotion. This behavior makes Drosophila a robust system for identifying genes important for nociception. In a genetic screen using tissue specific microarray analysis and thermal nociception assays, we identified genes enriched in nociceptive class IV neurons that are functionally important for thermal nociception. Of these genes, we identified a previously uncharacterized gene, which we named smoke alarm due to a hypersensitive behavioral response to noxious thermal stimuli upon tissue specific RNAi knockdown. Analysis of class IV neurons revealed hyperbranched dendrites and an increase in isoneural crossovers. Investigation of the expression pattern of smoke alarm revealed robust expression in class IV nociceptors, class I and class III sensory neurons, and chordotonal organs. Our research aims to understand the role of smoke alarm in dendrite morphogenesis of these sensory neurons.

553 The role of LNd clock cells in the regulation of circadian rhythms. N. Bulthuis, K. Spontak, B. Kleeman, D. Cavanaugh Department of Biology, Loyola University Chicago, Chicago, IL.

Most physiological processes exhibit daily oscillations under the control of an endogenous circadian clock, which allows animals to adapt to the 24-hr rhythms of light and temperature that result from the rotation of the Earth. The circadian
system consists of a central clock, input pathways responsible for transmitting environmental signals to the clock, and output pathways that connect the clock to behavioral rhythms. The central clock in Drosophila is comprised of ~150 clock neurons that each contains a cell-autonomous molecular clock. These clock neurons are divided into distinct subpopulations based on anatomical and functional properties. Much progress has been made in both identifying these populations and revealing the genetic components of the endogenous clock they contain. For example, among the clock neurons, the ventral lateral (LNv) neurons have been shown to be master oscillators that are necessary for the production of behavioral rhythms under constant environmental conditions. However, relatively little is known about the mechanisms by which circadian information is coordinated between clock neuron populations and translated into coherent behavioral outputs. In particular, it is unclear whether multiple clock cell populations transmit output information in parallel or whether they instead consolidate this information in a single population that serves as an output node of the clock. Here we test the role of non-LNv clock neurons in the production of rest:activity rhythms, assessing the contribution of the dorsolateral (LNd) clock neurons specifically. To accomplish this, we use restricted GAL4 lines to express a dominant negative construct that shuts off the molecular clock in certain cells and assay for effects on locomotor rhythmicity. We find that eliminating molecular clock function broadly in all non-LNv clock neurons strongly suppresses behavioral rhythms, even in the presence of functional LNv clocks. However, more restricted abrogation of clock cycling in subsets of non-LNv clock cells leaves rest:activity rhythms intact. This includes manipulations that, for the first time, selectively target all six LNd neurons. Our results indicate that LNv clock cells appear unable to drive rhythmic locomotor outputs in the absence of molecular cycling in other clock neurons. Our data further suggest that molecular clock function in LNd cells is not required for the production of coherent behavioral rhythms.

554 The circadian clock network constantly monitors environmental temperature to set sleep timing. Swathi Yadlapalli1, Pramod Reddy2, Edgar Meyhofer2, Orie Shafer3 1) Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI; 2) Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI.

Circadian rhythms are ~24-hour oscillations in behavior and physiology that are generated by endogenous clocks found in a majority of living organisms. The disruption of clocks has been linked to many human diseases, including diabetes, obesity, Alzheimer’s, and Parkinson’s. For effective maintenance of circadian rhythms, endogenous clocks must be entrained to external environmental cues such as light and temperature. Interestingly, circadian clocks display a remarkable combination of imperturbability and sensitivity with regard to temperature: the clock has a periodicity that is temperature-insensitive, while retaining the ability to synchronize to low-amplitude temperature cycles. While significant progress has been made in elucidating the role of light in circadian entrainment, the neural and molecular mechanisms by which temperature regulates the circadian clock remain poorly understood. Further, recent studies have also revealed a key role for both environmental temperature and body temperature rhythms, which are clock-controlled, in the duration and timing of sleep in many species, including humans. Therefore, it is critically important to understand how clocks process temperature changes, and in turn control sleep/wake behavior.

Here, we elucidate how the Drosophila circadian clock network processes changes in environmental temperature. Using in vivo calcium imaging techniques, we demonstrate that the DN1s, a discrete subset of clock neurons, constantly monitor changes in environmental temperature. We find that these neurons are acutely inhibited by heating and excited by cooling, an unexpected result given the strong correlation between temperature and light in the environment and the fact that light excites clock neurons. We demonstrate that the DN1s rely on peripheral thermoreceptors located in the chordotonal organs and aristae. Finally, we show that the DN1s and their thermosensory inputs are required for the normal timing of behavioral rhythms in the face of natural ramping temperature cycles. Our work reveals how a circadian clock modulates sleep and activity by constantly integrating temperature signals into its neural network. The duration and timing of sleep are closely correlated to both environmental temperature cycles and clock-controlled body temperature rhythms in many species. We therefore expect that the mechanisms reported here will be broadly relevant to animal sleep, including sleep in humans.

555 Transmitter synthesis and release machinery in glia influences Drosophila alcohol sedation. Kristen Lee1, Mike Grotewiel1,2 1) Neuroscience, Virginia Commonwealth University, Richmond, VA; 2) Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA.

Many studies in mammals have investigated the responses of central nervous system glia to alcohol administration. Few studies in any species, however, have explored whether glial cell function directly influences alcohol-related behaviors. We have begun to address this issue in Drosophila. Starting with genes known to be expressed in Drosophila glia, we performed a targeted screen in which we assessed the consequences of altered glial gene expression on alcohol sedation. One of the genes identified in this screen was tyrosine decarboxylase 2 (Tdc2). The Tdc2 gene product converts tyrosine to the transmitter tyramine in a well-established biochemical pathway, which also produces the transmitter octopamine. Tyrosine is also the precursor for the transmitter dopamine. Over-expression and RNAi-mediated knock-down of Tdc2 in glia during adulthood (via steroid inducible GliaGS) blunted and increased, respectively, alcohol sedation in flies. These data suggested that altered tyramine, octopamine or dopamine synthesis within adult glia might influence the initial sedative effects of alcohol. In follow-up studies, we found that expression of RNAi targeting tyramine β hydroxylase (TβH), which encodes the
enzyme that converts tyramine to octopamine) and tyrosine hydroxylase (TH, which encodes the enzyme responsible for converting tyrosine to L-Dopa, the rate limiting step in dopamine synthesis) in adult glia did not impact alcohol sedation. These results suggest that synthesis of tyramine, but not octopamine or dopamine, in adult glia regulates alcohol sedation sensitivity in flies. Interestingly, in subsequent studies we found that adult-specific expression of RNAi targeting the vesicular monoamine transporter (VMAT, which packages catecholamines into vesicles for release) in glia also increased alcohol sedation sensitivity. Additionally, we found that expression of RNAi targeting Tdc2 and VMAT specifically in CNS astrocytes, but not other CNS glial subtypes, increased alcohol sedation sensitivity. Taken together, our data support a model in which synthesis and vesicle-mediated release of tyramine in/from adult astrocytes dynamically regulates alcohol sedation in Drosophila.

556 Assessment and selection of Drosophila melanogaster directional orientation behavior. Kristin L. Latham-Scott, Taylor James, Eli Zachary, Spicie Davis, Stephanie Torrez, Natalie Wallace, Mariah McKechnie, Rachel Mendazona, María Franco Ramos, Tori Crumrine, Michael J. Baltzley Biology, Western Oregon Univ, Monmouth, OR.

Previous studies suggest that Drosophila melanogaster are able to orient using Earth-strength magnetic fields in the presence of UV light. However, the specifics of Drosophila magnetic-orientation ability are inconsistent across studies, and most insects that orient using Earth-strength magnetic fields appear to use UV-independent mechanisms. To investigate the underlying mechanisms of magnetoreception, we subjected a wild-caught population of Drosophila melanogaster to a sequential Y-choice maze with choice points to the magnetic north or south. We selectively bred flies for 15 generations to generate two strains that potentially expressed higher sensitivity to Earth's magnetic field. We analyzed generation zero (non-selected flies) for innate ability to orient directionally and generation 15 north- and south-selected flies to determine if we had selected for increased orientation ability. Using the same protocol, we selected and bred populations of positively- and negatively-phototaxic flies to distinguish any bias in the Y-maze. We demonstrate no significant difference between generation zero, north-selected, and south-selected flies, suggesting that adult male and female Drosophila melanogaster cannot orient using the Earth's magnetic field. We did find that light-selected flies have a significantly stronger preference for light than our dark-selected flies (p < 0.05), thus our ability to select for a trait under known genetic control was robust. This study adds to knowledge on magnetic detection and orientation shown in other insects and a wide variety of animals.

557 Complex visual processing during action selection in Drosophila melanogaster. HyoJong Jang1, David Goodman1, Brennan McFarland1, Linda Solomon2, Catherine von Reyn1,2 1) School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA; 2) Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA.

Action selection often requires sensory integration across both brain hemispheres. However, we know little about how neural circuits integrate bilateral sensory information to drive behavioral responses. Here, we take advantage of neurogenetic tools, accessible neural circuits, and easily quantifiable escape behaviors of the fruit fly Drosophila melanogaster to investigate bilateral sensory processing. In response to an approaching predator, perched D. melanogaster perform takeoff escapes regardless of the direction of the predator’s approach. Similarly, two sensorimotor neurons that drive escape behaviors, the giant fibers (GF), respond to ipsilateral, contralateral, and bilateral visual stimuli. Previous research identified visual projection neurons that convey ipsilateral angular velocity (LC4) and looming (LPLC2) information to the GF circuit. The neurons that provide visual information from the contralateral hemisphere, however, remain unknown. In D. melanogaster, GF dye couple giant commissural interneurons (GCI/AMMC-A1) that arborize in the antennal mechanosensory and motor center and receive mechanosensory information from the Johnston’s Organ neurons in antenna. By using a MultiColor FlpOut technique, we support that GCI dendrites arborize within the posterior ventrolateral protocerebrum (PVP) where ipsilateral LC4 axons terminate. Additionally, GCI axons project to the contralateral hemisphere and terminate in gorget and PVP regions adjacent to GF dendrites. GCI are therefore prime candidates for transmitting contralateral visual information to the GF circuit. Repeating previous dye fill experiments, we found GCI to be gap junction coupled to GF. To test functional connectivity, we used optogenetics to activate GCI while recording GF responses using whole-cell patch-clamp in tethered, behaving flies. GCI activation resulted in significant GF depolarizations as compared to control flies (t-test, p<D. melanogaster escape behavior as an ideal model to study how neural circuits are interconnected, how they integrate sensory input, and how they guide action selection in response to sensory stimuli.

558 RNA pseudouridylation sites in the Drosophila transcriptome. Wan Song1, Ram Podicheti2, Douglas Rusch2, Dan Tracey1,3 1) Biology, Indiana University Bloomington, Bloomington, IN; 2) Center for Genomics and Bioinformatics, Indiana University Bloomington, Bloomington, IN; 3) Linda and Jack Gill Chair of Neuroscience, Indiana University Bloomington, Bloomington, IN.

Pseudouridylation is the most common post-transcriptional RNA modification. Pseudouridine has been well studied as a modification found to rRNA, tRNA and snRNA and has recently been discovered in many mRNAs in mammals and in yeast. The isomerization of uridine to the C5-glycoside isomer pseudouridine (Psi) is catalyzed by six families of pseudouridine synthases which function either as a guide RNA directed ribonucleoprotein complex or as stand-alone proteins. Although
pseudouridine synthases appear to function ubiquitously, the *Drosophila* gene RluA-1 has been found to be specifically expressed in multiple dendritic (MD) neurons of the peripheral nervous system in embryos and in larvae. Thus, we have tested the role for this RNA modification enzyme in nociception pathways that are known to depend on md neurons. We found that either deletion of RluA-1 or targeted degradation of RluA-1 transcripts via RNAi in Class IV neurons results in hypersensitive thermal nociception phenotypes. This indicates that RluA-1 plays a role in negatively regulating the nociceptive response to noxious heat. This suggests that substrates of Drosophila RluA-1 may include as yet to be identified mRNAs functioning in the MD neurons. In order to identify potential targets for RluA-1 dependent pseudouridylation we have carried out an RNA-seq based approach (pseudo-Seq) that allowed us to detect Psi residues that are present in the *Drosophila* transcriptome. Our preliminary data indicate reproducible and potentially widespread patterns of Psi modification in the *Drosophila* transcriptome. These results on wild type and RluA1 mutant RNA will be presented.


Noxious stimuli result in avoidance and stress behaviors in organisms ranging from paramecia to humans. This avoidance behavior is often used as a readout of memory of the noxious stimuli. For almost half a century electric shock has been used to train Drosophila melanogaster with little understanding of how this noxious stimuli itself is represented in the brain of the fly. We do know a significant amount about how this noxious stimuli is integrated with other signals such as olfactory cues to remember and avoid different cues. However, little is known about the genes involved in shock detection itself or if electric shock overlaps with other types of nociception in higher brain regions. Previous work has demonstrated that the gene *NinaA* is downregulated following memory formation in dorsal-anterior-lateral (DAL) neurons that are key in learning and memory formation. Upon further investigation of *D. melanogaster* with decreased levels of *NinaA*, we found that these animals failed to avoid electric shock yet still maintained their jump response to the stimulus. *NinaA* is known to be important for trafficking *Rhodopsin 1* (*Rh1*) in the eye and *Rh1* is also involved in temperature preference, leading us to hypothesize that *NinaA* is acting through *Rh1*. With this hypothesis, we investigated the role of *NinaA* in general nociception by running a broad range of temperature avoidance assays, such as the global heat assay in larvae. Given that *Drosophila* larvae behaviors are well stereotyped, we used established heat assays to determine whether or not the lack of functional *NinaA* produced temperature deficits in larvae. In addition, we also compared global changes in gene expression in the brain to noxious heat and electric shock in wildtype as well as *NinaA* mutants. Interestingly, preliminary data suggests significant overlap in higher order processing of both noxious thermal perception and electric shock perception. Overall, this work investigates the connection as well as the autonomy of the nociception pathways for non-ethologically relevant stimuli, such as shock, and naturally significant stimuli, like temperature.

560 IRE1α inhibition suppresses sleep in *Drosophila*.  S. Ly¹, E. Strus¹, S. Berlas², J.A. Williams², N Naidoo¹  1) Center for Sleep and Circadian Neurobiology, University of Pennsylvania, Philadelphia, PA; 2) Department of Neuroscience, University of Pennsylvania, Philadelphia, PA.

The Unfolded Protein Response (UPR) is a cellular process that regulates protein homeostasis in response to endoplasmic reticulum (ER) stress. UPR activation occurs when misfolded proteins accumulate in the ER and leads to the downregulation of protein synthesis, upregulation of molecular chaperones, and increased protein degradation. In the following study, we examined the role of the UPR sensor protein inositol-requiring enzyme 1 (IRE1) in regulating sleep and wake behavior. Using pharmacological and genetic approaches in *Drosophila* we investigated the effect of IRE1 inhibition on behavioral state. Drug administration of the IRE1α inhibitor STF083010 significantly reduced sleep time in wildtype *Drosophila* compared to vehicle controls. We also utilized the *Drosophila* GAL4/UAS GeneSwitch system to transgenically express IRE1α RNAi in adult neurons in the *Drosophila* brain. RNAi knockdown of IRE1α mimicked the behavioral effect observed following drug treatment; flies expressing IRE1α RNAi in neurons displayed significantly reduced amounts of total sleep. We further confirmed that IRE1α inhibition reduces the levels of xbp1 splicing in *Drosophila* heads. In ongoing experiments, we are evaluating effects of IRE1α inhibition on stress-induced sleep and survival. The preliminary results suggest that the UPR protein IRE1α promotes sleep. It is possible that during wake, upregulated protein synthesis in the brain leads to a gradual increase in UPR activation that may contribute to a sleep-promoting signal. Both dysregulated sleep and sustained UPR activation have been implicated in the pathophysiology of numerous neurodegenerative diseases. Thus, there is strong incentive to understand the mechanisms underlying UPR-mediated sleep regulation.

561 Evolutionarily conserved “genetic toolkit” drives animal sociality.  I.M. Chin, C. Vernier, Y. Ben-Shahar  Department of Biology, Washington University in St. Louis, St. Louis, MO.

The prevalence of analogous social traits across the animal kingdom suggests the existence of a conserved “genetic toolkit” that drives the genetic networks supporting interactions between animals and their social environments. The goal of this project is to identify specific genes that play key, conserved roles in driving social behaviors across long evolutionary distances, from humans to flies. Specifically, we are investigating the potential contributions of several candidate *Drosophila melanogaster* genes that are homologous to loci implicated in Williams-Beuren Syndrome (WBS), a human developmental
disorder caused by a hemizygous deletion of ~28 genes on Ch. 7. WBS-affected individuals are stereotypically hyper-social, indicating that WBS gene dosage plays a role in human sociability. Remarkably, the majority of WBS genes are conserved in the fly genome. We hypothesized that some of these WBS-related genes contribute to the regulation of social traits in the fly. To test our hypothesis, we used an in vivo neuronal RNAi screen for the impact of gene knockdowns on adult behavior by using a “social-space displacement” paradigm. These studies demonstrated that the knockdown of at least two *Drosophila* WBS homologs, *fz* and *elF4H1*, have a significant impact on sociability in flies. The data suggest that at least some of the WBS-related genes play a conserved role in the development and/or regulation of the neural circuits underlying sociality across mammals and insects.

562 A unique switch in thermal preference in *Drosophila* larvae depends on rhodopsins/lipases/TRPA1 signal pathway. T. Sokabe¹, C. Montelli² 1) Division of Cell Signaling, National Institute for Physiological Sciences, Okazaki, Aichi, JP; 2) Neuroscience Research Institute, University of California, Santa Barbara, CA.

All organisms seek better temperature environment at every stage of their lifetime and many temperature-sensitive TRP channels have been identified as a primary internal thermometer. We found that *Drosophila* larvae displayed a dynamic shift in their temperature preference within a comfortable range during development, where no thermosensitive TRP channels are activated. In a thermal gradient condition ranging from 18°C to 28°C, early 3rd instar larvae [72 h after egg laying (AEL)] distributed in the comfortable range (18-24°C) with a mild peak at 24°C. They started to migrate to lower temperatures in the following 48 hours, and the majority of late 3rd instar larvae (120 h AEL) accumulated in an 18°C zone, which was right before a wandering stage.

We identified two rhodopsins, *rh5* and *rh6*, which play a major role in this thermal preference switch together with *trpA1* channels. Rhodopsins are dominantly expressed in light-sensitive Bolwig organ, but Bolwig organ was dispensable for the thermal preference. Instead, *rh5*/*6* expressed in thermo-sensitive, *trpA1*-positive neurons appeared to be important. *trpA1*-A/B and C/D isoforms were expressed together with *rh5*/*6* in a subset of neurons in the Brain and peripheral md neurons, respectively. *rh5*/*6* couple to *Gqa* and *plc*, and all of them were required for the switch in thermal preference. Furthermore, we also found functional involvement of lipases including *inaE*, a DAG lipase that catalyzes diacylglycerol to generate monoacylglycerol and fatty acid.

We concluded that *rh5*/*6*/*Gqa*/PLC/DAG lipase/TRPA1 cascade is essential to develop the shift in thermal preference in larvae. TRPA1 plays a critical role in sensing temperature higher than 25°C by its direct activation, whereas the upstream cascade from *Rh5*/*6* to lipases may convey temperature information in the comfortable range (18-24°C) to regulate TRPA1. Multiple opsins have been reported to participate in sperm thermotaxis in mammals, suggesting that this unconventional role of rhodopsins in thermotaxis may be evolutionally conserved.


MicroRNAs are implicated in diverse brain functions, including development, cognition, and synaptic plasticity. These powerful regulators are small noncoding RNAs that can bind specific recognition motifs in multiple target mRNAs and silence their expression through post-transcriptional mechanisms, such as translational repression or transcript destabilization, enabling them to serve as master regulators of transcriptional networks. Expression-profiling studies indicate that alterations in miRNAs occur in the brains of Alzheimer's Disease (AD) patients, but the functional implications of these changes remain unclear.

Our research has shown that a specific microRNA, miR-219, is downregulated in AD and Primary Age Related Tauopathy (PART). In a transgenic *Drosophila* model of tau induced neurodegeneration, we have observed that miR-219 reduces tau toxicity, suggesting that miR-219 is part of a vital regulatory mechanism that prevents the deposition of tau. Moreover, we are currently investigating the role of miR-219 in the regulation of circadian rhythm and learning, which disruption is associated with impaired cognition and AD. We have observed aberrant activity in miR-219 knockout flies during normal L/D cycles, which might involve defective core clock operation, altered clock output, or perhaps aberrant environmental perception and that we are investigating. These results implicate miR-219 as a potential target for the treatment or prevention of AD and other tauopathies. Therefore, these studies are starting to unravel mechanistic roles for microRNAs in neuronal tau physiology and pathology.

564 Effect of the human cathelicidin antimicrobial peptide, LL-37, on Aβ42 neurotoxicity in a *Drosophila* model of Alzheimer's disease. Brandy Baird¹, Roger Huynh¹, Kaylee Marrow¹, Tari Kurman¹, Joselin Perez¹, Annelise Barron², Jeremy Lee¹ 1) Department of Molecular, Cell, & Developmental Biology, University of California, Santa Cruz, CA 95064; 2) Stanford University, School of Medicine, Department of Bioengineering, 443 Via Ortega, Stanford, CA 94305.

Alzheimer's disease (AD) is a chronic neurodegenerative disease and the leading cause of dementia worldwide. AD is characterized by the presence of senile plaques, predominantly composed of the Alzheimer's disease-related protein, amyloid-beta (Aβ42). Though AD was first discovered in 1906, the mechanism of disease is still poorly understood. Recent
evidence links AD to innate immunity, suggesting Aβ42 functions as an antimicrobial peptide (AMP). Furthermore, evidence suggests that other antimicrobial peptides interact with Aβ42. Identifying possible binding partners of Aβ42 as a result of an immune response could be helpful in enhancing our understanding of the disease and identifying potential targets for preventative or therapeutic intervention.

Oligomerization is an important mechanism in the antimicrobial activity of some innate immune peptides including the human cathelicidin peptide, LL-37, which has some similar biophysical characteristics to Aβ. Recent in vitro studies have shown that LL-37 inhibits normal Aβ42 fibril formation (De Lorenzi, E. et al., 2017). Since Aβ42 aggregates are neurotoxic, the observed effects of LL-37 on Aβ42 fibril formation suggest that LL-37 could affect Aβ42’s neurotoxicity.

To investigate whether LL-37 interaction with Aβ42 affects Aβ42’s toxicity in vivo, we generated Drosophila that express and secrete both Aβ42 and human LL-37 peptides in the CNS, using the elav-Gal4 driver. LL-37 has no analogues in Drosophila. In a longevity assay, we compared these co-expressing flies to flies that express each peptide independently as well as non-expressing controls. Longevity data showed there was a slight but significant amelioration of the deleterious effects of Aβ42 when LL-37 was co-expressed. Interestingly, despite its ability to partial rescue the effects of Aβ42 expression, LL-37 expression alone had a small but significant negative impact on longevity, as compared to controls. It has been reported by others that LL-37, at elevated levels, can have cytotoxic effects on mammalian cells.

Additional experiments will be conducted to elucidate the mechanism of this partial rescue; i.e. whether it is the result of a direct interaction of Aβ42 and LL-37 in vivo. We will also conduct behavioral assays to determine whether LL-37 ameliorates the behavioral abnormalities caused by Aβ42 expression.


Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons. Patients gradually lose control of muscle movement as the motor neurons deteriorate, which leads to the atrophy of muscles. ALS also correlates with defects in metabolism as patients display weight loss, hyper metabolism, and hyperlipidemia. TAR DNA binding protein (TDP-43) is a key player in ALS as it is found within cellular aggregates in 97 percent of ALS patients. Our lab has previously developed a Drosophila model of ALS expressing human TDP-43 that recapitulates multiple aspects of the disease. To investigate the relationship between ALS and cellular metabolism, we overexpressed the neuronal human glucose transporter 3 (GLUT3) and fed the flies a high sugar diet. An increase in glucose availability improved locomotor dysfunction caused by the expression of TDP-43 in the central nervous system. To test whether increased glucose availability improves defective neuromuscular connections in our model of ALS, we analyzed the neuromuscular junction (NMJ) of the flies that overexpressed GLUT3 in the context of TDP-43. Our data show that overexpressing glucose transporters mitigates TDP-43 dependent phenotypes and results in a greater number of boutons per muscle area. This suggests that increased glucose availability improves locomotor dysfunction and mitigates the NMJ architecture in a Drosophila model of ALS.

566  Identification of common biological pathways that protect against neurodegeneration associated with Amyotrophic Lateral Sclerosis models in D. melanogaster. Mathieu Bartoletti, Kit Mocarski, Kristi Wharton Brown University, Providence, RI.

ALS is a progressive neurodegenerative disease whose pathogenesis is still not understood. ALS symptoms involve a disruption in the function of both upper and lower motor neurons leading to a loss of voluntary movement and a loss in diaphragm function, which is the primary cause of death. ALS causing mutations have been identified in a number of genes and while the symptoms and progression of the disease are very similar, these genes act in very different cellular pathways. TARDBP and FUS both encode RNA-binding proteins, SOD1 encodes a superoxide dismutase known to be involved in the protection against ROS release during tissue damage, and C9orf72 encodes a protein involved in trafficking and autophagy. The overexpression of human genes harboring ALS mutations (TDP43[M337V], FUS[R521C]) and an expanded hexanucleotide repeat[30xG4C2] found in the C9orf72 gene in the eye (GMR-Gal4) has been shown to result in eye degeneration, reflecting degeneration of the photoreceptors. A screen using known transposon insertions identified mutations for their ability to suppress the ALS-related eye degeneration in each model. We next tested lines that suppress all three overexpression models for their ability to alleviate phenotypes associated with a knock-in Sod1[G85R] model. The Sod1[G85R] related phenotypes we assayed included defective locomotion at two stages of Drosophila development, as well as shortened adult lifespan. We identified 31 P-element insertions that modified Sod1[G85R] phenotypes. The affected genes fall into a handful of functional categories including metabolism, stress response, cell polarity, mitosis and chromatin regulation. Further genetic analysis indicates that two modifiers, one affecting stress response and the other metabolism, exhibit a genetic interaction that enhances their ability to rescue the neurodegeneration phenotypes caused by some but not all four ALS models. Moreover, this analysis allowed us to separate the different ALS models into two categories: 1) ALS models where modifiers of stress response and metabolism interact to alleviate neurodegeneration-related phenotypes, and 2) ALS models where
Thus, Troponin excess elicits transcriptional upregulation of TnI, Par3/Bazooka and Dlg, but not for that of aPKC or Scribbled. In neuroblasts, the polar localization of Miranda requires proliferation and antagonizes the overgrowth due to these oncogenic mutations. TnI is required for the growth of Drosophila TNNI1, which encoding Troponin I.

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Amyotrophic Lateral Sclerosis (ALS) is a fatal disease that causes progressive neurodegeneration of motor neurons. TAR DNA Binding Protein (TDP-43) has been implicated in the progression of ALS, as well as at the level of pathology. TDP-43 is an RNA-binding protein that is known to regulate many steps of RNA processing. However, little is known of TDP-43's role in the dysregulation of translation. Several eukaryotic initiation factors (eIFs) have been identified in TDP-43-positive stress granules. Here we show that changing expression levels of several eukaryotic initiation factors (eIFs) to reduce translation is neuroprotective in a Drosophila model of ALS. Specifically, when various eIFs are co-ordinated in the context of human TDP-43 in motor neurons of Drosophila, locomotor deficits and retinal neurodegeneration are suppressed. Results from quantitative PCR demonstrate that TDP-43 alters levels of eIF mRNA transcripts in motor neurons. Furthermore, results from Western blotting suggest that eIFs affect the level of TDP-43 protein found in whole larvae. We will further explore the interaction between TDP-43 and these eIFs in patient-derived lymphoblastoid cells. As we identify specific translational mechanisms that are dysregulated by the presence of cytoplasmic TDP-43, new targets will emerge for the development of novel therapies for ALS.


Epigenetic gene control mechanism that utilize histone acetylation are crucial for neurogenesis and long term neuronal health. Misregulation of epigenetic molecules, including histone acetyltransferase (HAT) Tip60, have been implicated in Alzheimer’s Disease (AD). In the AD fly model there is a decrease in Tip60 and an increase HDAC1/2 (Rpd3) that leads to transcriptional repression of synaptic plasticity genes and a subsequent decrease in learning and memory function. Increasing Tip60 in the AD fly brain restores Tip60 and HDAC1/2 (Rpd3) balance that relieves synaptic gene repression and restores learning and memory ability. Although different diagnosis, neurodegenerative diseases have similar hallmarks including decreased cognitive ability. While familiar forms of these diseases are distinct, the more common sporadic forms may endure similar disease progression mechanism. Here, we elucidate the role of the HAT Tip60, previously characterized to decrease HDAC binding and increase neuroplasticity gene expression profiles in AD and improve cognitive function in the AD fly, in both Huntington's Disease (HD) and Parkinson's Disease (PD). Using qPCR, we demonstrate that in the HD and PD fly model, there is a decrease in Tip60 expression and misregulation of a set of Tip60 target synaptic plasticity genes. Using a larval cognitive function assay, we demonstrate that in the HD and PD fly, there is an early learning and memory deficit that is partially rescued with increased Tip60 levels. Our data suggest that targeting of HAT Tip60 for the treatment of neurodegenerative disease can be applied not only to AD, but to other diseases including HD and PD.

Active wingless vampirization by glioblastoma network leads to brain tumor growth and neurodegeneration. M Portela, I Venkataramani23, M Fahey-Lozano1, F Winkler12, F Casas-Tinto1 1) Cajal Institute, Madrid, Madrid, ES; 2) Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg, INF 400, 69120 Heidelberg, Germany; 3) Clinical Cooperation Unit Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany; 4) Institute for Anatomy and Cell Biology, Heidelberg University, 69120 Heidelberg, Germany.

Human tumors of various tissue origins show an intriguing over-expression of genes not considered oncogenes, such as that encoding Troponin-I (Tnl), a well-known muscle protein. Out of the three Tnl genes known in humans, the slow form, TNNI1, is affected the most. Drosophila has only one Tnl gene, wupA. Here, we studied excess- and loss-of-function of wupA in Drosophila, and assayed TNNI1 down regulation in human tumors growing in mice. Drosophila Tnl excess-of-function increases proliferation and potentiates oncogenic mutations in Ras, Notch and Lgl genes. By contrast, Tnl loss-of-function reduces proliferation and antagonizes the overgrowth due to these oncogenic mutations. Tnl is required for the polar localization of Par3/Bazooka and Dlg, but not for that of aPKC or Scribbled. In neuroblasts, the polar localization of Miranda requires Tnl. Troponin-I defective cells undergo Flower- and Sparc-dependent cell competition. Tnl can localize to the nucleus and its excess elicits transcriptional up-regulation of InR, Rap1 and Dilp8, which is consistent with the increased cell proliferation. Thus, Troponin-I reveals a novel function in cell proliferation of interest in certain types of cancer.
Multi-level misregulation of Parkin and PINK1 revealed in Drosophila and cell models of TDP-43 proteinopathies. Xing Sun12, Yongjia Duan12, Caixia Qin1, Jiangxia Ni12, Kuili Tian1, Yuanping Deng1, Ang Li1, Yanshan Fang1 1) Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China; 2) University of Chinese Academy of Sciences, Beijing, China; 3) Guangdong-Hong Kong-Macau Institute of CNS Regeneration, Guangdong Medical Key Laboratory of Brain Function and Diseases, Jinan University, Guangzhou, China.

Background: Transactivative response DNA-binding protein 43 (TDP-43) is a predominantly nuclear RNA-binding protein that forms ubiquitinated inclusion bodies in a spectrum of neurodegenerative diseases, especially amyotrophic lateral sclerosis (ALS). However, molecular mechanisms underlying TDP-43 proteinopathies remain elusive. The E3 ubiquitin ligase Parkin and the PTEN-induced putative kinase 1 (PINK1) are important mitochondrial quality control proteins associated with Parkinson's disease (PD). Moreover, recent studies have shown that Parkin and PINK1 are genetic modifiers of FUS-induced neurodegeneration and that Parkin may ubiquitinate TDP-43, suggesting that Parkin-PINK1 may also be a common pathway involved in ALS pathogenesis.

Methods: Using transgenic flies that express human TDP-43 (hTDP-43) in the adult Drosophila neurons, we investigated whether Parkin and PINK1 modified TDP-43-induced degenerative behavioral phenotypes. Next, in the fly head, primary mouse neurons and human cells overexpressing hTDP-43, we examined the mRNA levels, protein abundance, subcellular localization, and solubility of Parkin and PINK1 by quantitative RT-PCR, Western blot, immunocytochemistry and biochemical fractionation. Finally, we evaluated the proteasome function of the cells by an in vitro proteasomal activity assay.

Results and Discussion: Surprisingly, we found that although Parkin or PINK1 both modified the degenerative phenotypes of the TDP-43 flies, they went in the opposite directions – upregulation of Parkin but downregulation of PINK1 suppressed TDP-43-induced neurodegeneration, whereas upregulation of PINK1 enhanced the neurotoxicity. We further revealed that the differential effects of Parkin and PINK1 were because excessive TDP-43 reduced the mRNA and protein abundance of Parkin but promoted cytosolic accumulation of cleaved PINK1 protein. Interestingly, in addition to the previously reported regulation of Parkin pre-mRNA by binding to its long introns, we found that TDP-43 also regulated the mRNA abundance of Parkin by an unidentified, intron-independent mechanism. Finally, we showed that excessive TDP-43 moderately impaired the proteasomal activity, which was sufficient to alter the turnover and the subcellular localization of PINK1 but not Parkin.

Conclusions: Together, our data from the Drosophila and cell models have demonstrated that Parkin and PINK1 are misregulated differentially at RNA and protein levels in TDP-43 proteinopathies, which may contribute to the pathogenesis of ALS and related diseases.

Testing the Factors Affecting Ethanol Sedation and Tolerance of Drosophila melanogaster. Moshan Guo, Alex Heisler, S. Tariq Ahmad Biology, Colby College, Waterville, ME.

Repeated consumption of ethanol causes alcohol dependency and tolerance in humans. Drosophila are affected by alcohol in a similar manner to humans, developing both ethanol sedation and a tolerance response over time. Variations in responses are linked to mating status, gender, and Drosophila genotype. The purpose of this study is to test the ethanol sedation and tolerance of flies of two wild-type strains: w1118 and Canton S. 1 and 2 week-old flies were separated by gender and mating status (mated or virgin). Flies are sedated with 500 μL of 100% ethanol and the time it takes for 50% of the flies to be sedated (ST50) is measured. The same experiment is performed on the same set of flies 1 day and 7 days after initial ethanol sedation to measure the development of tolerance. Based on the preliminary ST50 data, mated flies of both genders generally have more initial tolerance to ethanol exposure than virgin flies of both genders. Male and female flies from both strains also show an increased tolerance to ethanol both 1 day and 7 days after initial sedation. This study will further knowledge of factors that affect ethanol-induced behavior, as well as investigating the length of time tolerance can continue to affect ethanol response.

The Effect of a Hyperglycemic Diet on Alzheimer's Disease in Drosophila melanogaster. R.L. Hawkins, A.N. Fuhrman, B.E. Paddock Biology Department, Arcadia University, Glenside, PA.

Alzheimer’s Disease (AD) and Diabetes are progressive, age-related diseases that afflict 5.3 million and 29.1 million Americans, respectively. AD is a multifactorial neurodegenerative disease characterized by abnormal protein aggregates, loss of synaptic function, and decrease in memory function, all worsening over time. Though many genetic and environmental factors are associated with the development and progression of AD, the hallmark glucose and insulin dysregulation of diabetes mellitus type 2 (T2DM) has been demonstrated to increase the risk of AD. To test the role of high circulating sugar levels in amyloid-associated dysfunction, a Drosophila model of AD co-expressing hAPP and hBACE was reared on a 1M sucrose diet. This high sugar diet, which has previously been demonstrated to result in T2DM-like dysregulation of insulin sensitivity in Drosophila, resulted in alterations in behavior, as measured by an increase in reorientation behaviors.
Furthermore, we demonstrate that co-expression of hAPP and hBACE within the nervous system of Drosophila alters in vivo glucose regulation. These findings support the hypothesis that a positive feedback relationship between amyloid production and hyperglycemia.

573 **highroad** is a *Drosophila* carboxypeptidase induced by retinoids and clears mutant Rhodopsin-1 in *Drosophila* Retinitis Pigmentosa models.  
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The light detecting protein, Rhodopsin, requires retinoid chromophores for their function. In vertebrates, retinoids also serve as signaling molecules, but whether these molecules similarly regulate gene expression in *Drosophila* remains unclear. Here, we report the identification of a retinoid-inducible gene in *Drosophila*, **highroad**, which is required for photoreceptors to clear folding-defective mutant Rhodopsin-1 proteins. Specifically, we identified **highroad** through an in vivo RNAi based genetic interference screen with one such folding defective Rhodopsin-1 mutant, *ninaE*G69D. CRISPR-Cas9-mediated deletion of **highroad** results in the stabilization of folding-defective mutant Rhodopsin-1 proteins, and acceleration of the age-related retinal degeneration phenotype of *ninaE*G69D mutants. Elevated **highroad** transcript levels are detected in *ninaE*G69D flies, and interestingly, deprivation of retinoids in the fly diet blocks this effect. Consistently, mutations in the retinoid transporter *santa maria* impairs the induction of **highroad** in *ninaE*G69D flies. In cultured S2 cells, **highroad** expression is induced by retinoic acid treatment. These results indicate that cellular quality control mechanisms against misfolded Rhodopsin-1 involve regulation of gene expression by retinoids.

574 **Synthetic Lethal Interactions Associated with Polyglutamine disease and Amyotrophic Lateral Sclerosis.**  
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Neurodegenerative disorders such as Polyglutamine (polyQ) diseases and Amyotrophic Lateral Sclerosis (ALS) lead to progressive loss of neurons that affect motor function. Mechanisms of pathogenesis for these diseases are complex and have been areas of considerable study. One approach focuses on identifying genetic factors that contribute to disease pathogenesis through genetic screens in model organisms and genome wide association studies. However, it remains largely elusive of how neuronal cell death is induced. In addition, genetic variants may act only in combinations. We used a synthetic lethal screen in *Drosophila melanogaster* to find strong genetic modifiers that interact with polyQ repeats and hexanucleotide repeats associated with a subtype of ALS. Synthetic lethality represents essential interactions where co-expression of two mutated genes results in lethality, unlike their single mutations that are viable. We report four *Drosophila* alleles that are synthetic lethal when co-expressed with polyQ repeats and two alleles when co-expressed with hexanucleotide repeats associated with ALS using the GMR-Gal4/UAS gene expression system. We have further identified most of these overexpression alleles to be transcription factors. Our data suggest that transcriptional deregulation strongly enhances neurodegeneration caused by expressing the toxic polyQ repeats and the ALS-associated hexanucleotide repeats, resulting in neuronal cell death.

575 **Identifying physiologically relevant targets of tdp-43 translational inhibition.**  
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The mRNA binding protein TDP-43 forms cytoplasmic inclusions as part of the pathogenesis of amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder affecting motor function and survival. A potential causative mechanism for the ALS phenotype is inhibition of key metabolic enzymes via translational repression [1]. The objective of the project was to identify physiologically relevant targets of TDP43 translational inhibition. We overexpressed TDP-43, either wild type or a mutant variant, in *Drosophila* motor neurons [2] then performed immunoprecipitation experiments to detect mRNAs enriched in TDP-43 complexes. Tagged Ribosome Affinity Purification, transcriptomics, and metabolomics were also employed to understand the molecular and metabolic changes caused by TDP-43 overexpression in *Drosophila* motor neurons. Several mRNA candidates linked to synaptic function and metabolism were identified as potential primary targets due to their high association with TDP43 (log fold change>2 and pad).

References:

Acknowledgements:
Ernesto Manzo, Josh Paree, Alyssa Coyne, Mathew Scadura, and Ben Zaepfel. Funding was provided by NIH NS091299, MDA RG “Metabolic Dysregulation in ALS” and Sandra Harsha Estate.
576 Specific depletion of Dipeptide Repeat Proteins in a fly model of C9ORF72 mediated ALS/FTD. K. Moulding, K. Cunningham, T. Lloyd Dept of Neuroscience, Johns Hopkins University, Baltimore, MD.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by degeneration of motor neurons which ultimately leads to paralysis. The most common genetic cause of ALS is a hexanucleotide (GGGGCC)n repeat expansion within the first intron of the C9orf72 gene. These repeats are transcribed to form repeat containing RNA, which may contribute to toxicity via formation of RNA foci. The repeats are also translated via noncanonical, Repeat Associated Non-AUG (RAN) translation to form dipeptide repeat proteins (DPR) which may contribute to toxicity by a variety of mechanisms. A longstanding question in the field is whether repeat containing RNA or DPRs are the primary toxic species. In order to address this, we are developing a model in which we specifically target GFP-tagged DPRs for degradation using the DeGradFP system. In this system, GGGGCC repeat constructs are transcribed under control of Gal4-UAS but contain no translation start codon. Thus, all DPRs must be produced by RAN translation. The constructs then contain GFP fused in frame with one of the DPRs. These GFP tagged DPRs can be targeted for degradation by co-expression with DeGradFP, which functions as an E3 ubiquitin ligase directed against GFP by an nonbody. This model will allow us to remove specific DPRs while leaving repeat containing RNA and other DPRs intact. We and others have previously observed defects in nucleocytoplasmic transport and autophagy when C9 constructs are expressed. We will compare the severity of these phenotypes when GFP tagged constructs are expressed with and without DeGradFP. Thus, using this model may help resolve the relative contribution of RNA and DPR toxicity in ALS.

577 Tip60 HAT activity reverses early epigenetic alterations and reinstates cognition in multiple Drosophila neurodegenerative models. Priyalakshmi Panikker, Haolin Zhang, Mariah Beaver, Visha Parmar, Felice Elefant Department of Biology, Drexel University, Philadelphia, PA.

Impairment of cognitive ability is a debilitating hallmark during early pre-clinical stages of most neurodegenerative disease, yet causes remain elusive. Epigenetic mechanisms are fundamental early developmental step for orchestrating the dynamic gene expression profiles throughout the lifetime of a neuron and is critical for promoting cognitive function. Histone acetylation is one of the best-characterized epigenetic modification crucial for learning and memory, regulated by antagonistic activity of histone acetyltransferase (HAT) and histone deacetylases (HDAC). As histone acetylation homeostasis is critical for mediating epigenetic gene control throughout neuronal development, we hypothesized that its disruption is a common factor in multiple types of neurodegenerative conditions and contributes to early cognitive impairment. To test this hypothesis, we used 3rd instar larvae to look at early changes in the following four Drosophila neurodegenerative models: Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD) and Amyotrophic Lateral Sclerosis (ALS). Here, we show that disruption of Tip60 HAT/HDAC2 balance is an early event in multiple neurodegenerative disorders including AD, ALS and PD, and triggers epigenetic repression of certain subset of synaptic plasticity genes early in the disease. Moreover, larvae displayed significant defects in learning and memory function in an olfactory associative learning assay, as well as defects in locomotor abilities under neurodegenerative conditions. Our previous discovery that Tip60 plays a critical role in cognitive function led us to hypothesize that increasing Tip60 HAT activity restores early cognitive defects in multiple neurodegenerative Drosophila models. To test this hypothesis we used a robust GAL4 responsive Tip60;201Y model system that enables us to modulate Tip60 HAT levels in the mushroom body under different neurodegenerative conditions, in vivo. Remarkably, we found that increasing Tip60 HAT activity specifically in the mushroom body of the disease larvae restores learning, short-term memory and locomotor deficits in AD, HD, ALS and PD. Our results support a model by which Tip60 HAT/HDAC2 mediated epigenetic gene control is critical for cognitive function and its disruption is an early event in multiple neurodegenerative disorders.

578 Anesthetics influence mortality in a Drosophila blunt trauma model. Julie Fischer, Hannah Schifftman, David Wassarman, Misha Perouansky 1) Anesthesiology, University of Wisconsin-Madison, Madison, WI; 2) Department of Medical Genetics, University of Wisconsin, Madison, WI; 3) Feinberg School of Medicine, Northwestern University, Chicago, IL.

Exposure to anesthetics is common in the majority of early survivors of life-threatening injury. Whether and to what degree general anesthetics (GAs) influence outcome from major trauma is unknown. Trauma models in vertebrates almost invariably incorporate anesthesia during the infliction of trauma because of the requirement for humane treatment of laboratory animals. Potential confounding effects of GAs (drugs with numerous molecular targets) on outcome measures are commonly disregarded. We used a blunt trauma model with associated traumatic brain injury (Katzenberger et al. PNAS 2013; Jove 2015; Elife 2015; G3 2016) in Drosophila melanogaster to test the hypothesis that GAs modulate outcome from blunt trauma. We administered a standard dose (concentration x duration) of volatile anesthetics either before, during or after a high-impact acceleration-deceleration injury that was calibrated to result in a 24-hour mortality of 20-25% in 1-7 day old adult w+ flies. We found that isoflurane and sevoflurane reduced 24-hour mortality when administered before or concurrent with trauma. In contrast, administration of isoflurane but not sevoflurane after trauma increased 24-hour mortality. Furthermore, we found that the protective effects of volatile anesthetics were lost in starvation-selected flies with an obese phenotype (Masek et al. JExpBiol 2014).
We conclude that (i) general anesthetics are not neutral with respect to outcome after life-threatening injury, (ii) their effect is influenced by genotype, and (iii) results obtained in vertebrate trauma models may be influenced by the anesthetics used.

579 Identification of genetic modulators of TDP-43 production in a new autoregulatory TDP-43 Drosophila model. M. Pons1, L. Miguel1, C. Miel1, T. Avequin1, F. Juge1, T. Frebourg1,2, D. Campion1,3, D. M. Lecourtou1 1) Normandie Univ, UNIROUEN, Inserm, U1245, IRIB, Rouen, France; 2) IGMM, CNRS, Univ. Montpellier, Montpellier, France; 3) Rouen University Hospital, Department of Genetics, Rouen, France; 4) Centre Hospitalier du Rouvray, Sotteville-Lès-Rouen, France.

TDP-43 is a critical RNA-binding factor linked to numerous aspects of the mRNA life cycle, including transcription, pre-mRNA splicing, mRNA transport, mRNA stability, and mRNA translation. Numerous studies showed that perturbation of TDP-43 levels by either increasing or decreasing TDP-43 in animal and cellular models results in severe consequences. Thus in the physiological state, maintaining normal TDP-43 protein levels is critical for proper physiological functions of the cells. As such, TDP-43 expression is tightly regulated through an autoregulatory negative feedback loop. TDP-43 has been found to be a major disease protein in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar Degeneration (FTLD). The observations that the level of TDP-43 is elevated in tissue samples from patients, the fact that TDP-43 expression is tightly controlled by autoregulatory mechanisms, and the strong evidence that overexpression of TDP-43 protein level is detrimental to central nervous system cells and can cause cell degeneration, argue for a pathogenic role of elevated TDP-43 levels. Modulating of TDP-43 production might therefore provide a new therapeutic strategy. We recently developed a new transgenic Drosophila model recapitulates key features of the self-regulatory process of TDP-43 protein steady-state levels described previously in mammalian and cellular models, namely alternative splicing events, differential usage of polyadenylation sites, nuclear retention of the transcript and a decrease in steady-state mRNA levels. Using this new Drosophila model, we identified splicing factors as genetic modulators of TDP-43 production. Interestingly, our data indicate that RNA-binding proteins regulate TDP-43 protein production, at least in part, by controlling steady-state mRNA levels, using distinct molecular mechanisms. Characterisation of other genetic modulators of TDP-43 production and associated molecular mechanisms are on-going and will be also presented.

580 The effects of SOD2 antioxidant on LC3 localization in a Drosophila model of Machado-Joseph Disease. H. Ragoowski, J. Warrick University of Richmond, Richmond.

Spinocerebellar Ataxia Type-3 (SCA3) is one of the most common dominantly inherited ataxias. It is caused by an expansion of the CAG repeat in the ataxin-3 protein. The extended polyglutamine sequence causes the diseased protein to form aggregates and leads to neuronal dysfunction and death. It has been suggested that antioxidants may be helpful in slowing disease progress because they remove the damaging reactive oxygen species. However, our previous research has shown that increased SOD2 antioxidant expression increases the disease progression and toxicity. We believe this effect is due to reduced autophagy. In this experiment, we visualized ataxin-3 and LC3 (representative of the autophagosome membrane) in a Drosophila model of SCA3 disease. We increased the levels of SOD2 antioxidants in the flies and compared them to flies with endogenous levels of SOD2. We found that the LC3 and Ataxin-3 proteins co-localize. We had hypothesized that if the neuron was able to autophagocytize the mutant ATX-3, the LC3 to form a ring around the ataxin-3 protein aggregates. We found the co-localization of the two proteins to be enhanced in day old flies with upregulated antioxidant levels. This finding could signal a dysfunction in autophagosome formation and, consequently, function.

581 Notch target gene E(spl)mδ is a Mediator of Methylmercury-Induced Myotoxicity in Drosophila. L.M. Prince, M.D. Rand Department of Environmental Medicine, University of Rochester, Rochester, NY.

Methylmercury (MeHg), a ubiquitous environmental contaminant, has been shown to induce Notch target gene expression in Drosophila cells and embryos. MeHg action on Notch signals aligns with the longstanding notion that MeHg is a selective neurotoxicant. However, we have recently shown that MeHg perturbs embryonic muscle formation, which parallels induction of the Notch target gene Enhancer of Split mDelta (E(spl)mδ). Subsequently, we have shown that, in contrast to some other E(spl) genes such as E(spl)My, E(spl)mδ is expressed primarily in the myogenic domain and that genetic upregulation of E(spl)mδ can disrupt embryonic muscle development. Furthermore, a recent GWAS with the DGRP panel revealed a number of core genes in pathways of myoblast fusion and muscle development that associate with MeHg tolerance and susceptibility in fly development. Here, we tested the hypothesis that developing muscle is targeted by MeHg via upregulation of E(spl)mδ using genetic modulation of E(spl)mδ expression in combination with MeHg exposure in developing flies. Developmental MeHg exposure is seen to cause a decreased rate of eclosion that parallels gross disruption of indirect flight muscle (IFM) development in the pupal stage. An MeHg-induced increase in E(spl) expression across the pupal stages, with preferential E(spl)mδ upregulation occurring at early (p5) stages, is also observed. E(spl)mδ overexpression in myogenic lineages under the MeF2 promoter was seen to phenocopy eclosion and IFM effects of developmental MeHg exposure; whereas reduced expression of E(spl)mδ shows rescue of eclosion and IFM morphology effects subsequent to MeHg exposure. In contrast to expression under the MeF2 promoter, E(spl)mδ expression targeted to neural and gut tissues showed no effect on eclosion rate. Our data indicate that muscle development is a target for MeHg and that E(spl)mδ is a likely muscle-specific mediator of
this myotoxicity. In addition to identifying target pathways that potentially mediate susceptibility to MeHg toxicity, our results highlight the endogenous role E(spl)m8 in adult muscle morphogenesis.

Drosophotoxicology: Toxicity mechanisms of methylmercury in a Drosophila model of Minamata Disease. M.D. Rand, D. Vorojieikina, L.M. Prince Department of Environmental Medicine, University of Rochester, Rochester, NY.

Drosophila use in toxicological studies is growing rapidly due to its potential to unveil and characterize conserved mechanisms of toxicity. Here, we show the utility of flies in deciphering fundamental properties of the ubiquitous environmental toxicant methylmercury (MeHg), the agent responsible for neurological syndromes in Minamata disease. First, we show how overt MeHg toxicity assessed at various life stages reveals that eclosion behavior is most susceptible. The erratic wing and uncoordinated motor phenotypes seen in developmentally exposed adult flies are consistent with the perturbation of adult indirect flight muscle morphogenesis seen with MeHg in pupa. Kinetics of larval uptake and elimination of MeHg in two strains, Canton S (susceptible) and Hikone R (tolerant), reveal that MeHg levels (body burden) are inversely correlated with eclosion rate. MeHg exposures targeting windows of larval development demonstrate that the eclosion phenotype is most sensitive to exposure leading up to pupariation, and MeHg exposure restricted to the L1/L2 larval stage has no effect on eclosion rate. The reliance of MeHg susceptibility on toxicokinetic properties was further evaluated by targeted induction of glutathione (GSH) synthesis. GSH being a mediator of MeHg detoxification via xenobiotic excretion pathways. Ubiquitous expression of the glutamate-cysteine ligase catalytic subunit (UAS-GCLc), the rate limiting enzyme for synthesis of GSH, by Actin-GAL4 yielded flies that were exceptionally tolerant to MeHg, and had a corresponding reduction in MeHg body burden. GCLc targeted to muscle (Mef2-GAL4), neurons (ELAV-GAL4) or gut (NP1-GAL4) also showed greatly increased MeHg tolerance yet, in contrast, no corresponding decrease in MeHg body burden was seen. These latter findings unveil a robust toxicodynamic property capable of buffering the MeHg insult internally. Among 20 DGRP lines, previously characterized for their MeHg tolerance or susceptibility (10 lines each), the susceptible lines, as a group, accumulated higher levels of MeHg compared to the tolerant lines. Thus, genetic variation in toxicokinetic pathways is likely a major determinant of MeHg susceptibility.

Characterization of dSod1 knock-in mutations associated with ALS. K. Russo1, A. Held2, K. Wharton1,2 1) Neuroscience, Brown University, Providence, RI; 2) Molecular and Cell Biology, Brown University, Providence, RI.

Amyotrophic lateral sclerosis (ALS) is characterized by a loss of motor control that results from the degeneration and death of motor neurons. There are a variety of genes that are involved in the development of ALS, and most of these genes are known to be involved in pathways such as apoptosis, oxidative stress, and RNA processing. However, it is not yet clear how mutations in these genes cause ALS. The mutations could represent a loss of gene function, a gain of function, or a mix of both. In fact, it is also not clear that motor neurons are the only cells affected.

Mutations in the superoxide dismutase-1 (SOD1) gene represent 20% of familial ALS cases, second only to those resulting from a hexanucleotide repeat expansion in the human C9OrF72 locus. To gain a better understanding of the cells and cellular processes affected by ALS-associated mutations, we generated synonymous mutations in the endogenous Drosophila melanogaster dSod1 locus, using CRISPR-Cas9 technology, for the disease-causing SOD1-G85R and SOD1-A4V mutations. dSod1G85R and dSod1A4V exhibit distinct phenotypes. dSod1G85R homozygotes exhibit motor circuitry problems as well as motor neuron degeneration, which manifests in larval locomotion defects and failure to eclose from the pupal case. While preliminary studies indicate that dSod1A4V homozygotes fail to show defects in larval locomotion and eclosion, they exhibit uncoordinated movements in adulthood. We are currently performing lifespan analyses and adult locomotion assays to better quantify lifespan and locomotor deficits in dSod1A4V mutants. Unlike the dSod1G85R model, the dSod1A4V model will allow us to assess the cellular and physiological basis of motor loss. We will also be assessing genetic characteristics, such as dosage, and the subsequent behavioral consequences. Comparative analyses of dSod1G85R and dSod1A4V associated dysfunction may inform us of different stages of disease progression.

An inducible expression system to delineate developmental versus homeostatic defects in photoreceptor neurons. J. Rylee, S. Mahato, A. Zelhof Department of Biology, Indiana University, Bloomington, IN.

Misexpression of human genes in Drosophila photoreceptors has become a popular model for studying neurodegenerative disease. By driving UAS constructs of these disease-causing genes with retinal Gal4 drivers, their expression is limited to a simplified circuit, and neuronal degeneration can be easily scored. In a quick survey of recent publications employing this model, we found that many used GMR-Gal4 to drive UAS constructs of human genes. While this approach has been fruitful in revealing potential factors involved, and potential therapeutic targets for these diseases, extreme and rapid degeneration can limit the observations that researchers are able to make. Additionally, this means of transcriptional activation fails to separate out developmental versus homeostatic defects due to misexpression during the entire development of the photoreceptors and surrounding support cells. We have developed two photoreceptor-specific, inducible systems using Geneswitch to drive expression of transgenes post-eclosion, after the circuit has been established, using the upstream regulatory sequences of Pph13 and NinaE. We are using these systems to induce expression of a suite of human genes involved in neurodegenerative disease including HTT, MJD, and APP as well as protein variants specific for retinal degeneration in adult fly retinas. We believe
this approach will better separate developmental defects from degeneration. In addition, by limiting expression to only photoreceptors, we can take advantage of the relative simplicity of this circuit to study the characteristics of the resulting degeneration unique to each of these genetic disorders.

585 Wingless, a mediator of crosstalk between Amyloid-beta 42 expressing and wild-type neurons in Alzheimer's disease. A. Sarkar, J. Kofier, M. Kango-Singh, A. Singh 1,3,4, 1) Department of Biology, University of Dayton, Dayton, OH; 2) Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA; 3) Premedical Program, University of Dayton, Dayton, OH; 4) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH; 5) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN.

One of the hallmarks of Alzheimer's Disease (AD), an age related progressive neurodegenerative disorder, is formation of the Amyloid-beta 42 (hereafter Aβ42) plaques, which trigger oxidative stress due to aberrant signaling and finally resulting in the death of neurons. The exact mechanism underlying cell death is still not well understood. We misexpressed high levels of human Aβ42 polypeptide in the developing fly retinal neurons, which mimics AD like neuropathology. Using these Aβ42 expressing transgenic flies, in a forward genetic screen, we identified members of highly conserved Wingless (Wg) signaling pathway as the modifiers of Aβ42 mediated neurodegeneration. Misexpression of agonists of Wg signaling enhanced the Aβ42 mediated neurodegeneration. However, antagonists of Wg signaling suppressed the Aβ42 mediated neurodegeneration by reducing the number of dying cells and restoring the axonal targeting from the retina to the brain. Blocking transport of Wg signaling from Aβ42 expressing cells rescued the neurodegenerative phenotype. In order to determine the mechanism by which the Wg signaling triggers neuronal death, we have developed a two-clone system to generate two cell populations by somatic recombination viz., GFP tagged Aβ42 misexpressing cells and the wild type cells lacking GFP. Surprisingly majority of the wild-type neuronal cells failed to survive whereas the Aβ42 cells were alive. These wild-type cells exhibited robust upregulation of Wg and their nuclei were TUNEL positive, a marker for cell death. Thus, this death signal is emanating from Aβ42 expressing cells to kill wild-type cells. Using our established drug-screening regimen, we validated our results by blocking wg transcription and Wg signaling using chemical inhibitors. We analyzed Wg signaling in tissues of genotyped human Alzheimer's patients to validate our studies in Drosophila. Our studies demonstrate use of fruit fly model to study complex signaling crosstalk among neurons in human disease.


Innate immunity is a conserved process that provides a defense to the organism against microbial invasion. Emerging evidence suggests that this highly organized response is central to the pathophysiology of neurodegenerative (ND) disorders, but what that role is remains murky. Here, we used a Drosophila p35/Cdk5 model of neurodegeneration to show that upregulation of the innate immune response is responsible for loss of dopamine (DA) neurons with age in the mutant conditions, and that the upregulation of immunity occurs as a consequence of disrupted autophagy. Deregelation of Cdk5 activity has been linked to Alzheimer's and Parkinson's in humans. In flies, we find that both knockout (KO) or overexpression (OE) of p35, a neuronal-specific activator of the Cdk5 protein kinase, altered cellular homeostasis and enhanced sensitivity to oxidative stress, along with causing shifts in the transcriptome. Surprisingly, principal component analysis of transcriptome data revealed that overexpression of genes related to immunity, particularly antimicrobial peptides (AMPs), drives the difference in gene expression between Cdk5-altered and wildtype flies, even before the onset of overt degeneration. Moreover, dopaminergic (DA) neurons in the brains of both KO and OE flies uncovered a precipitous decline with age. qPCR data from head tissue shows that Cdk5-associated neurodegeneration is accompanied by massive upregulation of AMPs as compared to wild type. We observed neurodegeneration from targeted overexpression of AMPs and, conversely, protection from degeneration by reducing AMP expression using a Relish mutant. Together, this provides strong evidence that hyperexpression of AMPs is necessary and sufficient for DA neuron degeneration in this Cdk5/p35 model of neurodegeneration. To discover why altered Cdk5 activity stimulates AMP expression, we recalled that in some systems disruption of autophagy is associated with increased AMP expression. We therefore, first, assayed autophagy and found that altering Cdk5 activity disrupts autophagy efficiency resulting in high levels of Ref(2)P, the Drosophila p62. Next, we found that this disruption of autophagy in Atg8a mutant is sufficient to produce hyperexpression of AMPs. Furthermore, moderate upregulation of the canonical regulator of autophagy, TFEB (Mlfl), in the brain of KO Drosophila leads to normalization of Ref(2)P level along with decreased AMPs and rescue of DA loss. Altogether, these results suggest the model that altered Cdk5 activity in neurodegenerative conditions disrupts autophagy which hyperactivates AMPs, and that in turns causes DA cell loss. Given the dysregulation of Cdk5 in human neurodegeneration, and the conserved role of the kinase in regulation of autophagy in humans, these data likely have direct application to the sequence of events in humans ND disease.

587 Deciphering the role of key metabolic genes regulating lipid homeostasis in Drosophila model of Huntington's disease. A. Singh, K. Aditi 1) Department Of Zoology, University of Delhi, New Delhi, India; 2) Department Of Zoology, University of Delhi, New Delhi, India.
Huntington's disease (HD) is a dominantly inherited, late-onset, progressive neurodegenerative disease caused by an unstable expansion of the homopolymeric polyglutamine (polyQ) tract within the huntingtin protein (Htt). In addition to the neuronal degeneration of specific brain areas, HD patients and animal models exhibit a highly dysregulated energy homeostasis as evident by excessive weight loss and impaired systemic metabolism.

The transgenic Drosophila model of HD exhibits significant increase in weight and lipid content during initial stages of the disease while exhibiting an extreme loss of weight and lipid content at the terminal stages. Moreover, the distribution of lipid droplets (LDs) in fat body cells in diseased condition modulates from an initially large, unilocular form to a terminally small, multilocular one. To understand the reason underlying altered lipid metabolism in diseased condition, we monitored the expression of two key lipid metabolic genes namely brummer; a key TAG lipase and lipin; a major lipogenic protein. Interestingly, our preliminary results displayed that brummer is transcriptionally upregulated throughout disease progression suggesting an enhanced brummer mediated lipolysis. Lipin is found to be upregulated at disease onset and downregulated at terminal stages implying a transition from an initial lipogenic state to a terminal lipolytic one. Our results point towards the role of brummer and lipin in regulation of lipid metabolism throughout the course of disease. Based on our results, we envisage that therapeutics aimed at regulation of the lipid metabolism might abate disease progression and improve the quality of patient's life.

588 Impairment of circadian oscillations in core clock genes in Drosophila model of Huntington's disease. K. Singh, I. Malik, N. Agrawal, V. Kumar Department of Zoology, University of Delhi, New Delhi, IN.

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder, mainly caused by augmentation of polyglutamine repeats within Htt protein. HD condition affects specific regions of brain leading to progressive behavioral, physiological and cognitive decline. In addition to cognitive and psychiatric symptoms; there are other important non-motor symptoms in HD, including sleep and circadian abnormalities. It is possible that sleep and/or circadian disturbance in HD patients might be contributing towards symptoms and disease progression. Circadian clocks sustain temporal homeostasis in molecular, cellular and physiological functions by generating daily output rhythms. Therefore, there is a need to understand whether loss of rhythm is the consequence of the disease and/or loss of rhythm is the causative factor in disease progression.

In order to evaluate role of core clock genes like period, timeless and cryptochrome and their regulators in HD pathogenesis, we used transgenic Drosophila as a model and expressed mHtt in neuronal population. We monitored expression of these clock genes in head and entire body of diseased flies at different ages from day 1 to 13 to cover entire course of the disease from onset to progression.

We found that transcriptional oscillations of clock genes period, timeless and cryptochrome was comparable to their age matched control at disease onset (day1) but significantly reduced as the disease progressed (day7). Interestingly, the decline in transcription of these core clock genes was independent of their regulators (vrille & clockwork orange) and activators (clock & cycle). These results suggest role of clock genes in Huntington's disease progression, thereby restoring their expression might be a key towards management of this devastating disease.

Based on these results, we further propose that as the clock serves as an orchestrator of a multitude of biological processes, there is great potential for clock-targeted therapeutics to simultaneously ameliorate multiple pathologic aspects of complex neurodegenerative diseases.


Aging is the greatest risk factor for neurodegenerative (ND) but the connection between the two processes remains opaque, largely for want of a rigorous way to define physiological age, as opposed to chronological. Here we develop a comprehensive metric for physiological age based on genome-wide expression profiling, and apply this metric to a model of adult-onset ND. Cdk5 has been linked to multiple ND diseases, including Alzheimer's and Parkinson's, and its altered activity causes ND in Drosophila. Cdk5 activity requires interaction with activator subunits; in flies, the sole activator is p35. We show that loss (null) or overexpression (OE) of p35 in flies induces degenerative phenotypes earlier than typically observed in controls. Specifically, we see impaired autophagy, brain ND, motor defects, and shortened lifespan, all of which are observed in human ND diseases. To test if altered p35 accelerates the rate of aging en route to causing ND, we used microarray to measure RNA expression with age in heads and thoraces of control flies, hypothesizing that genome-wide appraisal of expression changes can provide a metric to quantify physiological age; we also identify expression changes in presymptomatic null and OE flies. Ultimately, we find that young null and OE flies resemble older controls more so than age-matched controls. First, there is significant overlap between p35-affected genes and aging-related genes, and gene ontology shows p35 levels affect biological processes involving mitochondria, metabolism, proteostasis, and immunity, which are all affected by aging. Second, analysis of mean expression of genes altered by both aging and p35 reveals that null and OE samples more strongly correlate with older controls than age-matched controls; the increased correlation is true regardless
Copper-mediated oxidative stress correlates to memory deficits in a Drosophila model of Alzheimer's Disease. AM. Sullivan, AN. Fuhrman, BE. Paddock Arcadia University, Glenside, PA.

Alzheimer's Disease (AD) is a multifactorial neurodegenerative disease characterized by abnormal protein aggregates, metal dyshomeostasis, and a decrease in neuroanatomical areas, especially those associated with memory function, all worsening over time. Heavy metal dyshomeostasis is closely linked to AD pathogenesis, with Cu(II) found within amyloid plaques and contributing to oxidative stress, but the role of these metals in anatomical and behavioral deficits associated with AD remains unclear. To test the role of copper-mediated oxidative stress in amyloid-associated neuronal dysfunction, a Drosophila model of AD was reared on media containing either CuSO4 or a copper chelator (BCS). Here, we demonstrate that copper availability is directly related to both memory dysfunction and neuroanatomical deficits associated with co-expression of the amyloidogenic proteins hAPP and hBACE. Taken together, these data support the hypothesis that copper availability exacerbates deficits associated with in vivo amyloid accumulation.

Do reactive oxygen species contribute to neurodegeneration in a Drosophila model of Machado-Joseph Disease. Y. Wong, J Warrick Biology, University of richmond, Richmond, VA.

Machado-Joseph disease (MJD) /Spinocerebellar Ataxia Type 3 (SCA3) is an inherited neurodegenerative disease caused by a polyglutamine domain expansion of the ataxin-3 protein (ATXN3). It is believed that oxidative stress contributes to the pathogenesis of MJD disease. To determine the role of oxidative stress and reactive oxygen species (ROS) in disease pathology we are using a transgenic Drosophila melanogaster model, in which flies were fed paraquat with and without SOD2 antioxidant over expression. Mutant MJD alleles (Q84) that encode pathologic expanded polyglutamine proteins are expressed in the eyes of flies and compared with Q27 non disease (wild type) alleles. Compared with Q27 ATXN3 flies, mutant Q84 ATXN3 flies have generally deformed ommatidia and decreased number of rhabdomeres for each ommatidia. Q84 flies treated with paraquat (increased oxidative stress) have generally smaller, more deformed rhabdomeres than Q84 flies on control media, but the difference in number is not statistically significant. It suggests that the excessive oxidative stress has a minimal contribution to neurodegeneration. Surprisingly, Q84 flies with SOD2 gene up regulated on control media show more neuron degeneration than the ones treated with paraquat. Our results show that increased antioxidative capacity of Q84 ATXN3 + SOD2 flies is not only unable to rescue degeneration, but increases the degeneration in our MJD model. Our results suggest that although excessive amounts of reactive oxygen species (ROS) causes damage to flies, they may also be involved in other pathways that are related to neuron protection.

Mitochondrial dysfunction associated with a SOD1-ALS knock in model. Beatrice Steinert1, Kristi A. Wharton1,2 1) MCB Department, Brown Univ, Providence, RI; 2) Brown Institute for Brain Science, Brown Univ, Providence, RI.

Many neurodegenerative diseases such as ALS, Parkinson's Disease, and Huntington's Disease have been associated with defects in mitochondrial morphology and function. A growing body of evidence has suggested that these mitochondrial defects are a central contributor to the ultimate loss of motor neuron function in familial amyotrophic lateral sclerosis (FALS) linked to mutations in the antioxidant enzyme superoxide dismutase 1 (SOD1) [1, 2]. Studies in overexpression models of mutant human SOD1 have demonstrated a range of mitochondrial defects in neurons, such as altered axonal transport, changes in fission/fusion dynamics, abnormal distribution, ultrastructural differences, and bioenergetics [3, 4, 5]. It is not yet fully appreciated whether these mitochondrial defects result from the cellular stress associated with the overexpression of mutant proteins, or if they are associated with the disease phenotype. In a Drosophila mutant dSod1 knock-in model where the endogenous levels of SOD1 are maintained, we have also detected defects in mitochondria. Interestingly, not all cell types in these mutant animals exhibit mitochondrial defects, suggesting a differential response of cells to the ALS-associated mutation. We have identified several genetic suppressors of the dSOD1-ALS model, and we are exploring how the observed defects in mitochondria morphology and possible changes in mitochondrial function associated with mutant dSOD1 respond to genetic suppression. (This work was supported by the ALS: Finding a Cure Foundation.)


Alzheimer's disease (AD) is a chronic neurodegenerative disease characterized by Aβ plaques and dementia. Symptoms can include short-term memory loss and early death. It was recently discovered that AD is accompanied by reduced histone acetylation in the brain. The antagonistic activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) determines “histone-acetylation-code” that we hypothesize underlies AD pathogenesis. Notably, HDAC inhibition improves learning and memory in AD mouse models, underscoring its therapeutic potential for AD. Unlike HDACs, the correlation of HATs and AD remains unclear. Tip60 is the most abundant MYST-type HAT in the mammalian hippocampus, inferring its importance in hippocampal dependent cognitive functions. In line with this assumption, our previous study demonstrated that amyloid precursor protein (APP)-induced immediate-recall mating memory deficits were effectively rescued by Tip60 HAT overexpression specifically in the fly mushroom body. To confirm and extend these studies, here we investigate whether Tip60 HAT function is compromised in a Drosophila AD model designed to enhance Aβ plaque formation. Here, we show that Tip60 and Tip60 mediated histone acetylation levels (H4K5Ac, H4K12Ac and H4K16Ac) were significantly reduced in the neurodegenerative Aβ42 Drosophila brain. To test whether increasing Tip60 levels would restore Aβ42 induced deficits, we generated a UAS-Aβ42;UAS-Tip60 transgenic fly line that enables us to increase levels of Tip60 in cell types of choice in an Aβ42 neurodegenerative background. We show that in a larval olfactory short-term memory assay, Tip60 overexpression improved memory performance in Aβ Drosophila. Moreover, Tip60 restoration also rescued the early death in Aβ Drosophila in survival assay. Tip60 is thought to rescue Aβ pathology by transcriptional mechanism. To determine whether Tip60 transcriptionally targets Aβ pathology-associated neuroprotective genes, ChiP-qPCR was performed and revealed Tip60 enrichment at Neprilysin gene which encodes the Aβ cleaving protein. However, the disturbed Tip60 binding on these genes under Aβ conditions and the restoration of Tip60 binding by Tip60 overexpression (Elav>Aβ42;Tip60) are still to be determined. Our findings will uncover the associations between AD pathology and Tip60 HAT and support novel neuroprotective roles for HATs in AD pathology.

Stress granule assembly disrupts nucleocytoplasmic transport. Ke Zhang1, J Gavin Daigle2, Kathleen Cunningham3, Alyssa Coyne2, Kai Ruan1, Jonathan Grima4, Kelly Bowen1, Harsh Wadhwa1, Jeffrey Rothstein1,3,4, Thomas Lloyd1,3,4 1) Department of Neurology, Johns Hopkins University, School of Medicine, Baltimore, MD; 2) Brain Science Institute, Johns Hopkins University, School of Medicine, Baltimore, MD; 3) Cellular and Molecular Medicine Program, Johns Hopkins University, School of Medicine, Baltimore, MD; 4) Department of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD.

Nucleocytoplasmic transport (NCT) defects have been identified as a key pathogenic mechanism in C9orf72-mediated amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), the most common familial forms of these devastating neurodegenerative diseases. Recently, NCT disruption has also been implicated in Huntington's disease and tauopathies (e.g., FTD and Alzheimer's disease), suggesting a common pathogenic mechanism underlying cytoplasmic protein aggregation diseases. However, how cytoplasmic protein misfolding disrupts NCT is unclear. Using Drosophila and cell culture models, we find that diverse cellular stressors causes NCT defects. Interestingly, we find that multiple exogenous (e.g., arsenite) and endogenous (e.g., TDP-43) stressors lead to mislocalization of proteins required for NCT, including Ran, importins, exportin-1, and nucleoporins that constitute the nuclear pore complex. Moreover, these NCT defects can be suppressed by inhibitors of stress granule assembly, implicating stress granule formation in the regulation of nucleocytoplasmic transport. As stress granules are implicated in ALS pathogenesis, we next tested whether stress granule proteins contribute to NCT defects observed in C9orf72-mediated ALS/FTD. Indeed, we find that molecules inhibiting stress granule assembly suppress NCT defects as well as neurodegeneration in fly and/or iPS models of C9orf72-mediated ALS/FTD. Hence, our studies identify a novel link between stress granule formation and nucleocytoplasmic transport, two fundamental cellular processes implicated in the pathogenesis of neurodegenerative diseases.

Thermal injury results in nociceptive sensitization in D. melanogaster. G.M. Dion, C. Brann, G. Ganter Biology, University of New England, Biddeford, ME.

Fruit flies are used to observe the process by which thermal injury results in sensitization of nociceptive neurons. Because pain sensitization perpetuates chronic pain and not enough is known about its mechanisms, we have established a model of nociceptive sensitization induced by thermal injury. A third-instar larva is placed into a groove cut in an aluminum plate that rests on a tray of ice. Then the larva is covered with a 0°C water bubble and touched with a surface heated to 85°C (experimental group) or 25°C (control group). Larvae then undergo only one of two sensitization tests. To test for allodynia, a nocifensive response to a normally innocuous stimulus, larvae are tested 24 hours post-injury with a probe heated to just below the nociceptive threshold, at 41°C. Sensitization is indicated if the larva performs a nocifensive roll in response to this normally innocuous stimulus. The second assay is performed eight hours after the injury. To test for hyperalgesia, a stronger response to a normally noxious stimulus, larvae are tested 8 hours post-injury with a probe that is heated to 45°C. Once again, sensitization is indicated by a nocifensive roll. The researcher testing for sensitization is blinded to the treatment so no biases will occur when assessing nocifensive behavior of the larva. Injured larvae can also be dissected for imaging under a
confocal microscope. The larvae's dissected body walls are treated with anti-fasciclin antibodies and fluorescently tagged secondary antibodies, allowing for visualization of the epidermal damage caused by the thermal injury. These larvae also express nociceptor-specific Td Tomato, a red fluorescent protein, which allows assessment of the larva's nociceptive neurons. Results reveal that larvae injured with a hot surface undergo nociceptive sensitization, compared to larvae mock-injured with a 25°C surface. This could help to better understand through what pathways the fly's nociceptor neurons become sensitized after injury. This knowledge could provide insight into how human patients who have sustained extensive bodily burns experience chronic pain even after their injuries have fully healed. Novel pathways identified using this method of experimentation may represent new targets for medications that will more effectively treat chronic pain.

596 Characterizing the neuronal and cognitive defects of histone demethylase kdm5 mutants in a Drosophila model of intellectual disability. H.A.M. Hatch, S. Zamurrad, C. Drelon, H. Belalcazar, J. Secombe Albert Einstein College of Medicine, Bronx, NY.

Intellectual disability (ID) disorders affect 2% of the population and are characterized by an IQ score lower than 70. Mutations in over 400 genes contribute to ID disorders, with patients often presenting with seizures, learning and memory impairments, increased anxiety and abnormal social behaviors, short stature, and increased aggressive tendencies. Mutations in kdm5, a chromatin modifier responsible for recognizing and demethylating trimethylated lysine 4 of histone H3 (H3K4me3, a mark associated with active promoters) have been reported in families presenting with heritable forms of ID disorders. Previous studies in rat cerebellar granular neurons and mice pyramidal neurons of the basolateral amygdala demonstrate that mammalian kdm5SC is essential during dendritic growth and spine morphogenesis. Additionally, kdm5SC-KO mice display several behavioral deficits similar to those observed in ID patients. However, despite these findings, little is known regarding the mechanism or temporal and spatial requirements of KDM5.

Here, we demonstrate that Drosophila, which possess a single kdm5 ortholog, serves as a suitable and genetically tractable model to investigate the molecular, cellular, and behavioral defects associated with ID disorders. Flies strains harboring mutations in evolutionarily conserved residues of KDM5 recapitulate several of the syndromic features observed in ID patients with analogous KDM5 mutations. Using genetic tools such as Mosaic Analysis with a Repressible Cell Marker (MARCM) and targeted RNAi knockdown, we are able to probe the neuronal requirements of KDM5 in vivo. Additionally, based on recently generated transcriptome and protein-protein interaction datasets, we are investigating the molecular mechanism of KDM5 function in the CNS. Here we demonstrate that kdm5 ID mutants (1) display short- and long-term memory impairments, (2) bear gross morphological guidance and growth defects of the mushroom body kenyon cells, and (3) show a reduction in neuronal protein synthesis, a requirement for long-term memory formation.

597 Down syndrome kinase minibrain control dendritic morphogenesis in Drosophila. K. Lee1,2, D. Kwon1,2, I. Cha3, K. Yu1,2, S. Lee3 1) BioNano Research Center, KRRIBB, Daejeon, KR; 2) Department of Functional Genetics, University of Science & Technology, Daejeon, KR; 3) Department of Brain & Cognitive Sciences, DGIST, Daegu, Korea.

Phosphorylation events have emerged as a crucial regulatory mechanism in the nervous system to regulate the activity of substrates involved in dendritic and synaptic morphogenesis, function and plasticity, particularly during changes in neuronal activity. Human Dyrk1A is a serine/threonine kinase implicated in autism spectrum disorder and Down syndrome. Here we identified that minibrain (Mnb), a Drosophila homologue of Dyrk1a, is involved in dendritic morphogenesis. We find that co-expression of Mnb nicely rescued the dendritic branching defect caused by overexpressing abrupt in dendritic arborization (DA) neuron. Mnb also rescued the morphological defects of wing and eye in abrupt overexpressed flies. Based on these genetic interactions, we find that phosphorylation level of abrupt increased by Mnb-dependent manner. We also confirmed that Mnb inhibits abrupt through promoting protein degradation. These results indicate that Mnb kinase is critical for determining the dendritic patterning of DA neuron through regulating the protein stability of abrupt.

598 Traumatic injury induces Stress Granule Formation and enhances Motor Dysfunctions in fly models of ALS. Eric Anderson1, Lauren Gochnauer1, Aditi Singh1, Rogan Grant1, Krishani Patel1, Jane Wu2, Udai Pandey1 1) Pediatrics, University of Pittsburgh Medical Center, Pittsburgh, PA; 2) Northwestern University.

Traumatic brain injury (TBI) has been predicted to be predisposing factor for Amyotrophic lateral sclerosis (ALS) and other neurological disorders. Despite the importance of TBI in ALS progression, our understanding of the underlying cellular and molecular mechanisms is still an enigma. We examined the contribution of TBI as an extrinsic factor and investigated if TBI influences the susceptibility of developing neurodegenerative symptoms. To evaluate the effects of TBI in vivo, we applied mild to severe trauma to Drosophila. We found that TBI leads to the induction of stress granules (SGs) in the brain and the levels of SGs induction are hit repeat-dependent. We observed that the level of mortality is directly proportional to the number of hit repeats and trauma. Interestingly, SGs are ubiquitin, p62 and TDP-43 positive, and persistently remain over time suggesting that SGs might be aggregates and toxic in our fly model. Intriguingly, TBI on animals expressing ALS-linked genes increased mortality and locomotion dysfunction as compared to controls, suggesting that repeated mild form of TBI might aggravate ALS symptoms. Furthermore, we found elevated levels of high molecular weight ubiquitinated proteins in
animals expressing ALS-causing genes with TBI, suggesting that TBI may lead to defects in protein degradation pathways. Finally, we observed that treatment with rapamycin (an autophagy inducing agent) enhanced the clearance of SGs and promoted survival of flies in vivo. Together, our study demonstrates that trauma can induce SG formation in vivo and might enhance motor dysfunctions in fly models of ALS.

599 Reduced presenilin activity alters insulin signaling in a Drosophila model of Alzheimer’s Disease. 

Meridith Toth, Thomas Jongens University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA.

Alzheimer’s Disease is a highly prevalent age-onset neurodegenerative disorder for which there are no effective treatments. The majority of Alzheimer’s research in the past has focused on the well described histopathology, namely the extra-neuronal accumulation of amyloid beta plaques and the intra-neuronal accumulation of hyper-phosphorylated tau tangles which are associated with the later stages of the disease. More recently, it is believed that identifying the causes and earlier physiological changes associated with the disorder may provide more effective targets for treatment aimed at prevention rather than reversal.

In order to identify early changes associated with Alzheimer’s Disease, we have chosen to model a rare form of the disorder termed familial Alzheimer’s Disease (FAD) which is caused by autosomal dominant mutations identified in PS1, PS2, and APP. Since the majority of mutations identified have been found in PS1, and these mutations have been shown in a variety of models to be loss of function, we have chosen to use flies that are heterozygous for presenilin loss of function mutations (psn-hets) to model this disorder. In previous studies, we have shown that the psn-hets have age-onset defects in memory, supporting their use in studying memory loss in Alzheimer’s Disease.

In our current studies, we have identified altered insulin signaling in the brains of young psn-hets which occurs before the onset of memory loss. Our data show that this effect is likely dependent upon the role of presenilin in the y-secretase complex, and may occur through an interaction with the amyloid precursor protein. These findings are significant considering the critical role that insulin plays in memory formation, and suggest that memory loss in the psn-hets may result from age-onset brain insulin resistance.

600 High-volume functionalization of human autism PTEN variants in multiple models including Drosophila. P. Ganguly1, T. Lian1, K. Post1, B. Young1, F. Melili1, M. Belmadani2, B. Callaghan2, R. Dingwall1, M. Edwards1, T. McDermid3, W. Meyers1, S. Rogic2, P. Pavlidis2, C. Loewen1, C. Rankin3, S. Banji1, K. Haas1, T. O’Connor1, D. Allan1 1) Department of Cellular and Physiological Sciences, The University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Psychiatry, The University of British Columbia, Vancouver, British Columbia, Canada; 3) Department of Psychology, The University of British Columbia, Vancouver, British Columbia, Canada.

Clinical exome and genome sequencing is identifying variants in gene sequence at an increasing pace. However, the development of methods to determine whether variants are function-altering lags far behind and around ~50% of identified variants for any gene are designated as VUS (Variant of Unknown Significance). Our group is developing a multi-platform approach using numerous model systems, including Drosophila, Saccharomyces cerevisiae (yeast), C elegans, rat and HEK293 cells to experimentally determine whether coding sequence variants are function-altering. We will present our analysis of ~100 human PTEN (hPTEN) variants that include established loss and gain of function mutants to calibrate assays, and also variants identified from non-diseased individuals, and those with either Autism Spectrum Disorder or cancer. These have all been integrated into flies as UAS-hPTEN transgenes into the attp2 locus by phiC31 integrase, and expressed in yeast for Synthetic Genetic Array analysis. Variants of interest are then examined in other models. In flies, ubiquitous overexpression of wildtype UAS-hPTEN resulted in phenotypes including a ~2-4 day delay to eclosion. By comparing eclosion delay for overexpressed UAS-hPTEN variants, we have assigned variants as wildtype, gain of function or loss of function (amorphic or hypomorphic). We will present these data and also comparisons of hPTEN functionalization data between the multiple model systems. Our work will demonstrate the utility of Drosophila as a powerful platform for high volume screening for the relative function of large numbers of human gene variants from healthy and diseased individuals.


The social environment can affect behaviour by altering molecular processes and neuronal function. For example, in the fly Drosophila melanogaster, social isolation affects complex behaviours such as aggression(1), courtship, mating, foraging, learning and memory, and as social spacing (2). Furthermore, changes in the expression of many genes in response to social experience have been found (i.e. 1, 3). Here we report that the expression of Drosophila Neurilig 3 (dnlg3), an autism-related post-synaptic cell adhesion protein (4), is also altered in response to social isolation. In Drosophila, there are four dnlg genes, among which dnlg2-4 are expressed in the CNS. Furthermore, dnlg2 and dnlg4 have been shown to be involved in adult social interactions (5, 6). The DnLG3 protein is involved in correct synaptic differentiation at the neuromuscular junction (7), however its role in the adult brain has not yet been investigated. We not only found that DnLG3 expression is modulated by social experience, but also that its expression is different in males and females. Additionally, mutations in dnlg3 lead to
abnormal behavioural spacing, although only in response to isolation in a sex-specific manner, or with age. This sex specific gene-environment interaction is particularly interesting, as such mechanisms might be conserved, and could underlie some of the complex aetiology of the multifactorial autism spectrum disorders.


602 Actin reorganization in a photoreceptor model of polyglutamine repeat disorders. A.S. Haberman, Annie Vu, Tyler Humphries, Sean Vogel. Biology Department, University of San Diego, San Diego, CA.

Drosophila melanogaster has proven to be a valuable system for studying the cellular pathology of polyglutamine repeat neurodegenerative disease genes. These genes, including Huntington (HTT) for Huntington's Disease and Ataxin 3 (ATXN3) for Spinocerebellar Ataxia type 3, encode short stretches of repeating glutamine residues. In disease-causing alleles, these polyglutamine tracts are expanded, causing protein misfolding and aggregation. Expression of the human disease-causing alleles in Drosophila neurons recapitulates much of the cellular pathology of these disorders. By expressing these human genes in an orthologous system, we can compare the cellular activities of different disease genes, removing the complication of differential expression in various parts of the human nervous system. We have found that expression of expanded HTT or ATXN3 alleles in Drosophila photoreceptor neurons induces a massive, progressive rearrangement of actin filaments that would normally localize to the light-sensing rhabdomere, as well as a dramatic accumulation of Rhodopsin. Coexpression of a constitutively activated Rac rescues the actin phenotype, implicating Rac in a pathway affected by polyglutamine repeat disorders. Activated Rac has also been shown to rescue dendrite morphology affected by polyglutamine repeat genes in dendritic arborization neurons, suggesting that photoreceptor rhabdomeres may be useful proxies for dendrite biology in these disorders.

603 New Drosophila models of repeat expanded diseases generated by CRISPR/CAS9 technology. H. Tricoire, E. Martin, V. Monnier, M. Russi. Unité de Biologie Fonctionnelle et Adaptative (BFA, UMR8251) Univ Paris Diderot/ CNRS, Sorbonne Paris Cité, PARIS, FR.

Repeat expanded disorders (RED) account for at least 20 rare and severe inherited neurological diseases. The repeat expansion can be either in the coding region of the gene, like in the dominant Huntington disease (HD), or in the non-coding part of the gene, like in the recessive Friedreich ataxia (FA) disease. Drosophila models have emerged as an attractive complementary approach to mammalian models to study REDs with fast in vivo studies, to find genetic modifiers, screen for active compounds and test therapeutic strategies. However the current models present some limitations in fully modeling the pathological situations. Taking advantage of the strong conservation in Drosophila of two human genes linked to REDs – the huntingtin (Htt) and the frataxin (fh) genes –, we attempted to generate drosophila models of HD and FA, based on repeat insertions in the fly orthologs of Htt and fh genes by homologous recombination, using the CrispR/Cas9 system.

We shall report the progress in the generation of these new knockin models of REDs. Notably we will describe the first data for a new Drosophila model of FA, based on the insertion, in the intron of the fly fh gene, of a portion of the first intron of the human FXN gene carrying GAA triplet expansions. At molecular level, this Drosophila model recapitulates some key feature of the human disease, while the Drosophila flies exhibit temperature dependent lethality, strong locomotor defects, short lifespan and cardiac dysfunction. This new Drosophila fh-GAA model is a significant advance to study physiopathological mechanisms involved in FA, and to identify and evaluate therapeutic compounds, in a context that mimics closer the situation in human patients compared to RNAi models.

We will discuss the use of CRISPR generated REDs models to evaluate in vivo innovative gene therapies based on repeats deletion by the CRISPR/Cas9 machinery.

604 Loss of scaffold protein RACK1 could suppress polyQ induced cell death. J. Xie1,2, T. Wang1 1) College of Biological Sciences, China Agricultural University, Beijing, Beijing, CN; 2) National Institute of Biological Sciences, Beijing, Beijing, Beijing, CN.

Polyglutamine (polyQ) diseases are autosomal dominant diseases caused by CAG repeat expansion in mutant genes. The
translated polyQ tracts tend to form oligomers or aggregates both in cytosol and nucleus, leading to degenerative cell death. Despite many demonstrated pathways polyQ tracts might affect, the molecular mechanisms of how those mutant proteins lead to cell death are still under discussion. Here we established a polyQ disease model in drosophila eye through overexpressing 63 CAG repeats under GMR promoter (GMR-63Q). Pigment cell loss were seen at day 1 63Q fly eye. Based on this phenotype, we combined mosaic systems with EMS screening to find suppressors of polyQ induced cell death. 44 suppressors were found, among which 22 are dominant suppressors. The scaffold protein RACK1 has been screened as one of the suppressors and rack1 mutation could reverse pigment cell lose in 63Q fly. Overexpress RACK1 have no effect on 63Q and rack1 mutation could not lead to cell death. Further experiment showed that rack1 mutants could not reduce 63Q aggregates, meaning the suppression effect of rack1 mutants was not through influencing polyQ aggregates. Rack1 mutation could also suppress cell death in SCA3 disease model, another polyQ diseases. The suppression of cell death by rack1 mutation is not through a general mechanism, as rack1 mutation could not alleviated other misfolded disease model, like Alzheimer's disease. The molecular mechanisms of how RACK1 as a suppressor of polyQ needs more work to be done.

605 Using Drosophila models to study PNPO deficiency and epilepsy. W. Chi1,2, M. Albersen1, W. Liu4, M. Bosma3, M. Wells2, N. Verhoeven-Duif1, X. Zhuang1,2 1) Committee on Genetics, Genomics & Systems Biology; 2) Department of Neurobiology, The University of Chicago, Chicago, Illinois, USA; 3) Department of Medical Genetics, University Medical Center (UMC) Utrecht, Utrecht, The Netherlands; 4) Department of Environmental Health, School of Public Health, China Medical University, Shenyang, P.R. China.

Pyridox(am)ine 5'-phosphate oxidase (PNPO) converts inactive forms of Vitamin B6 in the diet to the only active form PLP, which is a co-factor for more than 140 enzymes including those required for the synthesis of dopamine, serotonin, and GABA. In humans, PNPO deficiency (MIM# 610090) causes neonatal epileptic encephalopathy (NEE). Based on the conditional lethal phenotype, we have previously identified a hypomorphic dPNPO mutant, sglf38. The conditional lethal phenotype of sglf38 or sglf knockdown flies is correlated with low PLP levels and seizure-like behavior. These phenotypes can be rescued by either wild-type (wt) dPNPO or wt human PNPO, demonstrating that PNPO is functionally conserved between humans and flies. Since 2005, when Mills PB et al identified the first PNPO mutation in NEE patients, PNPO mutations have been increasingly reported. To functionally and systematically characterize human PNPO mutants, we generated a spectrum of PNPO deficiency Drosophila models using a CRISPR/Cas9 based knock-in approach. We found that severe PNPO deficiency affects the development of flies. In addition, we found that one mild PNPO mutation altered the cellular localization of PNPO, suggesting that residual enzyme activity is not the only factor that contributes to PNPO deficiency. These Drosophila models represent promising approaches to study the pathogenesis of NEE caused by different PNPO mutants in humans.

606 Investigation of genetic interactors with julius seizure, a bang-sensitive locus. D.M. Dean1, S. Amin1, H. Weinstein1, B. Karno1, N. Metaferia1, A. Viswanathan2, V. Hsu2, D.L. Deitcher2 1) Biology, Williams Col, Williamstown, MA; 2) Neurobiology and Behavior, Cornell University, Ithaca, NY.

Drosophila mutant for julius seizure (jus) exhibit seizure-like behavior in response to mechanical- or cold-shock. Previously we had established that jus expression is required during early pupal life to affect the bang-sensitivity of adult flies. This suggests that jus has a developmental function as opposed to affecting adult neuronal physiology in the more direct sense. The jus protein exhibits no significant homology to other known proteins, but is predicted to have two transmembrane domains and an extracellular loop. A transgene expressing Jus with a GFP tag at the C-terminus shows axonal expression in a subpopulation of neurons, while a MIMIC GFP-tag in the extracellular domain causes mislocalization of Jus to the corresponding cell bodies. The mechanism behind jus function is unclear and is complicated by its novel sequence. In an effort to elucidate its function, we screened for genetic interactors using RNAi of candidate genes in jus-expressing neurons as well as mutant alleles for these same loci. Expression reporters for HR38, a transcription factor that is highly-expressed in active neurons, was used as a marker for neuronal excitability before and after seizures in jus mutants.

607 Regulators of BMP signaling control injury induced nociceptive sensitization. C.L. Brann, J.K. Moulton, G.K. Ganter University of New England, Biddeford, ME.

Over 100 million people suffer from the effects of chronic pain in the United States alone. This burden also impacts the U.S. economy; 600 billion dollars annually is spent on medical care, medications, and lost productivity in the workplace. Current opioid treatments cause adverse effects including nausea, constipation, tolerance, and addiction liability. The neuroplastic process of pain sensitization is thought to perpetuate chronic pain, but too little is known about its mechanisms. Components of the pathways that connect injury and pain sensitization are likely to be valuable targets for novel medications for the relief or prevention of chronic pain. Utilizing the Drosophila melanogaster cell targeting and RNA interference toolkit, our lab investigates the Bone Morphogenetic Protein (BMP) pathway and its role in ultraviolet light (UV) injury-induced nociceptive sensitization. BMPs are well known as secreted developmental morphogens that control imaginal disc patterning by binding membrane bound receptors of target cells, but other functions are known. We have previously utilized a candidate gene approach to identify BMP signaling components used in nociceptive neurons to modulate injury-induced allodynia in Drosophila. The present study investigates the necessity of additional regulators of the BMP pathway in the formation of UV
injury-induced sensitization. The components of the BMP pathway are highly conserved and, because pain sensitization underlies chronic pain, these genes show potential to represent novel therapeutic targets in humans challenged by chronic pain.

608 Modeling Parkinson’s Disease in Drosophila: A Platform for Drug Testing. C.M. Frasik¹, M. Makarius¹, B. Gaynes², J. Jemc¹ 1) Loyola University Chicago, Chicago, IL; 2) Stritch School of Medicine, Maywood, IL.

Parkinson’s disease (PD) is the second most common neurodegenerative disorder present in the human population. Pathologically, the disease is believed to be linked to the overexpression and aggregation of the protein, alpha-synuclein, often observed post-mortem as Lewy bodies in the substantia nigra of the brain. The disease is associated with a number of symptoms, ranging from tremors and muscle rigidity to decreased visual acuity. These symptoms are a result of the progressive death of dopaminergic neurons, which in turn leads to decreased levels of dopamine. We are using a Drosophila melanogaster model of PD, in which we overexpress the human protein, alpha-synuclein, to model the disease and assess the efficacy of a variety of compounds as potential drug targets. First, we are evaluating the progressive degeneration of motor skills through a geotaxis assay, which tracks the climbing ability of flies over the course of twenty-one days. In addition, we are examining the progressive degeneration of rhabdomyeres in the eye via a pseudopupil analysis to better understand the basis of the ocular symptoms associated with PD. In conjunction with our methods of observing the motor and ocular symptoms of PD, imaging of brain tissues will be performed to determine whether aggregates of alpha-synuclein are forming utilizing immunohistochemistry. Following the analysis of controls and alpha-synuclein-expressing flies in each of these assays, we are beginning to test the efficacy of a variety of compounds for their ability to improve climbing ability, reduce rhabdomyere degeneration, and affect aggregate formation in the brain. Currently, we are focusing on polyphenolic compounds, as they have previously been demonstrated to inhibit alpha-synuclein aggregation and to promote its disaggregation in vitro. We are also analyzing the effect of L-DOPA, a common treatment for PD as a control, and black tea extract (BTE). The goal of these experiments is the identification of potential therapies that can be used to treat PD in humans.

609 Uncovering neuroregenerative mechanisms in the adult Drosophila brain. K.L. Crocker¹, K. Marischuk¹, G. Boekhoff-Falk¹,² 1) Genetics, University of Wisconsin-Madison, Madison, WI; 2) Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI.

Developing novel regenerative therapies depends on understanding endogenous repair mechanisms. We use an adult Drosophila brain injury model to dissect the cellular and genetic pathways underlying adult neurogenesis after injury. We use a sterile penetrating traumatic brain injury (PTBI) and test the extent to which adult flies can regenerate neural tissue in the central brain. We are focused on young adults that are thought to have a higher regenerative potential, and our model exhibits low levels of mortality. After PTBI, there is a significant increase in the number of proliferating cells, and some of the new cells give rise to neurons. Additionally, we observe an activation of glial cells after injury. To identify the trigger for glial activation, we conducted an RNA-seq experiment on heads from injured and control animals and found that the immune system was highly and rapidly upregulated post-injury. Preliminary studies indicate that the immune system plays a functional role in activating proliferation. Taken together, we have shown that neural regeneration can occur in the adult Drosophila central brain and that our model will allow us to dissect the mechanisms by which this occurs.

610 Identification of SAD and its glia-specific function in maintaining neural integrity during aging. S. Shu¹,², X. Cao¹,², Y. Deng¹,², Y. Deng¹, Y. Fang¹ 1) IRCBC, SIOC_Chinese Academy of Sciences, Shanghai, Shanghai, CN; 2) University of Chinese Academy of Sciences, Beijing, China.

Aging of the nervous system is a complicated process involving both neurons and glia. An integral nervous system is important for the longevity and healthy aging, but the mechanisms maintaining neural integrity during aging has not been fully understood. To reveal unknown genes and signaling pathways that are required for neural integrity during aging, we conducted a Drosophila genetic screen of brain-enriched genes and sought for the ones whose loss of function (LOF) would lead to shortened lifespan.

From the screen, we identified a novel gene, which we named SAD, whose adult-onset (Tubulin-GeneSwitch) downregulation not only significantly shortened the lifespan but also caused age-dependent neurodegeneration in the fly brain. Interestingly, despite that SAD was highly expressed in the fly’s nervous system, neuronal (elav-Gal4) downregulation of SAD showed no deleterious effect on the longevity or the brain integrity. In sharp contrast, glia-specific (repo-Gal4) knockdown of SAD resulted in striking neurodegeneration and significantly shortened the lifespan. Further investigation indicated that only the blood-brain barrier (BBB) glia and the cortex glia are involved in the aging function of SAD. Moreover, immunohistochemistry experiments and the Dextran injection assay indicated that the fly BBB and the glia matrix were severely damaged in the Repo-Gal4-RNAi-SAD flies.

To further investigate how SAD regulates glial functions and neural integrity, we have performed a RNA-seq analysis. The results suggest that SAD may function as a chromatin repressor in glia that keeps the innate immunity of the nervous system in check during aging. Without SAD repression, the immune response genes go unleashed, which, among other detrimental
consequences, leads to BBB disruption. In addition, our recent metabolomics data show abnormal lipid metabolism in the brain of the RNAi-SAD flies. Together, our study has revealed a glia-specific function of SAD in maintaining neural integrity during aging. Ongoing experiments are to establish the causal link of activated immune response with BBB disruption and lipid metabolism defects. By doing this study, we hope to cast new insights to the role and the mechanism of how glial regulation of innate immunity is involved in the process of aging of the nervous system.

611 Repair on the Fly: Trinucleotide Repeat Expansion during Homologous Recombination in Drosophila melanogaster. J. Blackmer, S. Dykstra, M. McVey Tufts University, Medford, MA.

Trinucleotide repeats are a source of genomic instability and cause a number of neurodegenerative diseases in humans, perturbing cellular metabolism on the DNA, RNA, and protein levels. Trinucleotide repeats can contract and expand during cellular processes such as transcription, replication, and DNA repair. Once expanded past a certain length, repeats cause neurodegenerative disorders, including Huntington's Disease. These diseases are highly heritable and exhibit genetic anticipation due to potentially large-scale expansions occurring in the germ line. When the DNA is single-stranded (during replication, transcription, or DNA repair), trinucleotide repeat expansions can occur. Homologous recombination creates ssDNA during the repair process. Homology directed repair of DNA double-strand breaks in the vicinity of trinucleotide repeats has been shown to cause expansions in yeast. In repeat-rich DNA sequences, recombination can cause hairpins to form in an attempt to stabilize the DNA. The hairpins can stall replicative polymerases involved in repair, leading to replication slippage and expansions.

CAG repeat expansions are thought to occur at an increased rate in the human male germ line, and are thus passed on in the expanded state to the next generation. Fruit flies serve as an ideal organism to observe germ line expansions. We have designed an assay to observe repeat expansion after induction of a site-specific DNA double-strand break and repair via homologous recombination. Using PhiC31 site-specific recombination, we inserted either 70 or 130 CAG repeats on chromosome 2. Experiments are underway to assess the mechanism of expansion during homologous recombination. In order to determine the roles of various repair proteins in repeat expansion, the assay can be performed in different mutant backgrounds, including translesion (TLS) polymerase mutants. In the absence of TLS polymerases, we anticipate increased repeat instability, as the replicative polymerases are not as adept at bypassing DNA hairpins as the TLS polymerases. Conducting these experiments in Drosophila, a metazoan, will provide novel insights into how trinucleotide repeat expansions occur in various tissue types, including the male germ line and as well as somatic tissues, and help to elucidate the molecular mechanisms that promote expansions.

612 Candidate Genes for Cocaine and Methamphetamine Preference in Drosophila melanogaster. C.A. Highfill, B.M. Baker, R.R.H Anholt, T.F.C Mackay Department of Biological Sciences, Genetics Program, W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695-7614.

In the United States, drug use accounts for monetary losses exceeding 193 billion dollars annually. Stimulant use disorder is characterized by drug-seeking behavior and consumption of psychostimulants in the face of deleterious effects. Our understanding of the mechanistic and physiological causes of stimulant use disorder is imperfect due to the difficulty in performing population genetic analyses in humans and vertebrate models. The Drosophila melanogaster Genetic Reference Panel (DGRP) consists of 205 sequenced inbred lines derived from a natural population. These lines are largely unrelated, highly polymorphic, and exhibit a rapid decay in local linkage disequilibrium – all favorable for genome wide association (GWA) mapping. We modified the Capillary Feeding (CAFÉ) assay to quantify consumption, preference, and tolerance for cocaine and methamphetamine relative to sucrose, and assessed variation in consumption, preference, and tolerance for 47 of the most genetically diverse DGRP lines. Briefly, each line was given a choice between sucrose and sucrose plus cocaine or methamphetamine for three consecutive days. Preference was scored as the proportion of drug consumed and tolerance as the difference in preference on days one and three. We observed significant genetic variation, including genetic variation in sexual dimorphism, for both traits, enabling us to perform GWA analyses for consumption, preference, and tolerance. We identified 830 variants in 411 genes at a nominal $P < 10^{-5}$. Several candidate genes were involved in dopamine signaling; others were novel. We used RNAi to knock down of gene expression for a sample of candidate genes with ubiquitous and brain-specific drivers and observed that a large proportion of RNAi targets affect preference and tolerance. Thus, Drosophila melanogaster can be developed as a powerful model system to identify evolutionarily conserved genes that affect drug-seeking behavior.

and amyotrophic lateral sclerosis, frontotemporal dementia, and Huntington's disease (HD) is the accumulation of misfolded proteins into insoluble aggregates, leading to appearance of inclusion bodies in regions of the brain undergoing neurodegeneration. Accumulation of misfolded proteins into insoluble aggregates in turn leads to reduced Complex V activity. Cells from subject 1 also exhibit a significant decrease in mitochondrial cristae.

ATP synthase, H+ transporting, mitochondrial F1 complex, δ subunit (ATP5D) is a subunit of mitochondrial ATP synthase that plays an important role in coupling proton translocation and ATP production, but it has not yet been implicated in human disease. Here we describe two individuals, each with homozygous missense variants in ATP5D, who presented with episodic lethargy, metabolic acidosis and hyperammonemia. Subject 1, homozygous for Pro82Leu (c.245C>T), presented with recurrent metabolic decompensation, while subject 2, homozygous for Val106Gly (c.317T>G), presented with acute encephalopathy in childhood. Fibroblasts from these individuals exhibited impaired assembly of F$_{1}$F$_{0}$ ATP synthase in turn leading to reduced Complex V activity. Cells from subject 1 also exhibit a significant decrease in mitochondrial cristae.

Knockdown of Drosophila ATPsynδ, the ATP5D homolog, in developing eyes and brains causes a near complete loss of the fly head, a phenotype that is fully rescued by wild-type human ATP5D. In contrast, rescue with the P82L and V106G variants, lead to flies with near normal head sizes that exhibit eye associated phenotypes seen in other models of oxidative phosphorylation deficiency. Our data establish ATP5D as a novel mitochondrial Mendelian disease associated gene featuring episodic metabolic decompensation.


A key pathological feature of neurodegenerative diseases [Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia, and Huntington's disease (HD)] is the accumulation of misfolded proteins into insoluble aggregates, leading to appearance of inclusion bodies in regions of the brain undergoing neurodegeneration. Accumulating evidence supports the hypothesis that pathogenic protein aggregates associated with neurodegenerative disease propagate through the brain with prion-like properties. Similar to infectious prions, these aggregates spread from cell to cell and template the conformational change of normally soluble versions of the same protein. We have previously demonstrated prion-like transmission of mutant huntingtin (Htt) aggregates associated with HD between olfactory receptor neurons (ORNs) and neighboring phagocytic glia in the Drosophila central nervous system (CNS) (Pearce et al., 2015, Nat Commun). Transfer of mutant Htt aggregates from ORNs to glia requires the phagocytic receptor Draper and results in aggregation of normally soluble, wild-type Htt in the glial cytoplasm. Thus, a proportion of neuronal Htt aggregates engulfed by glia can escape from Draper-dependent phagocytosis and effect prion-like conversion of cytoplasmic, wild-type Htt. We also demonstrate that mutant Htt aggregates spread from ORNs to the cytoplasm of their post-synaptic partners, projection neurons (PNs). Surprisingly, ORN-to-PN transfer of mutant Htt aggregates requires expression of glial Draper, suggesting that phagocytic glia mediate trans-synaptic prion-like spread of aggregates. Together, these findings demonstrate that pathogenic Htt aggregates can transfer between neurons and glia in intact brains and suggest a central role for the conserved Draper-dependent phagocytic pathway in both the clearance of potentially toxic aggregates from neurons and spreading of aggregate neuropathology across synapses.


TAR DNA-binding protein 43 (TDP-43) is a highly conserved DNA/RNA binding protein with primarily nuclear distribution. However, it translocates to the cytoplasm and forms pathological aggregates in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). Despite considerable efforts to investigate the physiological role of TDP-43, we still...
have a very limited understanding of the molecular and cellular mechanisms of pathogenesis underlying TDP-43 proteinopathies. A key challenge, therefore, is to identify critical proteins and pathways mediating neuronal degeneration. To shed light on this issue, we searched for genetic modifiers of neurotoxicity in transgenic flies expressing human TDP-43<sup>315T15</sup>, which display a very reliable phenotype in the eye. Thus, we crossed these flies with a library of 6,261 RNAi strains obtained from the Vienna Drosophila Resource Center. In a primary screen, we identified almost 300 modifiers of mutant TDP-43 toxicity. Then we verified the results of the primary screen to eliminate variability and account for different observers. We also examined the specificity of the enhancers by making sure that they do not induce abnormal eyes on their own. So far, we have confirmed 55 robust suppressors, 120 enhancers and 27 lethals. Interestingly, many suppressors are linked to transcription, mRNA elongation and splicing. We also confirmed the role of genes involved in nucleocytoplasmic shuttling. Furthermore, we also found a number of suppressors linked to other functions such as neurogenesis, syntaxin binding, mitochondrial transport, ubiquitin activity, phosphatidylinositol signaling, and protein quality control to name a few. In summary, this loss-of-function screen has led to the identification of several genes and molecular pathways not previously known to be associated with TDP-43 pathologies. This work was supported in part by grants NS096647 (DERL) and F058A204 (DERL and JL).

616 Modeling Chronic Myeloid Leukemia in Drosophila melanogaster. A. Al-Outa<sup>1</sup>, A. Bazarbachi<sup>1,2</sup>, M. El-Sabban<sup>1</sup>, * M. Shirinian<sup>3</sup>, * R. Nasr<sup>1</sup>, * Corresponding authors 1) Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine, American University of Beirut, Beirut, LB; 2) Department of Internal Medicine, Faculty of Medicine, American University of Beirut, Beirut, LB; 3) Department of Experimental Pathology, Microbiology and Immunology, Faculty of Medicine, American University of Beirut, Beirut, LB.

Chronic myeloid leukemia (CML) is caused by a balanced chromosomal translocation resulting in the formation of BCR-ABL fusion gene that encodes a constitutively active BCR-ABL tyrosine kinase which activates multiple signal transduction pathways leading to cancer. Several treatment modalities have been proposed using tyrosine kinase inhibitors; however, some mutations have proven elusive particularly the T315I mutation. Drosophila melanogaster is an established in vivo model of human disease including cancer. The targeted expression of the chimeric human/fly BCR-ABL in Drosophila eyes and central nervous system has been shown to result in detrimental effects. This study is designed to model human CML in Drosophila melanogaster as a platform for drug screening and to explore mechanisms of action. We will first characterize the phenotypic effects associated with the expression of human BCR-ABL (wild-type p210 and p210<sup>T315I</sup>). The expression of chimeric human/fly BCR-ABL and human BCR-ABL (wild-type p210 and p210<sup>T315I</sup>) in different Drosophila imaginal discs using the GAL4-UAS system resulted in abnormal development of Drosophila tissues at different developmental stages. Rough eye phenotype is one of the striking abnormalities observed upon transgene expression. We will further dissect the mechanisms involved in BCR-ABL pathogenesis, using genetic epistasis methodology, to identify pathways which can modify or attenuate the rough eye phenotypes observed. Our studies are poised to shed light on BCR-ABL induced CML pathogenesis and the underlying mechanisms governing it and provide an efficient in vivo model for drug screening.

617 Using the Drosophila melanogaster accessory gland as a model for prostate cancer. A. Box, J. Church, D. Hayes, P. Hakim, L. Buttitta Dept. of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Mi.

The D. melanogaster accessory gland (AG) is a secretory epithelium functionally analogous to the mammalian prostate. The use of D. melanogaster as a model for prostate cancer is advantageous due to its rapid life cycle and facile genetic manipulation. Prostate cancer risk is age-associated and the human prostate often grows and becomes hypertrophic with age. We measured the normal, physiological level of cell cycling in the adult D. melanogaster accessory gland along with tissue morphogenesis and growth. Similar to the human prostate, we find evidence of continued growth and age-associated cellular hypertrophy in the accessory gland. We developed a FACS protocol to analyze DNA content and examine aneuploidy/hyperploidy in this tissue and we can distinguish between the two epithelial cell types of the gland, the “main” cells and the secretory “secondary” cells. We have confirmed that a gene associated with prostate cancer, YAP (Yki) generates a hyperplastic mass in the accessory gland when a gain of function allele is expressed. To our knowledge this is the first demonstration of a tumor-like structure in the accessory gland of D. Melanogaster.

618 Pharmacological screens in a fly model of polycystic kidney disease. Cassandra Millet-Boureima<sup>1</sup>, Ramesh Chingle<sup>2</sup>, William D. Lubell<sup>2</sup>, Chiara Gamberti<sup>1</sup> 1) Biology, Concordia University, Montreal, PQ, CA; 2) Department of Chemistry, Université de Montréal, Montreal, Canada.

Polycystic Kidney Disease (PKD) is a genetic disorder that affects 12.5 million people world-wide for which there is no cure. PKD causes the formation of numerous cysts and progressive kidney degeneration. Without effective drugs, dialysis and kidney transplants are the only currently available treatments for PKD. In vertebrates, mutations of the Bicoid C (BicC) gene lead to cystic kidneys. We have recently published that BicC fly mutants display cysts in the Malpighian (renal) tubules as well as phenotypic and molecular hallmarks of PKD. Moreover, we demonstrated that BicC is downstream of the PKD1 gene which is implicated in 85% of all cases of autosomal dominant PKD. In murine PKD models, mimics of the second mitochondrial activator of caspases (Smac) reduced proliferating cystic cells.
Smac mimics were also found to induce apoptosis in cultured cancer cells. Using our first-in-kind *Drosophila* model of PKD, we have established pharmacological assays to probe a novel set of Smac mimics for their ability to modify the BicC mutant fly cystic phenotype. Similar to vertebrates, Smac mimics were found to rescue the BicC flies with improved cystic index and increased longevity. We are currently characterizing the mechanism of action of the active molecules. Our results add to a growing body of evidence that *Drosophila* can be effectively used to discover molecules with therapeutic potential.

619 Modeling pheochromocytoma and paraganglioma in *Drosophila*. J.M. Portilla, W.M. Deng  Department of Biological Science, Florida State University, Tallahassee, FL.

Pheochromocytomas and paragangliomas are rare catecholamine secreting tumors which can arise in multiple locations throughout the body. Typically pheochromocytomas originate in the adrenal glands and can occur extra adrenally, while paragangliomas can form in the head, neck, or abdomen. Symptoms can be fleeting and include headaches, hypertension, abdominal or chest pain, excessive sweating and a myriad of other ailments. Various mutations potentiate the formation of these tumors including insults to genes such as von Hippel-Lindau (VHL), rearranged during transfection (RET), neurofibromin 1 (NF1), and the four subunits of the succinate dehydrogenase complex (SDHx). These genetic causes have split pheochromocytomas into two clusters, with the first cluster consisting of SDH and VHL mutations and the second cluster consisting of RET and NF1 mutations. Mutations in the different subunits (SDHA-D) have been shown to be capable of spurring tumorigenesis via a pseudohypoxic pathway in which hypoxia inducible factor (HIF) is stabilized by the accumulation of succinate and generation of reactive oxygen species (ROS) interfering with its degradation. Although these tumors are rare, they present the possibility of further understanding the multitude of cellular contexts conducive to this tumor formation as each different subunit mutation is associated with tumorigenesis in a different area. The SDH genes are highly conserved between flies and humans, however no pheochromocytoma model has been established in flies. Utilizing the temporal and regional gene expression targetting (TARGET) system for temporal control of RNAi knockdown of first and second cluster mutations has yielded promising results. The majority of flies with any of the SDHB-D subunits knocked down die during pupation. In addition to the lethal phenotype, SDHC RNAi larvae accumulated dark colored masses of cells sporadically and clustered around the midgut. The study of pheochromocytoma and paraganglioma related genes in the fly model offers an opportunity to better comprehend the nature of the underlying mutations and defective pathways responsible for these unique neuroendocrine tumors.

620 NOTCH2-NOTCH3-DLL3-MAML1-ADAM17 signaling network is associated with ovarian cancer. Jesse Underwood, Dongyu Jia  Department of Biology, Georgia Southern University, Statesboro, GA.

Notch signaling has been well known for its role in regulating cell self-renewal and differentiation. Within the cancer research field, scientists have discovered that dysregulated Notch signaling is directly involved with various types of cancer. Although Notch signaling has been generally considered as oncogenic, it can sometimes act as a tumor suppressor, highlighting the complexity of Notch in cancers. Recently, many studies have linked Notch signaling components with ovarian cancer, but the associated clinical and molecular mechanisms were not well elucidated. In our study, we systematically analyzed the roles of Notch main components in ovarian cancer through large data portals, including PRediction of Clinical Outcomes from Genomic Profiles (PRECOG), Gene Expression across Normal and Tumor tissue (GENT), CSIOVDB and cBioPortal. We demonstrated that upregulated expression of Notch components in ovarian cancer was generally associated with poor overall survival, disease-free survival and increased cancer stages. In addition, Notch components were enriched in cancer tissues. These results led to our proposed NOTCH2-NOTCH3-DLL3-MAML1-ADAM17 network. Currently, we are utilizing *Drosophila* egg chamber system to manipulate *Drosophila* homologs of these Notch components to create ovarian cancer models. We aim to develop anti-cancer drugs to target the specific models, leading to Notch-related ovarian cancer treatment.

621 Probing intracellular pH dynamics during invasive migration in the *Drosophila* wing. V. Bui, B. Grillo-Hill  Biological Sciences, San Jose State University, San Jose, CA.

Directed cell migration requires many precise and coordinated steps, including early polarization of the leading edge. Following polarization, proteins are localized to the leading edge of migrating cells where they can coordinate and regulate the cellular machinery that facilitates migration. Inappropriate activation of cellular migration leads to developmental defects and diseases including metastatic cancer. One important protein with polarized localization is the ubiquitously-expressed plasma membrane resident sodium-proton exchanger NHE1, which maintains pH homeostasis. Increased pH is implicated in directed cell migration in cultured mammalian cells, however these ideas have not been tested in more complex tissues environments in vivo. We previously showed that over-expression of DNhe2 (the homolog of mammalian NHE1) caused increased pH in *Drosophila* eye and wing imaginal discs. We used the patched-GAL4 driver to express oncocgenic RasV12 in a stripe of cells in the wing imaginal disc. We found that co-expression of DNhe2 induced invasive cell migration, such that cells crossed the anterior-posterior compartment boundary. These RasV12+DNhe2-expressing cells displayed characteristic phenotypes of metastatic cells, including basal expansion, single cell invasion, and invasive streaming of cells. We are
KDM5 is required to regulate bouton number at the NMJ. Additionally, a demethylase and compared this function during development, we examined the architecture of the neuromuscular junction (NMJ) in mutant larvae specifically lacking histone demethylase activity. This revealed that the demethylase activity of KDM5 with consequences for cognitive and motor functions.

Since KDM5 proteins are transcriptional regulators, we hypothesized that these mutations affect the transcriptional function of KDM5 proteins in neurons. Likewise, it was demonstrated in C. elegans that rbr-2, the kdm5 orthologue, is necessary for axon guidance. In humans, mutations in three of the four kdm5 paralogs have been described in patients with neurodevelopmental disorders. Since KDM5 proteins are transcriptional regulators, we hypothesized that these mutations affect the transcriptional function of KDM5 with consequences for cognitive and motor functions.

Using qRT-PCR we found deviations in the maturation process of tRNAs. With inhibitory agent chloroquine abolished Daw RNAi-related beneficial effects on age-related cardiac phenotypes. Thus, our findings suggest that activin signaling regulates cardiac aging and autophagy through an mTOR complex 1 independent pathway, which will provide critical supporting evidence for the development of therapeutic interventions targeting TGF-beta/activin signaling for cardiac disease treatment.

Heart-specific activin signaling promotes cardiomyopathy and organonal aging through autophagy inhibition. K. Chang, Bai Hua Genetics, Development, and Cell Biology, Iowa State University, Ames, IA.

Age-dependent loss of cardiac homeostasis largely impacts heart performance, and dramatically increases the incidence of cardiac diseases. TGF-beta has been previously linked to age-related cardiac hypertrophy and remodeling, however, the mechanisms through which TGF-beta regulates cardiac aging remain largely unknown. Using Drosophila as a model system, I found that activin, a member of TGF-beta family, negatively regulates cardiac autophagosome number and promotes cardiac aging. Heart-specific knockdown of Daw, fly homolog of activin, delays age-dependent increases of arrhythmia and diastole, while heart-specific overexpression of activin receptor Babo results in premature cardiac aging. It is known that mTOR, especially mTOR complex 1 is the central regulator for longevity and autophagy. However, activation of mTOR complex 1 activity did not rescue the autophagy and cardiac aging phenotypes in Drosophila RNAi flies. Later we found that disruption of autophagy by knocking down Atg1 or feeding flies with lysosomal inhibitor chloroquine abolished Daw RNAi-related beneficial effects on age-related cardiac phenotypes. Thus, our findings suggest that activin signaling regulates cardiac aging and autophagy through an mTOR complex 1 independent pathway, which will provide critical supporting evidence for the development of therapeutic interventions targeting TGF-beta/activin signaling for cardiac disease treatment.

Creating Drosophila model of infantile hypertrophic cardiomyopathy to study the underlying mechanism of this pathology. Ekaterina Migunova, Marisa Mercadenter, Lian Duan, Luisa Göpfert, Jing Men, Chao Zhou, Edward Djabrovsky 1) Biology, Fordham University, Bronx, NY; 2) Electrical and Computer Engineering, Bioengineering, Lehigh University, Bethlehem, PA; 3) Hamburg University of Applied Sciences, Germany.

Hypertrophic cardiomyopathy (HCM) is a common inherited condition, which occurs in 1 out of 500 people. Certain recessive alleles of ELAC2 gene have been associated with especially severe form of HCM. Newborns homozygous for these alleles do not live past their first birthday and die from heart failure. ELAC2 is a highly conserved gene with homologs in all eukaryotes. It encodes RNase Z endonuclease that plays a critical role in maturation of tRNA molecules. Previously, we reported identification and analysis of dRNaseZ, the Drosophila homolog of ELAC2. Currently, we are working on creating a fly model for ELAC2-related cardiomyopathy. Two ELAC2 missense mutations, Leu423Phe and Thr520Ile, are conserved between human and fly proteins. To understand the molecular mechanisms of heart damage resulting from these mutations we created transgenic flies harboring dRNaseZ with HCM mutations in the background of endogenous dRNaseZ knockout. These flies have shown decreased viability and delay in development with most of them reaching adult stage. We are characterizing them at molecular, anatomical, and physiological level. Using qRT-PCR we found deviations in the maturation process of tRNAs. With histological sections, we observed changes in the heart wall thickness. Finally, we used a novel ultra-high-resolution optical coherence microscopy (OCM) imaging system to obtain in vivo, non-destructive imaging of the Drosophila heart. Time-lapse cross-sectional OCM images were acquired at a rate of 128 frames per second to capture fly heartbeat, with axial and transverse resolutions of ~1.5 and ~3.9 micrometers, respectively. First analysis shows changes in heart wall thickness and heart rate in HCM mutant flies. These data suggest the utility of the Drosophila model to study ELAC2-related cardiomyopathy.

Neurodevelopmental and transcriptional defects caused by KDM5 loss of function. H.M. Belalcazar, C. Drelon, S. Zamurad, J. Secombe Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, USA.

The KDM5 family of histone demethylases are key transcriptional regulators during development, especially for neuronal tissue. Studies in mice have indicated that KDMSC is required for correct dendritic pattern and spine density in pyramidal neurons. Likewise, it was demonstrated in C. elegans that rbr-2, the kdm5 orthologue, is necessary for axon guidance. In humans, mutations in three of the four kdm5 paralogs have been described in patients with neurodevelopmental disorders. Since KDM5 proteins are transcriptional regulators, we hypothesized that these mutations affect the transcriptional function of KDM5 with consequences for cognitive and motor functions.

In Drosophila there is a single orthologue of kdm5, initially called little imaginal disc (lid). In order to elucidate the effects of loss of function of kdm5, we have generated a null allele, kdm5, by imprecise excision of a P-element in the regulatory region. This strain displays developmental delay and pupal lethality. To define the neuronal defects associated with loss of KDM5 function during development, we examined the architecture of the neuromuscular junction (NMJ) in kdm5 null mutant larvae and compared this to larvae specifically lacking histone demethylase activity. This revealed that the demethylase activity of KDM5 is required to regulate bouton number at the NMJ. Additionally, a demethylase-independent activity is required to
regulate bouton size.
We are also examining the effects of alleles of Drosophila kdm5 that are analogous to mutations found in patients with neurodevelopmental disorders. Unlike the null allele, these strains are adult homozygous viable allowing us to determine the transcriptional defects associated with these alleles in the adult brain. Gene ontology and pathway analysis showed that disease-associated missense mutations in kdm5 cause alterations in cytoplasmic translation and neuromodulator signaling pathway. We are currently analyzing the possible direct targets of KDM5 in these pathways that might have a strong contribution in the development of neuronal circuitry.

625 Functional analysis of ANKLE2 in microcephaly using a genetic model system. N. Link1, P. Shah2, H. Chung1, N. Krogan3, H. Bellen1 1) Baylor College of Medicine, Houston, TX; 2) University of California, Davis, CA; 3) University of California, San Francisco, CA.

Microcephaly, or reduced head size, is often the result of a neurodevelopmental disease with associated cognitive and neurological defects. In a forward genetic screen identifying genes causing neurodegeneration or neurodevelopmental phenotypes, we identified a mutation in Ankle2 (Drosophila ankyrin repeat and LEM domain containing 2), which results in a small brain phenotype. To find a possible link between the human homolog, ANKLE2, and human disease, we surveyed the exome database of the Baylor-Hopkins Center for Mendelian Genomics (BHC MG) for mutations linked to rare human Mendelian disorders. Using this strategy, we identified compound heterozygous mutations in ANKLE2 in two patients that exhibit severe microcephaly as well as cognitive and neurological defects. Interestingly, our data suggest that loss of Ankle2 in Drosophila causes cell loss in the central nervous system, mimicking microcephaly phenotypes found in humans. Ankle2 mutants also exhibit defects in the peripheral nervous system and contain fewer neuronal stem cells that divide less frequently. We surmise that Ankle2 is required for progenitor cell maintenance, division, and survival in the nervous system. In addition, the recent outbreak of Zika has revealed that infectious microcephaly can mimic ANKLE2 associated disease. We are currently investigating the link between Zika infection and Ankle2 using Drosophila, and lessons learned here will translate to studies of genetic and infectious microcephaly and our understanding of neurodevelopmental disorders in humans.

626 A small molecule screen identifies defective prothoracic gland function in a fly model of N-glycanase 1 deficiency. J.D. Mast1, T. Portillo Rodriguez1, T. Hart2, E. Perlstein1 1) Perlara PBC, South San Francisco, CA; 2) BioMarin Pharmaceutical, San Rafael, CA.

Perlara PBC, is a public benefit company committed to discovering small molecule therapeutics for rare genetic diseases using genetic models such as yeast, worm, and flies in high-throughput drug discovery screens. This parallel, multi-model whole animal screening approach leverages shared evolutionary pathways and increases the probability of rapidly identifying potential therapeutics. We have worked closely with Grace Science Foundation to find such a therapeutic for N-glycanase 1 (NGLY1) deficiency, a disorder caused by mutations in N-glycanase 1 that leads to developmental delay, movement disorders, hypotonia, and peripheral neuropathy. Flies mutant for the ortholog of NGLY1 (pogl) are developmentally delayed during larval stages, exhibit partial pupal lethality, and are small as adults. To find chemical modifiers of pngl, we carried out a small molecule screen for compounds effecting the developmental delay phenotype in these flies. Of ~2,500 unique compounds screened, the steroid 20-hydroxyecdysone (20E) suppressed the small larva phenotype of the pngl mutant fly. Expression of human NGLY1 in the prothoracic gland, the site of synthesis for 20E precursors provided complete rescue of the pngl mutant's developmental delay. These data indicate that cell autonomous defects in the prothoracic gland, leading to diminished 20E levels, explain the developmental delay phenotype of the NGLY1 fly. These results are also consistent with the possibility that this pngl phenotype can be modified by other small molecules in a larger chemical screen.

627 The Involvement of the Myogenic Gene nautilus in Muscle Development. J. Cordova, R. Cripps  Department of Biology, University of New Mexico, Albuquerque, NM.

In vertebrates, MyoD belongs to a family of Myogenic Regulatory Factors (MRFs) that are essential for muscle differentiation. In Drosophila, the only MyoD homolog, nautilus, plays a role in the differentiation of progenitor cells into mature muscle fibers. However, there is controversy over the requirement for MyoD in this process. To resolve this controversy we will investigate the requirement and sufficiency of nautilus to promote muscle development. Individuals homozygous for nautilus mutations were examined at stage 16 of embryonic development so that the nautilus mutant phenotype could be characterized. These mutant embryos exhibited slight to severe alterations in a subset of muscles, indicating that a deficiency of nautilus leads to poor muscle development.

In order to visualize the importance of nautilus for myogenic conversion we transfected a line of Drosophila cells with cDNA expressing nautilus, daughterless, and Mef2, which allowed us to observe myogenic conversion of the cells. We hope to determine if nautilus is able to initiate myogenic conversion in tissue culture by itself or when coexpressed with other basic-Helix-Loop-Helix (bHLH) or MADS-domain box transcription factors. In addition, we hope to obtain a better understanding of how these transcription factors interact with one another, which will help us decipher the overall mechanism of muscle differentiation in Drosophila. In preliminary data, we found that the expression of nautilus, daughterless, and Mef2 was
sufficient to induce myogenesis. This will then give us a basic understanding of how orthologous genes for muscle development function in various organisms.

### 628 Study of Human Ovarian Development & Dysgenesis Mechanisms in a Drosophila Model.

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The orchestrated signal transduction mechanism and genes underlying ovarian development are poorly understood. XX-Ovarian Dysgenesis (XX-OD) is a rare heterogeneous genetic disorder characterized by underdeveloped, dysfunctional ovaries. Using homozygosity mapping and whole exome sequencing, combined with bioinformatics, we identified a novel homozygous missense mutation in the Nucleoporin107 (Nup107, c.1339G>A, p.D447N) gene as the candidate causative mutation for XX-OD in a consanguineous family with multiple affected females. Nup107 is an essential component of the nuclear pore complex.

Drosophila has proven to be a useful model for human diseases in which orthologous genes exist. To assess the possible role of Nup107 in ovarian development, we created Drosophila flies carrying the specific mutation corresponding to the human mutation found in the family. Strikingly, the transgenic Drosophila females were almost completely sterile with a marked reduction in progeny, morphologically aberrant eggshells and disintegrating egg chambers with increased apoptosis, indicating defective oogenesis. A closer look at the mutant ovaries demonstrated an ovarian dysgenesis like phenotype, where nearly 40% of the female mutant flies had either non-developed or under-developed ovaries. Upon analysis, we found that the larval gonads were fully present in the mutant larvae, indicating that the developmental failure must occur along the way and that the underlying genetic causes are already present at the larval or even embryonic stages. Consistent with this notion, we found that mutant larval gonads contained excess primordial germ cells (PGCs) and lacked intermingled cells (ICs), suggesting problems in the essential soma-germline interactions required for both growth and differentiation. These results suggest a pivotal conserved role for this nuclear pore gene in ovarian development.

Transcriptome analysis of WT and mutant larval and adult ovaries identified several dozen candidate genes whose expression is significantly affected by the mutation. In addition, we discovered a group of genes with unknown functions, all of whom are highly expressed in the larval gonad and not expressed in the adult ovary, suggesting them to be critical developmental genes. Most of our candidate genes have human orthologues, further underscoring their relevance to human ovarian development.

Future research using this XX-OD Drosophila model system will elucidate signaling pathways and new genes involved in ovarian development and dysgenesis.

### 629 The use of the Drosophila Genetic Reference Panel to map Genes and Gene Network Underlying High Fat-Diet induced Mortality.

B Danso Konadu, Matthew Talbert

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Diet-induced obesity does not guarantee a disease state in humans and animal models; however it considerably increases the risk of many diseases such as diabetes, cardiovascular disease, neurological decline, and cancer, increasing the risk of mortality in mammals. There is a great interest in unraveling the mechanisms that transition from “healthy obese” to “obese and diseased” in mammals. The genetic factors underlying obesity-induced mortality in humans cannot be easily investigated due to their naturally long lifespan. A resemblance to the mammalian obesity state is replicated in Drosophila melanogaster, a short life-span fruit-fly model that possesses parallel aspects of behavior and physiology with humans, especially when fed with High Fat Diet (HFD). The Drosophila Genetic Reference Panel (DGRP) was employed to allow for a genome-wide association study of lifespan upon a HFD. In our case, HFD involved supplementation of 4%, 3%, 2.6% w/v of yeast/sucrose/cornmeal medium, with 20% w/v coconut oil. Twenty mated female flies of each line from the DGRP (200 lines) were obtained by synchronously crossing virgin female of the lines with Bloomington stock 1 male flies over 2 days on normal media. Flies were kept on HFD at equal density and humidity at 23°C on a 12 hour light/dark cycle. Deaths were recorded daily to determine average lifespan. Adjustment of mean lifespan for block effect was performed and the means were submitted to the DGRP analysis pipeline. ANOVA were conducted utilizing chromosomal inversion status, Wolbachia infection status, and cryptic relatedness as covariates. There was significant variation in average lifespan (minimum of 7 days and maximum of 44 days). Wolbachia infection status was not associated with HFD, however one of the 5 major chromosomal inversions (In_3R_K) was correlated (P=0.002) with lifespan of DGRP lines fed on HFD. Polymorphisms and genetic loci linked with variation in lifespan on HFD via linear mixed model were identified. Genes such as Pvf3, which is associated with hemocyte migration, fumble for phosphorylation in coenzyme A synthesis, and others were isolated (P-values for top hits ranged from 10^-9 to 10^-10).
630  **Studying the in vivo effects of human pathogenic mutations in the mt-tRNA processing complex Mitochondrial RNome.**  M. Saoji, A. Sen, R. T. Cox  Biochemistry, Uniformed Services University of Health Sciences, Bethesda, MD.

Mitochondria have evolved endosymbiotically from alpha proteobacteria trapped within the eukaryotic cell. These organelles supply ATP and play a critical role in cell homeostasis, apoptosis and signal transduction. Mitochondrial dysfunction is implicated in several cardiovascular and neurological disorders. Owing to its bacterial origin, mitochondria have their own genetic material (mtDNA) which encodes for proteins of the electron transport chain along with ribosomal and transfer RNAs (mt-tRNA). Interestingly, even though only ~9% of the mtDNA encodes for mt-tRNAs, point mutations in mt-tRNAs cause a disproportionately large number of mitochondrial diseases, which have been shown to manifest specific symptoms like cardiomyopathy and hypertension. Like bacteria, the mtDNA is transcribed as a polycistron. To become functional, mt-tRNAs must be excised from the adjacent protein coding and ribosomal RNA region. The Mitochondrial Ribonuclease P (MRPP) complex processes the 5'end of mt-tRNAs in humans. The human MRPP is a three-protein complex composed of MRPP1, MRPP2 and MRPP3. MRPP3 is the catalytically active subunit while MRPP1 and 2 have been shown to be required for the catalytic activity of MRPP3. Evidence suggests that mutations in the MRPP complex cause myocardial infarction, coronary heart diseases and cardiomyopathy. Our lab studies the Drosophila homologs of the MRPP complex: Roswell (MRPP1), Scully (MRPP2) and Mulder (MRPP3) in order to dissect how defects in this complex affect mitochondrial function and mt-RNA processing. We demonstrated that each of these proteins co-localize to mitochondria and are required for survival. Loss of each of these proteins is associated with mitochondrial deficits partly due to reduced mt-tRNA processing. Currently, we are generating fly lines with human pathogenic mutations in MRPP to study their in vivo effect on mt-tRNA processing, mitochondrial function and tissue homeostasis. These studies will advance our knowledge of the mechanistic detail underlying mt-RNA processing and how processing defects cause human mitochondrial diseases.

631  **WRNexo protects against aging and oxidative stress in Drosophila.**  Elaye Bolsterstein1, Robert Salomon2, Mitch McVey1, Chris Corso1, Deirdre Cassidy1, Deborah Onofrei1, Charlotte Salameh1, Luhan Zhou1 1) Biology, Northeastern Illinois University, Chicago, IL; 2) Department of Pathology, Tufts University School of Medicine, Boston, MA; 3) Department of Biology, Tufts University, Medford, MA.

As cells age, they accumulate DNA damage that can lead to genomic instability and cancer. RecQ helicases, such as Werner protein (WRN), have essential roles preventing and repairing DNA damage caused by environmental, replicative, and oxidative stress. In humans, mutations in WRN cause Werner syndrome, an autosomal recessive disease characterized by patients’ increased risk of cancer and early onset of aging-related pathologies. While human WRN exhibits helicase and exonuclease functions, the Drosophila homolog of WRN, WRNexo, contains only the exonuclease domain, providing us with a unique model to study exo-specific functions largely uninvestigated in human cells. Our studies use flies containing a null allele of WRNexo, WRNexoΔ, which we have previously shown to be deficient in repairing replication-induced DNA damage. We have recently found that compared to age-matched wild type controls, WRNexoΔ flies exhibit shorter lifespans and higher tumor incidence. WRNexoΔ also show increased physiological signs of aging such as degeneration of the flight muscles and reduced locomotor activity. Although WRNexoΔ are not sensitive to hydrogen peroxide at larval, adult, and aged adult developmental stages, the mutants display increased total antioxidant activity that declines with age. These data suggest that WRNexo may play a role in preventing aging pathologies caused by increased oxidative damage. This and future mechanistic studies will contribute to our knowledge of DNA repair mechanisms and their role in cancer prevention.

632  **When You Give a Fly a Tuna Sandwich: Screen of Tox21 Compounds Identifies Methylmercury as an Intestinal Toxin.**  J. DiRusso, M. Johnson, M. Hammond, H. Dayton  Biology, University of Massachusetts Amherst, Amherst, MA.

The Drosophila intestine is emerging as a key model in understanding the response of stem cells to environmental stressors such as chemotherapeutics, fungi and bacteria. We sought to leverage the well characterized molecular responses of intestinal stem cells to insult as well as the tractability of the Drosophila intestine to develop a high throughput system to assess impacts of compounds on the health of stem cells and their surrounding microenvironment in vivo. As a proof of principle, we partnered with the National Institute of Environmental Health Science's Tox21 program to assay a set of 80 compounds on intestinal stem cell health. Of the 80 compounds tested, we identified methylmercury as having a stimulatory effect on stem cell proliferation. This is a surprising result because methylmercury is best known for its deleterious role as a neurotoxin in humans.

Our findings suggest that methylmercury is an irritant to the gut and induces stress responses leading to hyperproliferation of stem cells. Indeed, we find that the canonical evolutionarily conserved JNK stress and JAK-STAT inflammation pathways are induced by methylmercury. Given the role of inflammation in initial tumor development and the highly conserved nature of these pathways, our results suggest methylmercury may trigger proliferation in humans and act as a carcinogen,
corroborating studies performed in rodents showing methylmercury may drive carcinogenesis. Currently, epidemiological data is not strong enough to link methylmercury exposure to cancer, however our results suggest that these conclusions should be reexamined.

**633 Drosophila as an Essential Genetic Model for Kidney Stones.** S. Ghimire¹, P. Cabrero¹, S. Terhzaz², M. F. Romero ², S. A. Davies¹, J. A.T. Dow¹ 1) University of Glasgow, Glasgow, UK; 2) Physiology & Biomedical Engineering, Mayo Clinic, Rochester, MN.

**Introduction**

Nephrolithiasis is one of the most common kidney diseases with poorly understood pathophysiology. Most human kidney stones are composed of Calcium Oxalate and Calcium Phosphate (>70%), followed by Struvite and uric acid (8-10%) and cysteine or ammonium acid (1%). Complexity in the physiological system has made it difficult to use mammals as a model organism for this disease. Hence, we addressed this issue by using *Drosophila melanogaster* as an ideal model for renal diseases because of its genetic and functional similarities with the human kidney.

**Methods**

Here, we inhibited the function of the gene, *Sip1* (SRY-interacting protein 1), the homolog of human Na⁺/H⁺ exchanger regulatory factor (NHERF1), which resulted in an accumulation of stones in Malpighian Tubules. To confirm the physical and chemical properties of the stones, flies were dissected in the bathing solution of different pH, imaged and quantified using Image J software. Furthermore, flies were fed with allopurinol and the level of uric acid was measured using commercially available uric acid measurement kit. The mechanism behind stones formation was determined using immunohistochemistry techniques.

**Summary**

We found that mutation of *Sip1* gene led to abundant stones formation, in both the tubules of male and female flies. The solubility of the precipitated stones increased with increasing pH of the bathing solution and vice versa. No any stones accumulated in mutant tubules after feeding flies with allopurinol (an inhibitor of xanthine oxidase, and hence purine metabolism), suggesting that the stones were composed of uric acid. Uric acid levels in MTs of *Sip1* mutant flies (-/-) was 10 fold high as compared to wild types (+/+). In addition to that, we hypothesize that the co-transport of Na+/H+ is mediated by the exchanger NHE2; it is expressed in epithelial tissues and has a specialized role in ion transportation in tubules. However, further investigation is in progress.

**Conclusion**

Taken together, our results further increase the understanding of human kidney stone disease, which may lead to the identification of novel approaches to treatment.

**634 High-throughput drug screening and automation platforms in Drosophila.** T. Portillo Rodriguez¹, J. Mast¹, T. Hart², E. Perlstein¹ 1) Perlara PBC, South San Francisco, CA; 2) BioMarin Pharmaceutical, San Rafael, CA.

Perlara is a public benefit corporation that discovers small molecule therapies for orphan diseases. Our approach often begins with the use of simple, whole-organism models such as yeast, worm, flies in high-throughput drug discovery screens. Since our conception in 2014, we have focused on developing automated screening platforms in *Drosophila* that can be adapted for different disease phenotypes. Models of rare genetic disorders in *Drosophila* can lead to a variety of different phenotypes such as development delay during larval stages, pupal lethality, and behavioral phenotype in adult flies. We will present our fly high-throughput drug screening automated platform and our progress on discovering small molecules modifiers.

**635 A systematic, flexible approach to teaching human disease biology using principles from Drosophila genetics.** Linda Restifo¹²³ 1) Depts. of Neurology and Cellular & Molecular Medicine, University of Arizona Health Sciences, Tucson, AZ; 2) The Center for Applied Genetics & Genomic Medicine, UAHS, Tucson, AZ; 3) BIOS Collaborative Research Institute, University of Arizona, Tucson, AZ.

For several decades, as molecular biology techniques made gene identification easier, human geneticists have ‘discovered’ principles that practitioners of the Drosophila experimental system have long known. At the same time, the study of human genetic disease has revealed complex phenotypes, novel pathophysiological mechanisms not yet fully understood, and many therapeutic challenges. As part of ongoing efforts to bridge the cultural divide between laboratory science and clinical medicine, the author has developed a graduate-level course that presents human disease topics in a framework based on types of genetic causality. This provides an opportunity to introduce a wide array of examples from Drosophila genetics that demonstrate fundamental principles, as well as highlighting the extraordinary phylogenetic conservation of most aspects of cellular and organismal biology. The course has a modular design, with a systematic progression through increasing levels of complexity. In the first module, the emphasis is on single-gene Mendelian disorders with simple one gene-one disease
relationships (e.g., Duchenne muscular dystrophy, cystic fibrosis, fragile X syndrome). The second introduces genetic heterogeneity, in which a shared disease phenotype is caused by mutant genotypes in each of several or many different genes (e.g., hypertrophic cardiomyopathies, epilepsies, ALS). The transition from modules one to two allows the introduction of genetic interactions revealed by suppressor or enhancer alleles at a second locus. The third module deals with disorders that have high heritability due to alterations in one or more ‘risk genes,’ with the disease manifestations requiring environmental triggers or disease-promoting genetic backgrounds (e.g., diabetes mellitus, asthma, autoimmune diseases, cancer). Finally, the class tackles the most challenging module — disorders with high heritability, poorly understood pathophysiology (and even fluctuating diagnostic criteria), and evidence for environmental and/or polygenic effects (e.g., autism spectrum disorder, schizophrenia). Throughout the course, the use of Drosophila genetics for disease modeling and drug screening can also be addressed, along with discussions of how to assess the validity of disease models and cell-based assays.

636 Characterizing Drosophila Multidrug Resistance genes by CRISPR/Cas9 mediated Gal4 knock-ins. O. Williamson1, K. E. Kolbert1, J. DiRusso1, H. Dayton1, H. Bisbee1, A. Saftien1, M. Markstein1, S. Kondo2 1) Molecular and Cellular Biology, UMass Amherst, Amherst, MA; 2) National Institute of Genetics, Genetic Strains Research Center, Invertebrate Genomics Laboratory, Mishima, Japan.

50% of cancer patients with recurring tumors cannot be treated with current chemotherapeutics because their cancer cells express high levels of transmembrane pumps which efflux virtually every available drug out of the cell. Therefore, finding methods to inhibit these pumps, called Multidrug Resistance (MDR) pumps, holds great therapeutic promise. However, inhibitors which have powerfully reversed multidrug resistance in vitro, have proven too toxic in vivo, where they block not only MDR pumps in cancer cells, but also MDR pumps that play vital roles in organs such as the kidney and liver. This finding calls for alternative in vivo models to study MDR genes and to screen for MDR inhibitors.

MDR genes are highly conserved from Drosophila to human. We reasoned that the Drosophila posterior midgut could be an ideal system to study MDR pumps because of the ease performing chemical screens for effects on this tissue. We therefore set out to determine if MDR genes are expressed in the gut. To accomplish this, we used the CRISPR-Cas9 to create Gal4 knock-ins at the MDR49, MDR50, and MDR65 loci, creating over 5 independent lines for each. Here we report on the success of the knock-in approach on over 15 independently created lines and detail the expression pattern of each of the transporters in the fly intestine.

637 Genetic modifiers of NGLY1 deficiency, a rare deglycosylation disorder, identified by exploiting natural variation in Drosophila. E. Coelho, K. Owings, K. Peralta, C. Chow Human Genetics, University of Utah, SLC, UT.

Autosomal recessive loss-of-function mutations in N-Glycanase 1 (NGLY1) cause NGLY1 deficiency, the only known human disease of deglycosylation. Patients with NGLY1 deficiency present with developmental delay, movement disorders, seizures, liver dysfunction, and alacrima. NGLY1 is a conserved component of the endoplasmic reticulum associated degradation (ERAD) pathway. ERAD degrades misfolded proteins that accumulate in the lumen of the ER. NGLY1 deglycosylates misfolded proteins as they are translocated from the ER lumen to the cytoplasm for degradation. Patients with NGLY1 deficiency show a wide spectrum of severity, which is striking since all identified patients carry two null mutations. This phenotypic heterogeneity poses a great challenge to treating this disorder. We used a natural genetic variation screen to explore underlying inter-individual differences in severity of NGLY1 deficiency. We developed a Drosophila RNAi model of NGLY1 deficiency (Pnl1 is the Drosophila ortholog) and crossed it into 200 strains from the Drosophila Genetic Reference Panel (DGRP), a collection of wild-derived strains harboring polymorphisms present in a natural population. We assessed the effect of natural genetic variation in modulating the phenotypic impact of NGLY1 activity loss by quantifying the number of flies that survived to adulthood. There is a wide phenotypic spectrum among strains in survival in the absence of NGLY1. Some strains showed nearly 100% survival, while, others were completely lethal. Using an association analysis, we identified natural polymorphisms that modify survival in flies lacking NGLY1. Modifier genes fell into diverse categories not predicted to be involved in NGLY1 function, including ER Ca2+ homeostasis (CG31690), ER proteostasis (CG33012), ERAD (Hrd3), ubiquitination (hwi), and vesicle trafficking (Rab26). We will present functional evidence demonstrating how each modifier alters lethality associated with NGLY1 deficiency in our Drosophila model. The genes identified from this study have excellent biological support and serve as candidate modifiers of NGLY1 deficiency. This study represents an important step to understanding the pathogenesis underlying NGLY1 deficiency and provides a general framework for incorporating phenotypic variability, when developing personalized therapies for rare diseases.


Adhesion G-protein coupled receptors (GPCRs) are the second largest class of GPCRs, yet their functions and ligands remain poorly understood. Polymorphisms in the gene encoding the adhesion GPCR ADGRL3 have been associated with an increased risk for attention deficit hyperactivity disorder (ADHD) in a number of linkage and association studies. Variations in the gene
were found to predict the efficacy of stimulant treatment in patients, and a specific risk haplotype was shown to impact neurophysiological measures of cognitive response control. Previous studies have shown that disrupting the function of the ADGRL3 homologs leads to hyperactivity in three different model systems – zebrafish, fruit flies, and mice. ADGRL3 knockout mice have higher DA levels in forebrain motor regions as well as increased sensitivity to the stimulant cocaine, which acts at the DA transporter. Together with DA’s established role in mediating locomotion, these findings suggest that ADGRL3 modulates behavior by inhibiting DA signaling; however, a mechanistic link has yet to be established. We have replicated the hyperactive phenotype in fruit flies that carry a null mutation for the ADGRL3 homolog, dCirl. Our data also indicate that dCirl modulates psychostimulant-induced behavior. dCirl mutants exhibit decreased sensitivity to amphetamine whereas selectively restoring dCirl function in DA neurons increases the locomotor response to amphetamine, indicating a role for dCirl in regulating DA efflux. Preliminary imaging studies have revealed that dCirl localizes in the mushroom bodies – thought to play a role in associative olfactory learning, memory and motor output – as well as other brain regions innervated by DA neurons. Thus, ADGRL3 may present a novel mechanism for modulating neurotransmission with important therapeutic implications for the treatment of ADHD and other neuropsychiatric disorders that involve aminergic dysfunction, such as schizophrenia and addiction.

639 Developmental defects in Drosophila melanogaster caused by Twinkle mutations cannot be rescued by the alternative oxidase. A.P.C. Rodrigues, M.T. Oliveira Departmentamento de Tecnologia, FCAV, Universidade Estadual Paulista UNESP, Jaboticabal, SP, Brazil.

There are more than 40 mutations in the gene encoding the mitochondrial replicative helicase Twinkle that are associated with human neurodegenerative diseases, primarily autosomal dominant Progressive External Ophthalmoplegia (adPEO), with multiple deletions in the mitochondrial DNA (mtDNA). Two mutations analogous to those found in adPEO patients (W441C and A442P) and an active site mutation (K388A) were modeled in Drosophila melanogaster, leading to mtDNA replication defects, impairment of mitochondrial oxidative phosphorylation (OXPHOS), and developmental disorders. Transgenic expression of the mitochondrial alternative oxidase (AOX) from Ciona intestinalis (Tunicata: Asciidiacea) in mammalian and insect cells has been shown to be efficient in combating mitochondrial dysfunctions, mainly due to the electron transport bypass of OXPHOS complex III and IV that t

640 Staphylococcus aureus Tolerance to Antimicrobial Peptides. A. Page, K. Oppliger, A. Nuxoll Biology, University of Nebraska at Kearney, Kearney, NE.

Persisters are a subpopulation of dormant cells tolerant to antibiotic killing. Persisters are thought to be the underlying cause of many chronic and relapsing infections. Staphylococcus aureus is responsible for a number of chronic infections, including endocarditis, osteomyelitis, and biofilm-associated medical indwelling device infections. Recent work revealed persister formation in S. aureus is dependent on lowered ATP levels. Despite recent advances major questions remain unanswered, what is the underlying mechanism of persister formation and what is the significance of persisters in tolerance to components in innate immunity? Through whole genome screening, central metabolism was identified as an essential part of persister formation, specifically when the tricarboxylic acid (TCA) cycle was interrupted a significant increase in persisters was observed. Antimicrobial peptides (AMPs) are a key component of both the human and Drosophila innate immune system. Challenging S. aureus with the AMPs, LL-37 and hBD-3 revealed several logs of killing. Deletion of TCA cycle genes resulted in 10-fold more surviving cells compared to wild type. Currently, experiments are being performed was a Drosophila model for infection. Preliminary data suggests that persisters present a challenge for the immune system.

641 Flight and Jump muscles respond differently to experimental cachexia and impaired insulin signaling. Matthew Giedd, Maria Chechenova, Anton Bryantsiev Dept Molecular Cellular Biology, Kennesaw State University, Kennesaw, GA.

Cancer cachexia is a metabolic syndrome characterized by progressive wasting of muscle tissue in the presence of a tumor. In many cases, progressive muscle wasting becomes a primary cause of mortality in cancer patients, although molecular mechanisms of such phenomenon are not fully understood. In this study, we assessed cachexia sensitivity in the two largest but otherwise highly distinct types of muscle fibers belonging to indirect flight muscles (IFMs) and jump muscles (TDT).
Using a previously established model, we induced cachexia in adult flies by expressing a mutated transcriptional activator yorkie (yki) via the esg driver, in the midgut. Cachectic esg>yki flies progressively lost flight ability within two weeks after midgut neoplasia induction (82% flight-impaired flies), which suggested dysfunctional IFMs. Accordingly, succinate dehydrogenase (SDH) activity assay revealed reduced mitochondrial activity in 35% of IFM fibers in esg>yki flies, while 10% of IFM fibers were completely degenerated and lacked any SDH activity. We recapitulated the cachectic phenotype, without midgut neoplasia, in IFMs overexpressing a dominant-negative insulin receptor (100% flight-impaired flies, 11% degenerated IFM fibers at 2 weeks). In contrast to IFM data, TDT muscles did not demonstrate exacerbated fiber degeneration or significant functional decline (retaining 80% of jumping power and 100% of live fibers at 2 weeks) in esg>yki flies.

Our results indicate that muscles with high energetic expenditures, like IFMs, become most vulnerable to insulin signaling perturbations caused by collateral tumors. The comparative model of IFM and TDT muscles may reveal genetic factors that determine the difference in cachetic response.

642 Cancer, speciation, and chromosomes: how interspecies hybrids break somatic pairing. J.Guy. Baldwin-Brown Department of Biology, University of Utah, Salt Lake City, CA.

Homologous pairing (the physical matching up of homologous chromosomes) is a central cellular mechanism that must be executed correctly for organisms to survive. In most organisms, pairing must occur only during meiosis to allow for recombination, and accidental pairing at other times can lead to numerous problems, including aneuploidy, and many cancers are associated with pairing between small regions on chromosomes. Understanding the mechanism that allows aberrant somatic pairing will give insight into numerous diseases.

To study this type of pairing, we require a system that allows pairing to be manipulated, up-regulate, and down-regulated in different ways across different portions of the genome. Human cancer cells do not allow for these types of modifications, but the best studied somatic pairing model organism, Drosophila flies, do. Drosophila flies, unlike most other organisms, pair all of their somatic chromosomes all of the time; however, the offspring of one Drosophila melanogaster fly and one Drosophila simulans fly will lose pairing in a small number of replicable regions of the genome. This phenomenon is an opening that we can exploit to understand somatic pairing broadly. The wealth of genetic tools that exist in Drosophila allow us to manipulate pairing both through these interspecies hybrid crosses and through gene knockdowns that are known to upregulate and downregulate pairing.

The most common hypothesis for the loss of pairing in interspecies hybrids is loss of homology due to sequence divergence; however, the available evidence does not support an association between these. We can accurately measure pairing rates across the genome using the Hi-C sequencing technique. I have measured somatic pairing in normal individuals and interspecies hybrids, and compared the two to find the chromosome regions where pairing is lost. I used known annotated D. melanogaster genome features to identify features, such as genes and repeated DNA motifs, that are overrepresented in non-pairing regions. DNA features that are consistently found where pairing is lost are likely to be responsible for the loss of pairing. I show that these genome features are significantly associated with loss of pairing in interspecies hybrids. Further investigation into the mechanisms of somatic pairing will demonstrate their exact effects on localized loss of pairing.

643 Intragenomic conflict resulting from incomplete transposable element domestication. Anne-Marie Dion-Côté, Michael McGurk, Daniel Barbash Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Transposable elements (TEs) are selfish genetic elements that give rise to intragenomic conflicts due to their capacity to self-propagate to the detriment of their host. In some instances, TEs have been domesticated, potentially resolving this conflict. In Drosophila, the TEs Het-A, TAHRE and TART (HTTs) form the telomere instead of telomerase-dependent repeats, as in most other eukaryotes, and thus are generally considered as domesticated TEs. “Domesticated” HTTs differ from most active TEs as they rely on their neighbors for promoter activity and only transpose to chromosome ends. Yet, proteins involved in telomere maintenance are rapidly evolving, suggesting an ongoing arms race with telomeric TEs and thus incomplete domestication. If so, then variation in telomeric TEs should exist that affects their activity and thus copy number. We test this hypothesis by leveraging available population genomic data and the ConText pipeline that analyses TE variation from unassembled Illumina reads. We find that telomere length is extremely variable across the Drosophila Global Diversity Lines, and that HTTs are among the most variable TEs in copy number, consistent with their being currently active. As opposed to other TEs, polymorphism data from HTTs reveals a lack of population structure. Instead, we find that certain polymorphic positions in TAHRE are correlated with telomere length, suggesting the presence of “hyperactive” TAHRE variants. This is consistent with the model that the retrotransposase activity of TAHRE is used by Het-A, which is non-autonomous despite being the most abundant telomeric TE. We propose that HTTs variants are at least partly driving telomere length variation in Drosophila, consistent with the hypothesis of an ongoing genomic conflict between telomeric TEs and their host. We also have discovered host protein variants associated with telomere length variation in Drosophila. Our results suggest that the domestication of telomeric TEs in Drosophila is incomplete. Future work will aim to test whether HTT propagation is costly to its host, a key condition to formally establish intragenomic conflict.
The Roles of Nuclear-Encoded Mitochondrial Duplicated Genes in Spermatogenesis in Drosophila melanogaster.  M. Es lamieh, R. Parekh, C Hu, E. Betrân Biology, University of Texas at Arlington, Arlington, TX.

The analysis of nuclear-encoded mitochondrial genes (N-mt genes) in Drosophila melanogaster has shown that all of N-mt duplicated genes with tissue-biased expression are testis biased. These genes tend to be old, often relocated, and also have energy-related functions. Since males do not pass the mitochondria to the offspring and they are under intense pressures from male-male competition to fertilize females’ eggs, selection might favor different mitochondria in males. However, some of these duplicates might also evolve to compensate for mtDNA male-harming mutations that do not hurt females and cannot be selected against. Mitochondrial OXPHOS complexes seem to be good candidates to test these hypotheses because they are not only important in energy production but also they are the main place of interaction between factors encoded by N-mt and mt genes. To understand the functions of these new genes and address these hypotheses, we knocked down 12 OXPHOS complexes’ duplicated genes with testis-biased expression in D. melanogaster using different RNAi lines and Bam-Gal4. Some genes showed complete male fertility while others didn’t show any fertility effect. These results will be compared to the CRISPR/Cas9 knockout strains.

Identifying hybrid male sterility factors in Drosophila. R.A. Villegas, N. Weldon, G. Mavhezha, C.D. Meiklejohn School of Biological Sciences, UNL, Lincoln, NE.

Speciation occurs through the evolution of reproductive barriers that decrease gene flow between populations. Intrinsic postzygotic isolation, the inviability or sterility of hybrids due to genetic incompatibilities, is one of the many forms of reproductive barriers. In species with sex chromosomes, hybrid incompatibilities accumulate preferentially on the sex chromosomes; as a consequence, if hybridization results in the sterility of only one sex, it is the heterogametic sex (Haldane’s Rule). The underlying evolutionary forces driving this pattern are still unknown, but to address this problem it is essential to identify the genes that cause these hybrid incompatibilities to understand why hybrid male sterility factors (as opposed to hybrid female sterility or hybrid inviability factors) evolve first, and why they are enriched on the sex chromosomes. To initiate genetic analysis of hybrid male sterility (HMS), we introgressed a segment of the Drosophila mauritiana X chromosome into a D. simulans genetic background. This 4 Mb region (2P6) causes complete male sterility indicating it contains at least one HMS factor. We generated over 200 lines where the 2P6 segment is broken up through recombination, tested these lines for fertility and genotyped them across the 2P6 region. We find evidence of at least two HMS factors within the 2P6 region. At the proximal end, evidence suggests the existence of multiple additive incompatibilities that result in complete sterility only when combined. We designed a crossing scheme to investigate the presence of additional HMS factors in the middle of 2P6 and to resolve the factors we have already identified.

Microbiome influence on Drosophila melanogaster life-history evolution. R. Hughes1, J. Chaston1, A. Walters1, K. Schneider1, D. Lowder1, P. Schmidt2, S. Rajpurohit2, S. Rudman2, M. Berner2 1) Brigham Young University, Provo, UT; 2) University of Pennsylvania, Philadelphia, PA.

Drosophila melanogaster is a model for understanding how organisms adapt to changing environments. Recent work has established D. melanogaster rapidly and predictably evolves in response to seasonal selection. Here we focus on the microbiota as a variable influence on predictable life history phenotypes that evolve in response to seasonal selection in a Pennsylvania orchard. Drosophila-associated bacterial communities are known to influence Drosophila life history traits, such as lifespan and development rate, and the Drosophila microbiota, normally dominated by lactic acid (LAB) and acetic acid (AAB) bacteria, are readily manipulated. A genetically diverse Drosophila population was introduced to diet-controlled outdoor mesocosms where flies exclusively consumed a cornmeal-molasses diet that was freshly administered three times weekly. For three treatments, each prepared in triplicate, the diets were left undisturbed or inoculated with either an LAB or an AAB bacterial strain for 6 generations of selection. Preliminary culture-based assessments of bacterial communities in the flies and their diets confirmed that diet inoculation led to LAB- or AAB-dominated bacterial communities in the inoculated fly populations, respectively. We are currently working to confirm these findings with a 16S rRNA marker gene survey. Following outdoor selection, the F2 generation from each selected population was reared in the laboratory bacteria-free, or with a single species of LAB or AAB, and their development rate and starvation resistance were measured. We are currently analyzing the data to test if the microbiota manipulations altered the phenotypes of the adapted lines. If microbiota manipulation alters D. melanogaster adaptation, we expect to see differences in phenotype between adapted lines when the flies are reared under bacteria free conditions; and unique or shared responses to bacteria will be revealed by analysis of the same lines bearing an AAB or LAB strain. Our findings will be discussed within the context of established patterns of seasonal adaptation.


A large portion of many eukaryotic genomes is comprised of non-coding, repetitive DNA of unknown function. Satellite DNAs (satDNAs) are repetitive elements typically found in large tandem arrays in areas of reduced recombination (e.g.
centromeres and telomeres). These arrays evolve rapidly: their genomic positions can be drastically different even between closely related species, and different species can be dominated by different satellite sequences. SatDNA also evolves rapidly within species, as array sizes can be polymorphic. Direct study of satDNA loci is difficult, as their highly repetitive nature makes loci hard to assemble, resulting in incomplete or uncertain assemblies in these regions. Here we overcome this limitation using single molecule real time sequencing from Pacific Biosciences (PacBio), which enables us to improve assembly of satDNA loci in *Drosophila* and examine their structure in fine detail. We compare the evolutionary histories of two different complex satDNA families, the 1.688 g/cm family (which includes several members, such as 359-bp, 260-bp, and 353/356-bp) and the *Responder* (Rsp) satellite, which is well-characterized for its role in the male meiotic drive system *Segregation Distorter*. We create PacBio assemblies for the *D. melanogaster* reference strain and combine this with short-read sequence data from multiple *D. melanogaster* populations to demonstrate within-species variation in satDNA loci size and distribution. Small blocks of satDNA repeats are also present in the euchromatin, particularly on the X chromosome. We use a combination of long and short-read data to compare and contrast large pericentromeric satDNA loci with their smaller euchromatic counterparts. To compare across species we generated new PacBio assemblies for the three closely-related species of the *simulans* clade (*D. simulans, D. mauritiana*, and *D. sechellia*). We identified dramatic rearrangements in both heterochromatic and euchromatic satDNA clusters. The euchromatic satDNAs vary in both distribution and sequence composition. Our study lends insight into the evolutionary dynamics of satDNAs over short evolutionary time scales and has implications for chromosome evolution and speciation.

648 Unleashing cryptic sex chromosome conflict in *Drosophila melanogaster*. M. Mauger, M. Levine  Department of Biology and Epigenetics Institute, University of Pennsylvania, Philadelphia, PA.

In accordance with Mendel's first law of segregation, XY heterogametic males typically transmit their sex chromosomes at equal rates. Indeed, 1:1 progeny sex ratios are ubiquitous in nature. However, evolutionary theory predicts and many empirical examples demonstrate that X-linked elements can arise that distort sex chromosome transmission. These 'selfish' X chromosomes cause an excess of daughters over sons. Direct and indirect fitness costs of transmission distortion put pressure on the host genome to evolve unlinked suppressors that restore equal progeny sex ratios. Under this model of intra-genomic conflict, manipulation of suppressor loci will unleash currently cryptic selfish sex chromosomes. We harnessed this approach to awaken X chromosome distortion in *Drosophila melanogaster*. This tractable model system offers us the unique opportunity to uncover the still-elusive molecular mechanisms of X chromosome cheating, Y-chromosome resistance, and autosomal suppression. Here we report our progress dissecting two candidate autosomal suppressors encoded by the Heterochromatin Protein 1 gene family. These protein-coding genes are evolutionarily young, testis-enriched, and evolve adaptively across natural populations of *D. melanogaster*. We discovered that double mutant fathers suffer a fertility loss and, importantly, sire significantly more daughters than sons. The degree of progeny sex ratio skew varies across geographically diverse X chromosomes, with some X chromosomes causing a 1:2 son:daughter skew and others causing no skew at all. We also discovered that geographically diverse Y chromosomes vary in their degree of resistance to these selfish X chromosomes. Finally, the XY male genotypes associated with progeny sex ratio skew give rise to an excess of X0 sons as well as embryos with reduced hatch rates. Ongoing work aims to map the X-linked element(s) and to uncover the developmental stage and cell biological source of the dearth of sons. These data reveal that the HP1 gene family encodes not only selfish X-linked elements but also several autosomal-linked suppressors of sex chromosome conflict. Our discoveries suggest that sex chromosome conflict is rampant but cryptic across natural populations of *D. melanogaster*.

649 De novo evolved genes have essential roles in male *Drosophila* reproduction. E. Rivard1, E. Scott1, J. Schmitz2, K. Kelleher1, E. Bornberg-Bauer2, G. Findlay1 1) Department of Biology, College of the Holy Cross, Worcester, MA; 2) Institute for Evolution and Biodiversity, University of Muenster, Muenster, Germany.

*De novo* evolved genes arise from previously noncoding genomic material and have potential to develop integral functions within a relatively short evolutionary timeframe. Many *de novo* genes are expressed predominantly in male reproductive organs, suggesting roles in improving male fertility. Our previous pilot screen of 11 putative *de novo* protein-coding genes with male-biased expression identified two, *saturn* and *goddard*, which are essential for spermatogenesis and sperm function. Utilizing bioinformatics analyses, we have now comprehensively identified a total of 96 putative *de novo* genes with testis-biased expression in *Drosophila melanogaster* and have begun screening each for roles in fertility. One of these genes, *atlas*, is essential for male reproductive ability, since testis-specific RNAi-mediated depletion of *atlas* resulted in significantly reduced male fertility. Phase contrast visualization of *atlas* knockdown (KD) male reproductive tracts revealed the accumulation of morphologically abnormal sperm at the basal end of the testis. Through confocal imaging of GFP-labeled mature sperm heads in *atlas* KD testes, we observed disorganized spermatic bundle distribution. Due to the inability of *atlas* KD males to efficiently produce, organize, and store sperm, they also were unable to transfer many sperm to females. These phenotypes led us to seek potential roles for *atlas* in late spermatogenesis. We identified nuclear elongation abnormalities in the DAPI-stained developing spermaticds of *atlas* KD males. Additionally, phalloidin staining revealed normal actin investment cones associated with individualizing spermatids, but disorganized actin accumulating in the basal testis. We have recently generated mutant alleles of *atlas* using CRISPR/Cas9 and are replicating the above analyses, and we have begun to
characterize a fourth de novo gene with a major effect on male reproduction. These results establish that de novo genes can evolve quickly to modify essential reproductive phenotypes and implicate new gene creation as an evolutionary strategy for males facing intense sexual selection pressures.

650 Rapid evolution dictates population dynamics in orchard populations of Drosophila melanogaster. S.M. Rudman1, S. Rajpurohit2, S. Greenblum3, D.A. Petrov4, M.M. Turcotte4, J.M. Levine5, P.S. Schmidt1 1) Biology, University of Pennsylvania, Philadelphia, PA; 2) Ahmedabad University, Gujarat, India; 3) Biology, Stanford University, Stanford, CA; 4) Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 5) Environmental Systems Sciences, ETH Zurich, Zurich, CH.

Eco-evolutionary feedbacks, where rapid evolution alters an ecological parameter that then feeds back to drive further evolution, have such promise to enhance our understanding of both ecology and evolution that they have been called a ‘new synthesis’. Yet, we have little data from natural populations about whether these feedbacks occur and how profoundly they shape the fate of populations. We carried out a manipulative experiment to quantify the role of evolution in dictating population growth and fitness trait values of Drosophila melanogaster populations. We established a two treatments: 1) populations evolving to seasonal conditions 2) non-seasonally evolving populations. Non-seasonally evolving populations were maintained by collecting eggs from orchard replicates and replacing them with individuals from the founding population every two days. We measured population size, evolution of fitness traits, and changes in allele frequencies in each replicate over the duration of the 4 month outdoor experiment. Seasonally evolving populations showed an accelerated population growth trajectory and exhibited profound evolution in fecundity compared to non-seasonal populations, culminating in evolutionary rescue of all 8 replicates. Yet, the genomic basis of local adaptation was largely unique to each seasonally evolving replicate for the majority of the experiment, demonstrating limited parallelism in genomic basis of adaptation to warm temperatures and high density. We did observe parallelism across replicates in response to cold temperatures, providing evidence that the genomic basis of adaptation depends strongly on the agent of selection. Overall, our findings demonstrate the crucial role that evolution can play in population regulation and provide unique data on the repeatability of eco-evolutionary dynamics.

651 Recurrent turnover of the specialized retrotransposons that maintain Drosophila chromosome length. Bastien Saint-Leandre, Mia Levine Department of Biology and Epigenetics Institute, University of Pennsylvania, Philadelphia, PA.

Virtually all eukaryotes rely on telomerase to maintain chromosome length. Fruit flies are a widely studied exception. Instead of telomerase, Drosophila relies on domesticated transposable elements (TEs) that insert exclusively in telomeric DNA. This alternative mechanism of chromosome length regulation is widely hailed as an exemplary ‘molecular domestication’ event. However, the evolutionary stability of this host-TE relationship is poorly understood. We recently reported that Drosophila telomere packaging proteins evolve rapidly under positive selection, raising the possibility that this ostensibly stable ‘genomic symbiosis’ represents instead a thinly veiled intra-genomic conflict. Here we explore the evolutionary history of the retrotransposon side of this hypothesized conflict. We conducted an iterative BLAST search and de novo consensus-building approach on unassembled sequence reads from nine Drosophila species that span 30 million years of evolution. Validated by DNA-FISH and Sanger sequencing of head-to-tail arrays typical of telomere-lengthening TEs, we uncovered elements related to, but phylogenetically distinct from, the canonical retrotransposon lineages known from D. melanogaster and its closest relatives. Retrotransposon lineages appear to turnover recurrently – closely related species encode independently derived elements. Moreover, we discovered striking fluctuations in telomere-specialized retrotransposon copy number (up to 40-fold) and broad variation in the relative fraction of full-length or degraded copies encoded by a host genome. In the most extreme cases, we were unable to detect even one full-length telomere-specialized retrotransposon. This species’ telomeres, assembled from long-read sequencing, are instead riddled with degraded copies of non-telomeric retrotransposons reminiscent of pericentromeric regions. The ostensible death of retrotransposon-assisted chromosome length regulation in this species implicates alternative mechanism(s) of length regulation, including recombination-based lengthening documented in yeast, flies, and humans. These nine genomes reveal that telomere-specialized TEs are remarkably dynamic over both short and long stretches of evolutionary time. A telomeric TE ‘revolving door,’ combined with fast evolving telomere packaging proteins, suggests that Drosophila telomeres are maintained not by a stable genomic symbiosis but instead a dynamic, constantly re-negotiated truce.

652 Patterns of polymorphic duplications in Drosophila melanogaster. J Li1,2, C-T Ting1 1) Dept Life Sci, National Taiwan Univ, Taipei, TW; 2) Key Laboratory of Genomics and Precision Medicine, China Gastrointestinal Cancer Research Center, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China.

Duplications are an important source of evolutionary innovations. Notably, the newly arisen duplications within population has not been described carefully and their roles on regulatory elements has rarely been mentioned in previous studies. To
understand the evolutionary process of duplications within species, we took advantage of duplications with and without
divergence and examined their effects on genic in coding and regulatory elements regions. Thousands of unexpected
heterozygous sites found in *Drosophila* haploid genomes. Those heterozygous sites were associated with duplications by both
in silico and experimental methods, i.e. divergences between copies. Compared to *D. simulans* genome, those duplications
are slightly bias to newly-gained or quickly-evolved regions in *D. melanogaster* genome. Coding sequence duplications evolved
rapidly, and whole gene duplications even more rapidly which 50% were under positive selection by widely used statistics for
selection inference. Furthermore, duplications after some time duration in the population occurred less frequently at active
markers, suggesting negative selection worked on them. This comprehensive study on low copy duplications provides a
general perspective on the early evolution of duplications.

**653 Haploid selection model for new gene evolution.** J.B. Raíces, P.A. Otto, M.D. Vibranovski  Genetics and Evolutionary
Biology, University of Sao Paulo, Sao Paulo, Sao Paulo, BR.

New genes, those that have recently emerged in the evolutionary history of a taxon, are involved with the genetic basis for the
origin of novel phenotypes in several species and groups. Testis expression of new genes has been observed in a wide
phylogenetic range, such as *Drosophila*, mosquitos, plants and mammals, including humans. More specifically, mammals
and plants new genes are more expressed in later phases of spermatogenesis, the haploid phase of sperm morphogenesis
and maturation. Here, we propose a general model of haploid selection for the evolution of new genes. In this model,
adaptive recessive mutations emerging in a new gene would be fixed faster in a population if their phenotypes were
produced by a haploid rather than diploid genotype. The haploid populations would rapidly expose a low frequency adaptive
mutation, conferring immediate advantage to the organisms carrying it. Whereas a recessive mutation in diploid populations
would be hidden from natural selection in a heterozygous genotype, preventing or delaying its increase in frequency and its
fixation. Since the later stages of gametogenesis represent the only haploid cells in a diploid organism, this model explains
why new genes are more frequently expressed in meiotic and post-meiotic phases of spermatogenesis in several different
taxa. We tested our haploid selection model for the evolution of new genes by confirming its major predictions in *Drosophila*.
We showed that new *Drosophila* genes are more expressed in later phases of spermatogenesis which are enriched with
signature for positive selection.


**654 Genomics of parallel adaptation at multiple timescales in Drosophila.** Li Zhao, David J Begun  1) Laboratory of
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Understanding the process of adaptation is a fundamental question in evolutionary biology. Both local adaptation on short
timescales and the long-term accumulation of adaptive differences between species have recently been investigated using
comparative genomic and population genomic approaches in several species. However, the repeatability of adaptive
evolution at the genetic level is largely unknown. We attacked this problem by comparing patterns of long and short-term
adaptation in *Drosophila melanogaster* to patterns of adaptation on two timescales in a highly diverged congener, *Drosophila
hydei*. *D. hydei* and *D. melanogaster* shared a common ancestor about 50 million years ago and have highly diverged ecologies,
matings systems, and ancestral geographic ranges. While the recent spread of *D. hydei* to a cosmopolitan distribution is not as
well understood as that of *D. melanogaster*, the colonization of high temperate regions in North America by *D. hydei* is likely to
be recent, similar to the history inferred for *D. melanogaster*. We sequenced and annotated the genome, transcriptomes,
studied the population genomics of *D. hydei*, and then compared the population genetic signature of the *repleta* species
group and *melanogaster* species group. We found despite the fact that these species diverged from a common ancestor
roughly 50 million years ago, the population genomics of latitudinal allele frequency differentiation shows that there is a
substantial shared set of genes likely playing a role in the short term adaptive divergence of populations in both species.
Analyses of longer-term adaptive protein divergence for the *repleta* and *melanogaster* clades reveal a striking level of parallel
adaptation. This parallelism includes not only the specific genes/proteins that exhibit adaptive evolution, but extends even to
the magnitudes of the selective effects on interspecific protein differences.

**655 Testing the Bet-Hedging hypothesis: is intragenotypic variability in Drosophila thermal preference adaptive?** J.
Akhund-Zade, L. Pallares, J. Ayroles, B. de Bivort  1) Organismal and Evolutionary Biology, Harvard University, Cambridge,
MA; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

Individuals differ in their behavior – there exists a wide range of behavioral differences between individuals that are
consistent across environments and time in every species examined. We have found that these behaviors are manifested in
isogenized lines reared identically - we label these differences intragenotypic variability or IGV. Bet-hedging is an evolutionary strategy that is optimal in environments with high environmental variance. Under bet-hedging, a single genotype encodes for multiple phenotypes, which can be a plausible explanation for the existence of IGV. I am testing the hypothesis that thermal preference IGV is different across geographic regions in North America due to the differing advantages of bet-hedging strategy. We are gathering isofemale lines from different locations from across the U.S.A. and Canada with the help of collaborating labs in order to test this hypothesis. To elucidate the proximal causes of thermal preference IGV, we have performed an RNA-seq analysis on individual flies from an isogenic line and have found that genes whose expression is strongly anticorrelated with thermal preference are enriched for GO terms related to thermotaxis and thermosensation. In addition, we are performing a RT-qPCR on dTrpA1 to look at whether individual differences in the expression of this thermosensor within an isogenic line correlate with thermal preference. Identifying the molecular factors underlying differences in phenotypic variability will provide us with new capacity to understand the mechanisms of phenotypic evolution.

Variability in recombination rates as explanation for differences in levels of diversity among *Drosophila melanogaster* populations. Johnny Cruz Corchado¹, Josep Comeron¹,² 1) Interdisciplinary PhD Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Biology, University of Iowa, Iowa City, IA.

Recombination is a crucial biological process that also plays a key role in evolution. Under most conditions, meiotic recombination is essential for ensuring that organisms adapt to ever changing biotic and abiotic conditions and, as such, it shapes evolutionary change within and between species. Yet recombination is itself an evolving trait that varies between closely related species and among populations (and individuals) of the same species. However, population genetics models often assume that recombination rates are invariant within species leading to improper assessment of demographic and evolutionary events. In this study, we aim to determine inter-population variation in recombination rates in *Drosophila melanogaster* and assess whether the observed variation in recombination rates can explain the differences in diversity. We estimated genome-wide recombination rates in six *Drosophila melanogaster* populations (South Africa, Zambia, Rwanda, Ethiopia, France, and USA) based on population genomics data and determined the extent of variation in recombination rates across the genomes. We then quantified the levels of heterogeneity in recombination rates at different genomic scales and captured differences between populations using wavelet transformations. Also, we determined the correlation in recombination rates at multiple scales and the genomic regions with highest/lowest correlation to identify significant outliers. Finally, we analyzed correlations between recombination rates and the levels of nucleotide diversity across genomes, to ascertain whether differences in recombination landscapes play a significant role explaining population-specific differences in nucleotide diversity. Our results show that recombination rates not only change in total magnitude but also, and significantly, in their relative distribution within chromosomes (recombination landscapes). We find scale-specific patterns of variation with lower conservation levels at finer scales. Our results indicate that recombination rates are highly variable within species and that recombination is strong predictor of diversity at different scales and among the different populations. We also show that differences in recombination landscapes among populations play a significant role explaining population-specific differences in nucleotide diversity.

What’s the best way to sequence a *Drosophila* genome? - Applications for population and evolutionary genomics. K.C. Deitz¹,², P. Andolfatto¹,² 1) Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) The Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

How should one most accurately and efficiently sequence *Drosophila* genomes for population genomic studies? Methods employing long reads (such as PacBio) are prohibitively expensive for large sample sizes. On the other hand, the accuracy of less-expensive methods has not been evaluated. Full-sib inbreeding approaches are prone to residual heterozygosity maintained by segregating inversions or other factors. Align-to-reference approaches are prone to reference bias and have difficulty with insertion-deletion events. We use simulations and sequenced hybrids of Pacbio-quality *de novo* reference genomes to evaluate the efficiency and accuracy of four approaches to sequencing: 1. Sequencing haploid genomes by align-to-reference; 2. Sequencing outbred diploid genomes by align-to-reference; 3. Sequencing diploid genomes followed by subtraction of a known genome and 4. Sequencing outbred diploid genomes using 10X-genomics indexing. Each of these methods are evaluated in terms of cost, efficiency and accuracy.

Network constraints on local adaptation in *Drosophila melanogaster*. A. M. Early¹,², A. G. Clark¹ 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Infectious Disease and Microbiome Program, Broad Institute, Cambridge, MA.

Gene function is a major determinant of evolutionary rate, but it is not the only factor influencing a gene’s evolutionary trajectory. Past analyses in *Drosophila* have shown that expression level, protein length, and network position also affect species-level genetic divergence. Here, we sought to determine the extent to which such gene attributes also impact evolutionary rate on the population level. Using *D. melanogaster* genomes from five global populations, we studied patterns of diversity and population differentiation across 12,000 protein-coding genes. These genes were classified based on their network centrality (betweenness and degree) as well as phenotypic breadth (as estimated using published RNAi data). In
accordance with inter-species observations, we find that patterns of intra-species evolution correlate with both metrics, supporting a model in which network interactions amplify the strength and efficacy of purifying selection in functionally important genes. Compared to genes occupying more central positions, genes on the periphery of networks show evidence of less functional constraint: higher nucleotide diversity, higher derived allele frequencies (Fay and Wu’s H), and higher population differentiation. Similarly, genes with a larger phenotypic breadth experience a higher degree of purifying selection. These relationships remain after accounting for the effects of recombination rate, transcription level, and codon bias. Overall, these results show that our understanding of local adaptation can be strengthened by accounting for network structure in addition to more frequently considered factors like demographic history and recombination rate. We demonstrate how incorporating these gene properties into whole-genome scans can refine the selection of outlier genes and the interpretation of gene enrichment analyses.

659  The Influence of Migration on Adaptation in Natural Populations of Drosophila melanogaster.  O. Kiratli, P. Schmidt  Biology, University of Pennsylvania, Philadelphia, PA.

Environmental perturbations associated with climate change have created an urgent need to understand the process of adaptation, as rapid adaptation to changing environmental conditions could slow biodiversity loss. Although migration is one of the main driving forces of evolution, its influence on the adaptive process has been understudied even in the most commonly used model organisms, such as Drosophila melanogaster. In this research, we are conducting a set of laboratory and field-based experiments to investigate the effect of gene flow on the adaptation by monitoring the phenotypic and genotypic makeup of the population before and after experimental gene flow. We are using populations built from isofemale lines collected from Pennsylvania (PA), Florida (FL), and Maine (ME); with PA flies representing the local population and FL and ME are immigrant populations. In the laboratory, we are assessing how crosses between populations alter phenotypes to measure the effect of gene flow in the absence of selection. In an outdoor orchard experiment, we will track how populations evolve to seasonal conditions with experimentally manipulated amounts of gene flow to enhance our understanding of how gene flow and selection interact to influence the pace, magnitude, and direction rapid evolution. Here, I will be presenting the data we collected from the laboratory experiment to assess how gene flow alters phenotypes in the absence of selection. I will also discuss the plans for our outdoor research experiment to measure the influence of gene flow on adaptation in natural conditions.

660  Complex satellite DNA variation within and between populations of Drosophila melanogaster.  A. Kodza, D. Khost, A. Larracuente  Department of Biology, University of Rochester, Rochester, NY.

Satellite DNAs are large blocks of tandemly repeated sequences typically found in regions of the genome with low recombination (e.g. centromeres and telomeres). Satellites can turnover rapidly, causing species-specific profiles of repeats. This turnover in satellite DNAs may be important for speciation. Satellites also vary in copy number within species. Unequal crossing over and gene conversion shape both the distribution of copy number and sequence homogenization of satellites in the genome. Here we use deep short read sequencing to estimate the empirical copy number of complex satellite repeats in different natural populations of Drosophila melanogaster. Our focal satellite family is Responder (Rsp), a complex satellite repeat that consists of a dimer of two related 120-bp repeats. Rsp is well known for being the target of the selfish Segregation Distortion complex, a male meiotic drive system in D. melanogaster. We show a 10-fold variation in Rsp copy number within and between populations. We contrast this with variation at a satellite family that is not known to be involved in meiotic drive (260-bp). We find that 260-bp is much less variable within and between populations than Rsp. Using these empirical copy number estimates, we perform forward population genetic simulations and Approximate Bayesian Computation to infer rates of recombination shaping copy number variation at these loci. This combination of satellite modeling and empirical analysis lends insight into the evolutionary forces driving complex satellite DNA evolution in Drosophila genomes.

661  Filling the gaps in the Drosophila yakuba genome to obtain an accurate genome-wide high-resolution recombination map.  N. Pettie1, A. Llopart1,2, J. Comeron1,2  1) Genetics, University of Iowa, Iowa City, IA; 2) Biology, University of Iowa, Iowa City, IA.

Recombination is an important hallmark of meiosis with equally fundamental effects on evolution. Our understanding of the causes and consequences of recombination variation is, however, hindered by the limited number of high-resolution genetic maps that may be descriptive of a species, particularly under controlled conditions. In order to generate a genome-wide recombination map for Drosophila yakuba, we first improved the quality of the genome reference. The current version of the D. yakuba genome contains thousands of genomic gaps and, therefore, can bias estimates of recombination rate between non-continuous contigs. We utilized a 'hybrid’ sequencing approach with long-read Pacific Biosciences (PacBio) SMRT sequencing to fill gaps, followed by error correction with short reads from Illumina deep-sequencing of the Tai18E2 strain initially used to generate the reference genome. Using this approach, we fill more than 60% of the initial gaps, creating an improved version of the original reference genome with an additional 6.2 million bp (6%) of high quality euchromatic sequence. We then used multiplexed, whole-genome genotyping of more than 3,000 individual meiotic products using
Illumina sequencing to generate a detailed genetic map for this species. This first high-resolution recombination map for *D. yakuba*, combined with the improved genome sequences, will be important for evolutionary and genomic studies for this species and to address numerous aspects of the tempo and mode of recombination rate evolution.

**662 Subfunctionalization of SRPK—a new Y-linked gene family in the Drosophila simulans clade.** C. Chang¹, C. Meiklejohn², A. Larracuent³ ¹) Department of Biology, University of Rochester, Rochester, NY; ²) School of Biological Sciences, University of Nebraska, Lincoln, NE.

Non-recombinating Y chromosomes in *Drosophila* species are typically gene-poor compared to X chromosomes, and acquire most of their genes from autosomes. Most of what we know about *Drosophila* Y chromosome dynamics is based on studies of the ~20 Y-linked genes in *D. melanogaster*. We used long-read sequencing from Pacific Biosciences in *D. melanogaster* and 3 *simulans* clade species to assemble the Y chromosomes. Our Y-linked contig N50s range from 500 kb to 1.5 Mb. We estimated nucleotide variation using Pool-seq data in *D. melanogaster* and found that the Y chromosome is strongly conserved in a Pennsylvania population—we detect a very low nucleotide diversity (π = 0.0007 for Y chromosome, 0.004 for the X chromosome, and 0.005 for the autosomes) and no strong signal for positive selection (Tajima’s D = -0.39 and -0.45 on the autosomes and Y respectively). However, between species we discovered the rapid evolution of Y-linked gene copy number, intron size, repeat content, and gene order. We also reveal at least 56 independent *simulans* clade-specific duplications to the Y chromosome from elsewhere in the genome. Most notable of these is the Y-linked acquisition and amplification of serine-arginine protein kinase (SRPK), shared in the *simulans* clade (158, 45 and 51 Y-linked copies in our *D. simulans*, *D. sechellia* and *D. mauritiana* assemblies, respectively). SRPK is ubiquitously expressed in *D. melanogaster*, with roles in both oogenesis and spermatogenesis, suggesting a possible history of intralocus sexual conflict. One potential resolution of this conflict is through subfunctionalization following duplication to the Y chromosome—SRPK-Ys retained exons from a testis-specific isoform deleted in the autosomal copies. These SRPK-Ys evolve rapidly at the sequence and expression levels. SRPK-Ys have 3-fold higher protein evolution rate and are 20-fold overexpressed in the testes of *D. simulans* compared to their parental copy in *D. melanogaster*. Both gene upregulation and increasing the copy number of SRPK-Y contribute to the overexpression. These data suggest that sexual antagonism may have played a role in the evolution of SRPK-Ys. Interestingly, we find copy number differences in all known Y-linked genes, including SRPK-Ys. We hypothesize that gene duplications may accelerate Y chromosome evolution in *Drosophila* species and further suggest that subfunctionalization of SRPK and SRPK-Ys may contribute hybrid incompatibilities in *D. melanogaster* and the *simulans* clade.

**663 Comparative cytology of female meiosis among Drosophila species.** Ahmed Majekodunmi, William Gilliland Department of Biological Sciences, DePaul University, Chicago, IL.

The Muller F element can be found in many fruit fly species as a very small ‘dot’ chromosome, although in some species (e.g. *D. willistoni*) this chromosome has undergone a Robertsonian fusion. The function of this small chromosome is not known, however in *D. melanogaster* the dots move out onto opposite sides of the meiotic spindle during female meiotic prometaphase I, being positioned near the spindle poles while the exchange chromosomes stay at the metaphase plate. A previous manuscript from our lab identified a difference in dot chromosome positioning between *D. melanogaster* and *D. simulans*, where the dots in the former species move out around twice as far onto the spindle on average. It was speculated that the difference could correlate with the amount of heterochromatin in the genome (*D. melanogaster* has around 25% more than *D. simulans*) or with the abundance of inversions (*D. melanogaster* is polymorphic, while *D. simulans* is monomorphic).

To test these hypotheses, we have measured dot positioning in 12 additional species (6 monomorphic and 6 polymorphic for inversions). We found no significant correlation between either heterochromatin content or inversion type and the dot-dot distances among these species. However, we did discover over 10-fold differences in the apparent sizes of the dot chromosomes, and over 5-fold differences in the rates of chromosomes being out on the spindle, and these two factors are strongly correlated (r = 0.79). Because this data is from fixed images, we interpret these results to mean that the size of the dot chromosome is highly correlated with the length of time spent doing these meiotic prometaphase chromosome movements. Additionally, we will examine female meiosis in *D. willistoni* to characterize prometaphase in a species that lacks free dot chromosomes.

**664 Inversion polymorphism in populations with sexual antagonism and reproductive skew.** C. McAllester, J. Pool Laboratory of Genetics, UW Madison, Madison, WI.

Fixed differences in inverted DNA content between species and polymorphisms within species are well documented across life. Due to the generation of unbalanced gametes from heterozygotes in meiosis, inversions may be lost over time in neutral models, especially when the portion of gametes with unbalanced chromosomes has high impact on fecundity. The fixation of inversions in populations has been demonstrated in cases of linkage with beneficial alleles. In *Drosophila melanogaster*, paracentric inversions - inversions not crossing the centromere - are surprisingly common, under varying selective regimes (Corbett-Detig et al. 2012). However, polymorphic persistence of inversions on timescales longer than neutral expectation
requires some form of balancing selection. The benefit of linkage provided by inversions in rafting together alleles that share conditional benefit is fairly well evidenced in local adaptation, such as in ecotypes of *Mimulus guttatus* (Lowry et al. 2010). In this study we use a forward population simulation based on *Drosophila melanogaster* demography and ecology to demonstrate the potential for inversion polymorphism to arise stably when a population is under sexually antagonistic selection at multiple loci in a population with male reproductive skew. This seems more likely than ecological clines to explain the large number of inversions ubiquitous in African lowland populations at stable intermediate frequencies across broad geography. The model represents female choice on males by a representative quality score with a normal noise parameter. Alleles have additive quality score and multiplicative survival cost to both sexes. Here we present results demonstrating balancing selection on alleles with a range of antagonistic effects, the persistence of sets of such alleles when linked and the loss of portions of the set when unlinked, and finally the rise in frequency and stable persistence of inversions that establish linkage associations between such sets of sexually antagonistic alleles.

665  **Inversions and nucleotide diversity in Drosophila yakuba.**  Patrick F. Reilly¹, Mahul Chakraborty², Julie Z. Peng³, J.J. Emerson², Peter Andolfatto¹  1) Princeton University, Plainsboro, NJ; 2) University of California-Irvine, Irvine, California.

Inversion polymorphisms reduce recombination and can contribute to reproductive isolation between species as well as adaptation. Despite their predicted deleterious effects (gene disruption at breakpoints, increased rates of non-disjunction in heterozygotes), inversions often segregate at substantial frequencies in natural populations raising the question of what maintains them. We sequenced and assembled the genomes of two *Drosophila yakuba* isolates and identified the breakpoints of 4 polymorphic inversions segregating on chromosome 2. We estimate that one of these inversions (2Rj) is older than the *D. yakuba-D. santomea* species split (~400 KYA) and has a dramatic effect on structuring nucleotide diversity within *D. yakuba* and on patterns of differentiation between *D. yakuba* and *D. santomea*. The three other inversions are younger, but still have noticeable effects on structuring genetic diversity within *D. yakuba*.

666  **Quantitative evolution of gene activity follows many different mutational paths.**  D.W. Loehlin¹, S.B. Carroll²  1) Biology, Williams College, Williamstown, MA; 2) Howard Hughes Medical Institute and University of Wisconsin-Madison, Madison, WI.

Evolution of the quantitative output of genes is ubiquitous yet we know little of its rules. Does natural selection tune the activity of genes through hotspots such as catalytic domains and cis-regulatory enhancers, or are all parts of the gene potentially in play? To gain insight into this question, we dissected the genetic basis of adaptive changes in the model gene *Alcohol dehydrogenase (Adh)* among seven *Drosophila* species. Whole-gene recombinant transgenes were used to map functional divergence in *ADH* enzyme activity *in vivo*. We find that: 1) the sites responsible for activity evolution occur in many parts of the gene, not just in candidate regions such as enhancers or the coding sequence. 2) certain regions contributed predominantly to activity divergence in some lineages and not others. 3) amino acid changes were responsible for less than 25% of observed divergence in enzyme activity in each lineage studied. 4) both regulatory and coding changes of modest effect have demonstrable effects on flies’ resistance to ethanol toxicity. These observations suggest that the mutational target for quantitative evolution is broad.

Further supporting this observation, we determined the specific nucleotide changes involved in one pair of alleles, the classic *D. melanogaster Fast-Slow* polymorphism. These “alleles” actually consist of at least six linked causative substitutions, each of which changes gene activity in the same direction. Apparently, the typical path followed in quantitative evolution of *ADH* enzyme activity involves many different kinds of causative changes, with a relatively small contribution from the protein sequence itself.

667  **Quantitative genetics of ovipositor traits in the fruit pest Drosophila suzukii.**  E.N. Kim¹, J.I. Okoro¹, J. Pelaez², C.A. Rushworth¹, A.D. Gloss³, J. Ray³, N.K. Whiteman¹  1) Department of Integrative Biology, University of California, Berkeley, Berkeley, CA; 2) University and Jepson Herbaria, University of California, Berkeley, Berkeley, CA; 3) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 4) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

A major question in evolutionary genetics is the degree to which similar phenotypic traits share the same genetic basis. Across the Drosophilidae, ovipositors with dense pegs (teeth) have evolved multiple times, including in *Drosophila suzukii*. This species is an invasive pest that has recently spread worldwide. One of multiple *Drosophila* species that have transitioned to herbivory, *D. suzukii* uses its unique sawing ovipositor to attack living plant organs, piercing the skin of ripening fruit for egg-laying. A previous study in the drosophilid *Scaptomyza flav a*, which uses a similar sawing ovipositor to lay eggs in leaf tissue, found that variation in peg number ranges from 32-37 within two populations from New England, and has high narrow-sense heritability (H²=0.46). Pool-genome wide association mapping revealed four major genomic regions associated with heritable variation in peg number. We are conducting a similar study in *D. suzukii* and hypothesize that variation in ovipositor peg number shares a similar genetic basis in these two species, offering an example of convergent evolution. We phenotyped 795 female flies collected from two fruit environments: blackberry (N=266) and cherry (N=529). We measured length, width, and peg number, as well as wing chord length as a proxy for body size. Measurements were performed on either one oviscapt or
the average of two oviscaps, as values did not differ between oviscaps (p>0.05). We discuss heritability of ovipositor features, comparison of features between fruit types, and the implementation of next-generation genomic sequencing techniques to elucidate the genetic basis of this important trait underlying the transition to herbivory.

668 Understanding ovariole number regulation in Drosophila melanogaster using Quantitative trait loci (QTL) maps. T. Kumar1,2, CG Extavour1,2 1) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

The Drosophila melanogaster ovary consists of many functional units or ovarioles which comprise of the germaria and an ontogenetic series of ovarian follicles. Ovariole number, a quantitative trait, is linked to evolutionary fitness because of its positive correlation with fecundity. Understanding the evolutionary history of such traits requires a complete understanding of the genetic regulation of the development of the ovaries. Quantitative trait loci (QTL) mapping has been used to identify genomic regions associated with such phenotypes of interest by the correlation of segregating genetic markers with quantitative variations in a trait. A significant challenge to interpreting the results of QTL analyses is the limited distribution of such genetic markers, which define relatively large genomic regions of interest wherein all the genes could potentially be associated with ovariole number. Consequently, the functional relevance of genes contained within predicted QTL loci is unclear. An alternative approach has been an a priori candidate based approach, which reduces the number of genes under study but also lacks the strength of QTL mapping as an unbiased technique.

Here, we present an approach designed to systematically test the causal genetic hypotheses generated by QTL analyses of ovariole number. LOD (Logarithm of odds) scores, a likelihood ratio statistic, assigns significance to positions on the genome that contain a QTL. We use previously-generated QTL maps to identify the genes within the predicted loci with both high and low LOD scores, and determine their function in ovariole number regulation using the extensive toolkit that exists in Drosophila melanogaster. Because specification of ovariole number is largely dependent on somatic cell number within the ovary, we use the Gal4-UAS system to abrogate the function of each QTL gene in ovarian somatic cells to reveal novel candidates. This method thus allows us to make thorough use of the QTL maps developed over the years for studying quantitative traits like ovariole number.

Our preliminary results have identified genes within both low and high LOD score regions that affect ovariole number. We are currently comparing the absolute variations in ovariole number to see if the LOD score is a better predictor of the degree of variation in ovariole number rather than the presence or absence of genes within a QTL associated with phenotypic variation.

669 Fine-mapping the genetic basis of wing spot evolution between Drosophila elegans and D. gunungcola. Jonathan Massey1, David Stern2, Patricia Wittkopp1 1) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 2) Janelia Research Campus, Ashburn, VA.

Wing pigmentation has evolved rapidly over time and in a repeated manner between Drosophila species, with some species independently converging on the presence of a single, dark wing spot in male flies. This example of phenotypic evolution has been studied extensively, and previous studies reported that several candidate genes may be involved in the evolution of male-specific wing spots. We performed quantitative trait locus mapping in large backcross populations between the spotted species Drosophila elegans and the spotless species D. gunungcola to identify genes contributing to wing spot divergence. We identified a large-effect spot region on the X chromosome containing six genes that have not previously been implicated in wing spot evolution. This region appears to control the presence versus absence of wing spots in recombinant flies. Current work aims to identify which of these genes are responsible for the switching between wing spot presence and absence in D. elegans and D. gunungcola.

670 Parallel Evolution of Ethanol Tolerance found in Four Populations of Drosophila melanogaster. Q D Sprengelmeyer, J E Pool Laboratory of Genetics, University of Wisconsin-Madison, Madison, Wisconsin.

Ethanol tolerance in Drosophila melanogaster has been shown to increase with latitude and linked to different genes, most notably ADH. This study focused on ethanol tolerance of flies from their ancestral range (Zambia) compared to populations found in higher altitudes (Ethiopia, South Africa, and Uganda) and latitude (France). To test for ethanol tolerance we exposed the flies to an 8% ethanol solution and measured the survivability over a 12-hour period. Flies from low altitude Zambia were found to be the least ethanol tolerant, whereas the other three sub-Saharan high altitude populations had different levels of tolerance. The flies from France were extremely tolerant, having nearly 100% survivability. To ascertain candidate genes responsible for higher ethanol tolerance, bulk segregant analysis was performed to detect quantitative trait loci (QTL). Each of the ethanol tolerant populations had different QTL peaks and substantial differences were observed between strains from the same population as well. These data suggest that the loci for ethanol tolerance may not be fixed, or else that epistatic interactions significantly alter mapping results. To find evidence of selective sweeps, FST and the haplotype statistic \( \chi^2_{\text{HO}} \) were analyzed, comparing each of the higher tolerance population genomes with low-tolerance population Zambia. From
this study new insights can be gained in the genetics of adaption; in particular the polygenicity of a trait evolution and its genetic predictability between the different populations.

671 Misregulation of proteolytic genes and hybrid male sterility in crosses between \textit{D. p. pseudoobscura} and \textit{D. p. bogotana}. \textit{Doaa Alhazmi}1, Alberto Civetta2 1) Biology, University of Winnipeg, Winnipeg, MB, Canada; 2) Biology, University of Winnipeg, Winnipeg, MB, Canada.

The origin of prezygotic and postzygotic barriers serves as a mechanism to isolate divergent populations. Many studies on the genetic basis of speciation have focused on postmating postzygotic (i.e. sterility/ inviability of hybrids) reproductive isolation mechanisms and some general patterns have emerged from these studies. Commonly, there is a disproportinate contribution of the sex chromosome to heterogametic F1 inviability/sterility (the “large-X effect”) but epistatic interactions are needed for full establishment of isolation barriers. Less is known about how changes in gene expression can impact hybrid phenotypes. Despite some recent progress, the lack of information on differential gene expression and speciation is surprising given that eukaryotic genomes are mainly made of noncoding DNA that is likely to exert their effect through regulatory interactions. \textit{Drosophila} species pairs that show differences in fertility between reciprocal F1 hybrids (i.e. Darwin’s corollary to Haldane’s rule) can be used to identify candidate speciation genes whose expression changes uniquely in the hybrid male sterile (HMS) condition. Using RNAseq, we have identified proteolytic genes as the largest class of genes uniquely misregulated in the male sterile hybrids resulting from crosses between \textit{D. pseudoobscura bogotana} females and \textit{D. p. pseudoobscura} males. Here we 1) use a backcross approach to determine whether misregulation of gene expression uniquely associated with the sterile F1 male hybrid condition could be explained by lack of equivalent gene interactions between fertile and sterile hybrids (asymmetries), such as incompatibilities between maternally inherited factors and parentally inherited alleles or X-autosomal gene imbalances, 2) quantify levels of the proteolytic genes expression within the male reproductive tract, specifically in the accessory glands, testes, seminal vesicles and ejaculatory bulb, to further narrow down candidate misregulated HMS genes 3) use genetic introgressions to examine whether previously mapped major sterility gene alleles can modulate the level of expression of proteolytic gene candidates, thus identifying possible interactions and common pathways of sterility. Our results identify at least five misregulated proteases as direct candidates for HMS, one of them being a target of the major sterility gene \textit{Ovd}. Two other possible targets of \textit{Ovd} that do not directly affect hybrids’ fertility are also identified.

672 The genetic basis of female preference and incipient speciation. \textit{Dean Castillo}, Daniel Barbash Cornell University, Ithaca, NY.

The evolution of reproductive isolation is a critical step in the speciation process. To understand how reproductive barriers evolve we need to understand which traits confer reproductive isolation at the earliest stages of divergence. Determining which the genes contribute to female preference will reveal how this trait evolves, and how females perceive male mating signals. Races of \textit{D. melanogaster} (Zimbabwe [Z] and cosmopolitan [M]) show partial reproductive isolation and can be used as a model to understand how female preference evolves. A strong candidate gene, \textit{desat2}, has been previously proposed to contribute to female mate preference and pheromone differences between these races. \textit{desat2} is an important enzyme in the synthesis of cuticular hydrocarbons and produces precursors that determine the major compound that is specific to Zimbabwe genotypes. How it functions to contribute to female preference is unknown. Genes, like \textit{desat2}, that affect multiple traits may facilitate rapid speciation by linking female mate preference with mating signals, such as pheromones. Using genomic editing (CRISPR) and analysis of expression patterns, we are working to distinguish the effects of this gene on pheromone production, pheromone perception, and female choice. We are generating \textit{desat2} null mutations in multiple \textit{Z} backgrounds to analyze changes in pheromone production and sexual behaviors caused exclusively by \textit{desat2}. We are also generating mutants that no longer produce female pheromones, by targeting the female specific \textit{eloF} and \textit{desatF} genes, in the \textit{desat2} null background. Combined, these experiments will allow for a definitive test of whether pheromone production or pheromone perception, mediated by \textit{desat2}, is responsible for female choice. Previous work has identified a cline in female mating behavior and African vs European ancestry. We have identified several new candidate genes for female mating preference by looking at their allele frequency along a cline on the East Coast of North America. Knowledge of the molecular function of \textit{desat2} will reveal how females perceive and respond to sexual signals. Female mate preference is a complex trait and we will then be able to compare the mode of action for \textit{desat2} with other candidate genes.

673 Hybrid male sterility between subspecies of \textit{Drosophila willistoni}: A case of azoosperma. \textit{Hunter Davis, Alberto Civetta} Biology, University of Winnipeg, Winnipeg, Manitoba, Canada.

The fruit fly species \textit{Drosophila willistoni willistoni} was once believed to be a single species that spread from the southern United States to South America. We have recently found that \textit{D. w. willistoni} is subdivided into two subspecies that are reproductively isolated from each other; \textit{D. w. willistoni} in North America, Central America and northern Caribbean islands, and \textit{D. w. winge} in South America and southern Caribbean islands. When a female of \textit{D. w. willistoni} mates with a male of \textit{D. w. winge}, the resulting males are sterile, but the females are fertile. In the reciprocal cross, all offspring are fertile. We have also previously determined that the sterile hybrid males produce normal motile sperm but fail to place sperm within the female
reproductive storage organs after mating. Here we use different strains of the two subspecies (i.e. Guadeloupe, Puerto Rico, Uruguay and Saint Vincent) in a series of interrupted mating assays to track the fate of sperm and ejaculate within the female reproductive tract. We find that the sterile males manage to transfer an ejaculate that triggers the expected responses of elongation and expansion of the female uterus. However, the ejaculate is devoid of sperm. We identify a large mass of sperm forming a bulge at the basal end of the testes in sterile males. The sperm mass develops more prominently as the male’s ages. The sperm mass that forms at the basal end of the testes appears to impede the movement of the sperm towards the sperm pump, where sperm normally mixes with the ejaculate produced by accessory glands. Our results highlight a unique form of hybrid male sterility in Drosophila that is driven by a mechanical impediment to transfer sperm rather than by an abnormality of the sperm itself. Interestingly, this form of sterility is similar to a form of infertility in man (azoospermi) that is caused by the lack of sperm in the semen due to either lack of sperm production or blockages that impedes the sperm from reaching the ejaculate.

674 Introgression of Drosophila simulans alleles into D. sechellia alters female attractiveness. J.M. Gleason¹, D.R. Swartzlander² 1) Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS; 2) Molecular Biosciences, University of Kansas, Lawrence, KS.

Reproductive isolation between species is governed by the attraction of one species to the other and the resulting courtship effort. Cuticular hydrocarbons, acting as pheromones, play a large role in the attractiveness of many Drosophila species. Drosophila simulans and D. sechellia, sister species of D. melanogaster, are asymmetrically reproductively isolated; D. simulans males do not court D. sechellia females because they are repulsed by D. sechellia female pheromones. We have identified two genes, desatF and eloF, that are candidate genes for a species difference in female pheromones between these species. We introgressed the genes from D. simulans into D. sechellia and found that female cuticular hydrocarbons were changed as predicted by their function in D. melanogaster. Male D. simulans courted the introgressed females more than they did D. sechellia females, though much less than they courted D. simulans females. Thus, as few as two gene changes can affect reproductive isolation.

675 Determining if Ntu affects female rejection of heterospecific males in Drosophila. J.R. Isaacson, A.J. Moehring Department of Biology, Western University, London, N6A5B7, CA.

During courtship, it is extremely important for organisms to be able to recognize conspecifics because of the heavy costs associated with forming interspecies hybrids. Many organisms use species-specific cues to recognize potential mates. These cues are then perceived and evaluated via neural pathways. The underlying genetic basis of how species-specific cues are evaluated and subsequently processed into either receptive or rejectionary behaviour remains almost entirely unknown. This project aims to determine whether the gene Ntu, which is involved in neuron development in Drosophila species, is involved in species identification during courtship. I will use the CRISPR/Cas9 system to knock out either the Drosophila melanogaster or D. simulans allele of Ntu in interspecies hybrids and see if this results in melanogaster-like or simulans-like female mating behaviour. Complementary to this, I will silence each species’ Ntu allele using allele-specific RNAi that is expressed in particular subsets of neurons via the Gal4/UAS system in hybrids. Differences in behaviour would indicate that Ntu is involved in species recognition during courtship via the particular subset of neurons. If successful, this would be the first time that a gene has been linked to interspecies mate rejection and provide the first insight into which neurons contribute to that behaviour.

676 A conserved function for pericentromeric satellite DNA. M. Jagannathan¹, R. Cummings¹, Y. Yamashita¹,² 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) HHMI.

Satellite DNAs are simple tandem repeats that are ubiquitously present in the centromeric and pericentromeric regions of eukaryotic chromosomes. Unlike centromeric satellite DNA, whose function in chromosome segregation is well established, pericentromeric satellite DNA is often referred to as ‘junk DNA’ due to its apparent lack of function. As a result, while it is well known that pericentromeric satellite DNAs diverge rapidly among closely related species, the role of this divergence in speciation has remained untested. Pericentromeric satellite DNAs from multiple chromosomes are clustered into DNA-dense nuclear foci or chromatid complexes across eukaryotes. Although identified almost 50 years ago, the organizing principles and function of chromatid complexes remain unclear. Our data show that the orthologous proteins, Drosophila D1 and mouse HMGA1, containing multivalent satellite DNA binding domains bundle heterologous chromosomes together into chromatid complexes in both Drosophila and mouse cells. Chromatid disruption results in loss of unbundled chromosomes from interphase nuclei leading to micronucleus formation and cell death, suggesting that the bundling of pericentromeric satellite DNAs into chromatid complexes facilitates the encapsulation of all chromosomes in a single nucleus, a universal feature of eukaryotic genomes. Excitingly, we observe chromatid disruption and micronuclei in the inviable/sterile progeny of D. melanogaster and its nearest sibling species D. simulans, suggesting that divergent satellite DNA sequences in these closely related species likely trigger a cellular defect in hybrids. Moreover, our data show that the D1 satellite binding protein from D. simulans is unable to complement D1 function in D. melanogaster suggesting a potential mechanism for chromatid disruption in
hybrids. Thus, defective chromocenter formation as a result of satellite DNA divergence may partly account for the inviability/sterility phenotypes in hybrid species.

677 Functional evolutionary genomics of duplicated genes in the Drosophila melanogaster lineage. Alexandra Jones1, Louis Fortunato1, Jose Ranz2, Rob Kulathinal3 1) Department of Mathematics, Temple University, Philadelphia PA; 2) Department of Ecology and Evolutionary Biology, University of California Irvine, CA; 3) Department of Biology, Temple University, Philadelphia PA.

Duplication events can lead to the spread of new genes, novel genetic variation, and functional divergence in a population. If these duplicates provide a fitness benefit, they can quickly become fixed in a population and across the entire species. Here, we study lineage-specific duplicates that have recently evolved in the Drosophila melanogaster lineage. With sequenced genomes of D. melanogaster readily available from multiple locations, we mapped the origins and evolution of lineage-specific de novo genes by tracking the genealogies of recent duplicates observed in global D. melanogaster populations and not present in any of the sibling species including D. simulans, D. sechellia, and D. mauritiana. We find differences in the strength and direction of selection between parent and child genes, especially among de novo child genes enriched for male-specific expression. These findings in recent paralogs align with previous work showing that male reproductive orthologs are generally more diverged between species, with genes involved in sperm development harboring stronger signals of selection. Together, these results support the role of sperm competition or gametic selection in early population divergence and provide a greater understanding of the origin, proliferation, and functional divergence of de novo genes across recent evolutionary time.

678 Behavioral analysis of mate discrimination by Drosophila sechellia against D. melanogaster by using partial hemizygous hybrid females. M. Tomaru1, T. Akino2 1) Department of Drosophila Genomics and Genetic Resources, Kyoto Institute of Technology, Kyoto, Japan; 2) Department of Bioresource Field Science, Kyoto Institute of Technology, Kyoto, Japan.

Females of Drosophila sechellia rarely accept D. melanogaster males, whereas D. melanogaster females accept D. sechellia males fairly well. Since hybrid females accept D. melanogaster males better than D. sechellia females do, the discrimination by D. sechellia females against D. melanogaster males seems to be (partly) recessive. By using a third chromosome DrosDel deficiency kit, we found that several chromosomal regions in D. sechellia are candidates involving female discrimination factors; Hybrid females hemizygous for one of such chromosomal regions copulated with D. melanogaster males at 10% or less, while successful copulation was observed at about 40% in the control crosses. There were no remarkable differences in male courtship parameters, such as the rates and the latencies of courtship and copulation attempts, and also in the duration of courtship, between hemizygous hybrid females and the controls, suggesting that D. melanogaster males court hemizygous females and the control females in a similar fashion. Courtship sequences of unreceptive hemizygous hybrids were similar to those of the controls. This may suggest that courting males do not change their behavior even though they received rejection signals from the unreceptive hybrid females and/or that hemizygous hybrid females less discriminate against courting males. Although the candidate chromosomal regions do not involve elongases and desaturases, we found small differences in cuticular hydrocarbon profiles of females between hemizygous hybrids and the controls. At present, however, these differences do not seem to explain differences in copulation success.


In D. melanogaster, bag of marbles (bam) acts as the master switch for germline stem cell differentiation during oogenesis and plays a key role in regulating spermatogenesis. bam is rapidly evolving across the Drosophila genus and shows bursts of positive selection in D. melanogaster and D. simulans but not in D. ananassae, D. pseudoobscura, and D. mojavensis. We have resequenced bam in populations of the three closely related species in the yakuba complex—D. yakuba, D. santomea, and D. teissier. Our results indicate that like in D. melanogaster and D. simulans, bam in D. yakuba and D. santomea is evolving under positive selection. However, we do not see this signal at bam in D. teissier. What could be driving the episodic adaptive evolution of a gene essential for stem cell differentiation and fertility? One possibility is that bam's role as a differentiation factor in gametogenesis is novel to the melanogaster subgroup and is now under positive selection in these species. To evaluate this hypothesis, we are using CRISPR-Cas9 to generate bam null alleles in diverse Drosophila species and then testing for conservation of function. We have found that bam's core function in gametogenesis is conserved in D. melanogaster, D. yakuba, and D. ananassae. Together, our functional and population genetic data suggest that bam's function as a stem cell differentiation factor is not driving its adaptive evolution. The episodic signal of positive selection we observe at bam implies adaptation to pressures present in these particular lineages. Wolbachia is a maternally inherited endosymbiotic bacteria that transiently infects Drosophila species, manipulates host reproduction to ensure its propagation, and genetically interacts with bam during oogenesis in D. melanogaster. Epidemic natural infections with Wolbachia could result in genetic conflict with bam, leading to an evolutionary arms race for control of oogenesis. We are currently working to define the interaction between D.
fitness effects that vary over time. Studies that can be applied to autosomal or sex linked loci, easily adapted for other systems, and used for estimation of fitness effects that vary over time.

680 Variation in stress tolerance is associated with environmental differences in Drosophila americana group. Jeremy Davis, Leonie Moyle Biology, Indiana University, Bloomington, IN.

Trait-environment associations can reveal the selective pressures that drive adaptation and the traits that respond to these conditions. In Drosophila, desiccation and UV resistance are assumed to be important traits for survival in a diverse set of environments, although there are few direct analyses of their adaptive significance. Here we used members of the Drosophila americana species group—the xeric D. novamexicana and the two subspecies of the mesic D. americana—to investigate evidence for historical adaptation in these physiological stress responses. To do so, we quantified population differences in desiccation and UV resistance, and their association with population differences in climate based on values of 35 bioclimatic environmental variables. Pigmentation differences were also quantified to evaluate covariation with either physiological tolerance or climate variation—for which mixed evidence for these associations have been found in other species. We found that variation in all three traits is associated with abiotic climate variation—especially in UV radiation intensity—consistent with naturally selected responses to local environmental conditions. In particular, populations of the desert species D. novamexicana have heightened desiccation resistance relative to D. americana, and this is associated with variation in peak UV intensity in location of origin of each population. Our results link adaptive variation with underlying selective agents, and demonstrate the power of examining trait variation in the context of historical environmental conditions to reveal local adaptation in diverse Drosophila species.

681 X chromosome homozygosity in D. melanogaster females does not reduce lifespan as predicted by the unguarded X hypothesis. C.M. Kimber1, M. Brengdahl1, J. Maguire-Baxter2, U. Friberg1 1) IFM Biology, Linköping University, Linköping, SE; 2) University of Manchester, Manchester, UK.

Lifespan differences between the sexes are very common. Lifespan differs between the sexes in many species. Three genetic hypotheses to explain this interesting pattern have been proposed, involving different drivers: sexual selection, asymmetric inheritance of cytoplasmic genomes, and hemizygosity of the X (Z) chromosome (the unguarded X hypothesis). Of these, the unguarded X has received the least experimental attention. This hypothesis suggests that the heterogametic sex suffers a shortened lifespan because recessive deleterious X (Z)-linked alleles are expressed unconditionally. In Drosophila melanogaster, the X chromosome comprises an unusually large fraction of the genome (~20%), providing a powerful model for evaluating evolutionary theories involving the X. Here, we test the unguarded X hypothesis by forcing D. melanogaster females to express recessive X-linked alleles to the same degree as males, using females that are exclusively homozygous for the X chromosome. We find no evidence for reduced lifespan or egg-to-adult survival in females that are homozygous for the X. In contrast, males and females made homozygous for an autosomal both suffer similar, significant reductions in those traits. The logic of the unguarded X hypothesis is indisputable, but the degree to which recessive deleterious X-linked alleles depress performance in the heterogametic sex appears too small to explain general sex differences in lifespan.

682 Maximum likelihood estimation of sex-dependent fitness costs of a yellow mutant allele in Drosophila melanogaster. J. Liu1,2, J. Champer1,2, C. Liu1,2, J. Chung1,2, R. Reeves1,2, A. Luthra1,2, Y.L. Lee1,2, A. Clark1,2, P. Messer1 1) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Measuring fitness differences between allelic variants is a central goal of evolutionary studies, and several approaches have been developed for obtaining fitness estimates by tracking allele frequencies in experimental populations. However, these approaches typically assume equal fitness effects in males and females in autosomal genes, while little attention has been paid toward the possibility that fitness effects could often differ between the sexes. Disruption of the X-linked yellow gene in Drosophila melanogaster, for example, causes a characteristic pigmentation phenotype that is likely much more deleterious in males than females. In this study, we developed two maximum likelihood approaches for estimating the distinct fitness costs of yellow disruption in males and females, based on phenotype frequency trajectories observed in cage experiments. In our first model, the likelihoods of the different fitness parameters are multiplied across all generations, whereas in our second model each pair of consecutive generations provides independent fitness measurements, thereby allowing for changing fitness effects over the course of an experiment (e.g. when selection is frequency-dependent). We applied our inference framework to a set of three cage experiments in which we tracked yellow mutant frequencies in populations of ~3000 flies over the course of 5-6 generations. We obtained an average relative fitness of 0.29 for the yellow mutant allele compared to wild type in males. In females, we obtained a relative fitness of 0.68, indicating that yellow disruption is indeed more than twice as deleterious in males than females. The results from our second model further suggest that the fitness costs in males likely varied between generations. One possible explanation is frequency-dependent selection, which will be tested with further experiments. Our inference methods provide a flexible framework for estimating sex-specific fitness effects in cage studies that can be applied to autosomal or sex-linked loci, easily adapted for other systems, and used for estimation of fitness effects that vary over time.

melanogaster bam and Wolbachia, identify possible genetic interactions between bam hypomorphic mutants and Wolbachia in D. simulans and D. yakuba, and analyze patterns of evolution at bam in species with known Wolbachia infection histories.
683  Nuclear influence on mitochondrial DNA competition and transmission.  CY Chiang1, H Ma1, PH O’Farrell2  1) Gurdon Institute, University of Cambridge, Cambridge, GB; 2) Department of Biochemistry and Biophysics, University of California, San Francisco.

The genetics of mitochondrial DNA (mtDNA) is distinct from the nuclear genome in almost every aspect, from the uniparental inheritance to no recombination and the basic rules that underlie replication and segregation. The differences between the two genetic systems are probably a relic of evolution, but lead to fascinating biology that dictates the functional consequences of mtDNA mutations. Given that there are multiple copies of mtDNA within each cell, pathogenic mutations often arise among thousands of wild-type genomes. Selectivity in the transmission of functional versus pathogenic genomes in somatic cells impacts expression of the disease phenotype. While selective transmission in germline governs the heritance of mtDNA mutations, and thus its evolution. However, what influences the competition of co-existing genomes is not yet clear.

Recently, we have developed novel genetic tools in *Drosophila* and made it a powerful model for mtDNA studies. This helped to reveal two types of selection that influence mtDNA competition: 1) a purifying selection where the genome providing more function takes over; 2) a selfish selection where a bully genome gains over a wimpy genome if it replicates or transmits better (i.e. independent of function). Within an individual, when the gains in one selection are balanced by losses in the other, both genomes are maintained in a stable ratio for many generations. We have established such a line in *Drosophila*, and used it for a deficiency genetic screen to identify nuclear regions/genes whose genetic dose influences the mtDNA transmission. This screen found multiple such nuclear loci. Dissecting one locus revealed that the tamas gene, which encodes the catalytic subunit of the mitochondrial DNA polymerase, significantly enhanced purifying selection when its abundance is limited/halved. This example and the strong genetic interactions observed show the existence of suspected capacity of the nuclear genome to modulate mitochondrial competition.

684  A battle for mitochondrial DNA transmission.  Chieh-Yin Chiang¹, Hansong Ma¹, Patrick O’Farrell²  1) Gurdon Institute, University of Cambridge, Cambridge, GB; 2) University of California, San Francisco, US.

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685  Impacts of Recurrent Hitchhiking on Divergence and Demographic Inference in *Drosophila*.  J.D. Lange, J.E. Pool  Laboratory of Genetics, University of Wisconsin - Madison, Madison, WI.

In species with large population sizes such as *Drosophila*, natural selection may have substantial effects on genetic diversity and divergence. However, the implications of this widespread non-neutrality for standard population genetic assumptions and practices remain poorly resolved. Here, we assess the consequences of recurrent hitchhiking (RHH), in which selective sweeps occur at a given rate randomly across the genome. We use forward simulations to examine two published RHH models for *D. melanogaster*, reflecting relatively common/weak and rare/strong selection, respectively. We find that unlike the rare/strong RHH model, the common/weak model entails a substantial degree of Hill-Robertson interference, which has implications for the rate of beneficial mutation and for the simulation of RHH models. We also find that the common/weak RHH model is more consistent with our genome-wide estimate of the proportion of substitutions fixed by natural selection between *D. melanogaster* and *D. simulans* (19%). Finally, we examine how these models of RHH might bias demographic inference. We find that RHH has relatively minor effects on the inference of recent between-population demographic parameters, while having stronger effects on inference of longer term ancestral population parameters. Thus, even for
species with important genome-wide impacts of selective sweeps, neutralist demographic inference can have some utility in understanding the histories of recently-diverged populations.

Discerning the historical and genetic relationship between the endosymbiotic bacteria *Wolbachia* and the *Drosophila* germline stem cell gene bag of marbles. M. Wenzel, C. Aquadro Molecular Biology and Genetics, Cornell University, Ithaca, NY.

The *Drosophila* protein coding gene *bag of marbles* (*bam*) plays a key role in early male and female gametogenesis by regulating the differentiation of germline stem cells (GSCs). Although the regulation of GSC gene function is essential for reproduction, *bam* shows strong and episodic bursts of protein sequence diversification in many *Drosophila* lineages. Evolutionary conflicts that drive rapid evolution of reproductive genes are often found between males and females, yet these conflicts are not present in the GSC. Previous work in our lab suggests that one potential evolutionary driver of *bam* is the bacterial endosymbiont *Wolbachia*. *Wolbachia* is estimated to inhabit 40% of arthropods and can manipulate the reproduction of its host. *Wolbachia* partially rescues the reduced fertility of a *bam* hypomorphic mutant in female *D. melanogaster* as well as a hypomorphic mutant of another GSC gene, *sex lethal* (*sx*). These results have led us to hypothesize that *Wolbachia* may directly contribute to *bam*’s rapid evolution. One approach we are taking to evaluate this hypothesis is investigating the historical interaction between *Wolbachia* and *Drosophila*. *Wolbachia* can undergo lateral gene transfer, inserting pieces of its genome into its host’s genome. Over time, these insertions neutrally evolve and such “genomic fossils” can act as a proxy to identify past *Wolbachia* infections. To find such fossils, we are employing a k-mer matching program that has been useful in detecting evidence of past viral and transposable element infections in mammals. Thus far, we have run simulations to mimic a historic insertion event and preliminary results suggest we can detect historic *Wolbachia* insertions up to 7 million years ago, with consideration of the insertion size. Furthermore, we found that a k-mer size of 19 base pairs is sufficient to provide a signature with limited noise. A second approach we are taking to evaluate the role of *Wolbachia* as an evolutionary driver of *bam* is to elucidate the manner in which *Wolbachia* interacts with *bam* hypomorphs. The only existing hypomorph, which is rescued by *Wolbachia* infection in females, is located in a region where Bam binds to a key partner Bgcn. Using polymorphism and divergence data we have identified putative new hypomorphic alleles of *bam*. We are currently using CRISPR/Cas9 to generate these mutants in the w1118 background and test for *Wolbachia* rescue to determine if the *Wolbachia* interaction is specific to certain Bam regions.

Evolutionary Probability: A population-orthogonal framework for investigating ancient and contemporary selection. R. Patel, S. Kumar Institute for Genomics and Evolutionary Medicine, Department of Biology, Temple University, Philadelphia, PA.

The Evolutionary Probability (EP) method provides a Bayesian framework for determining the probability of observing an allele for a specified taxa given an evolutionary history. The method assumes no knowledge of the current state of the site, and utilizes the evolutionary history to provide a posterior probability distribution of observing any allele at the site. Low evolutionary probabilities indicate that an allele is unlikely to be found at a position based on allelic pattern observed in the evolutionary history of the site, and is likely to induce a significant functional change in the focal element (e.g., protein, regulatory). Thus, under Neutral Theory expectations, a low evolutionary probability allele is not expected to rise to high allele frequency in a population. The orthogonal nature of estimated EP and observed population frequency makes it a valuable tool to verify neutral evolutionary patterns and reveal signatures of adaptation. This can then be used to prioritize variants for downstream investigation. However, EP does not have to be limited to investigating polymorphic sites or observed residues. In fact, evolutionary probability can be calculated for unobserved alleles, or at sites that are monomorphic in a population, e.g., at an evolutionarily conserved position, EP can detect alleles that may have been previously driven to fixation due to positive selection. In these applications, we must first obtain reliable estimates of EP through sufficient taxonomy sampling. We present results to show that the robustness of EP estimation can be judged based on the resilience of EP to taxon sampling at fast evolving sites in an alignment. We provide a simple protocol to apply to sequence alignments to assess if they contain insufficient diversity to reliably estimate EPs.

Pervasive correlation of molecular evolutionary rates in the tree of life. Q. Tao1,2, K. Tamura3,4, F. Battistuzzi5, S. Kumar1,2 1) Department of Biology, Temple University, Philadelphia, PA; 2) Institute for Genomics and Evolutionary Medicine, Temple University, Philadelphia, PA; 3) Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan; 4) Research Center for Genomics and Bioinformatics, Tokyo Metropolitan University, Tokyo, Japan; 5) Department of Biological Sciences, Oakland University, Rochester, MI.

New species arise from pre-existing species and inherit similar genome biology and environment. This predicts greater similarity of mutation rates and tempo of molecular evolution between direct ancestors and descendants, which will cause autocorrelation of evolutionary rates in the tree of life. Surprisingly, molecular sequence data have not confirmed this expectation, possibly because available methods lack power to detect autocorrelated rates. Here we present an accurate machine learning method to detect autocorrelation of rates in large phylogenies. By applying this method to multigene and genome-scale sequence alignments from mammals, birds, reptiles, insects, metazoans, plants, fungi, and prokaryotes, we
discover pervasive and strong autocorrelation in molecular evolutionary rates throughout the tree of life in both DNA and protein sequences. These findings show concordance between molecular and non-molecular evolutionary patterns and will foster unbiased and precise dating of the tree of life.

689 Putting up with parasites: bruno reduces tolerance of transposition in the female germline. Erin S. Kelleher Kelleher, Uchechukwi Akoma, Jaweria Jaweria, Lily Ortega, Wenpei Tang Biology and Biochemistry, University of Houston, Houston, TX.

Transposable elements (TEs) are obligate genetic parasites that propagate in host genomes by replicating in germline nuclei, thereby ensuring transmission to offspring. This selfish replication not only produces deleterious mutations—in extreme cases, TE mobilization induces genotoxic stress that prohibits the production of viable gametes. Host genomes could reduce these fitness effects in two ways: resistance and tolerance. Resistance to TE propagation is enacted by germline specific small-RNA-mediated silencing pathways, such as the piRNA pathway, and is studied extensively. However, it remains entirely unknown whether host genomes may also evolve tolerance, by desensitizing gametogenesis to TE-induced genotoxic stress. In part, the absence of research on tolerance reflects a lack of opportunity, as small-RNA-mediated silencing evolves rapidly after a new TE invades, thereby masking existing variation in tolerance. We have therefore exploited the recent the historical invasion of the Drosophila melanogaster genome by P-element DNA transposons in order to study tolerance of TE activity in the absence of resistance. By performing genome-wide association on a panel of 1600 recombinant inbred lines that lack small-RNA-mediated silencing of P-elements, we uncovered multiple QTL that are associated with differences in tolerance of oogenesis to P-element transposition.

We took advantage of the classic phenomena of hybrid dysgenesis, in which crosses between naive females and males carrying genomic P-elements produce F1 offspring that suffer from unrestricted germline P-activity, due to the absence of maternally-transmitted piRNAs. The genotoxic stress imposed by P-elements disrupts oogenesis, and in extreme cases leads to atrophied ovaries that completely lack germline cells. We therefore examined broods of F1 offspring from dysgenic crosses involving >1000 RIL maternal genotypes for differences in the incidence of ovarian atrophy. We surprisingly uncovered continuous phenotypic variation, which we associated with multiple QTL on chromosomes 2 and 3. The most significant QTL explains 13% of phenotypic variation, and is localized to a small 260 Kb region in the euchromatic portion of chromosome 2L. The LOD peak resides in the bruno locus, which codes for a critical and well-studied developmental regulator of oogenesis. Surprisingly, we have discovered that multiple bruno loss of function alleles are strong dominant suppressors of ovarian atrophy, allowing for the development of mature egg-chambers in the face of P-element activity. In contrast, mutants that do not express oskar-mRNA, a critical target of Bruno protein throughout oogenesis, are strong enhancers of germline loss. Our observations reveal the potential for genetic variation in the developmental robustness of gametes to TE activity, which could minimize the fitness consequences of newly invading TEs.

690 Satellite Repeats are Associated with Host Tolerance of an Active TE. J. Lama, E. Kelleher University of Houston, Houston, TX.

Transposable elements (TE) are genetic parasites, which can move around in the genome during gametogenesis, causing DNA damage. Although host regulation of germline TE activity has been the focus of extensive research, less is known about host factors that could contribute to tolerance of TE activity. Because small-RNA mediated regulation of TEs is ubiquitous, tolerance is often masked, making it challenging to study. Hybrid dysgenesis systems in which TE regulation is short-circuited due to an absence of maternally deposited piRNAs, provide a unique opportunity to study tolerance. We used P-element dysgenesis in offspring produced from mating females from 617 Recombinant Inbred lines (RILs) obtained from Drosophila Synthetic Population Resource (DSPR) to males carrying P-element and assayed the ovaries of F1 females in two developmental time points. By Genome-Wide Association Study, we uncovered a complex quantitative trait locus (QTL) peak in the centromeric and pericentromeric region of chromosome 2, which influences female gametogenesis in the presence of P-element activity. Females harboring “tolerant” alleles of the QTL are more likely to produce gametes in the presence of P-element activity than those containing “sensitive” alleles. Pericentric heterochromatin is largely comprised of satellite repeats and TEs. They are therefore highly structurally variant and also are an important source of regulatory RNAs that silence homologous sequences across the genome. We discovered that strains carrying a tolerant allele produced significantly more piRNAs and siRNAs targeting responders (Rsp), a satellite repeat found in pericentric heterochromatin of chromosome 2. Further genomic analysis revealed a positive correlation of Rsp abundance with tolerance. It is wholly unexpected that the dosage of a satellite repeat with no homology to P-elements could be an important determinant of genomic tolerance of P-element activity. We propose that incomplete packaging of Rsp satellite repeats in dysgenic germline may enhance genomic instabilities triggered by P-activity, and thereby reduce tolerance.

691 Natural genetic variation in color perception in Drosophila. I. Reiss, J. Taylor, R. J. Johnston Biology, Johns Hopkins University, Baltimore, MD.

Color detection is an almost universal percept across metazoans and varies both between and within species. Currently, little is known about the genetic basis of intra-species phenotypic variation. Drosophila melanogaster is an excellent model to
study variation within species due to flies' reproducible and robust response to colored light. In a blue-green color preference test, flies navigate either toward blue or green light within a T-maze assay based on innate attraction to light. We analyzed natural variation in color preference using the Drosophila Genetic Reference Panel (DGRP). For each line, we calculated the ratio of flies that walked towards blue light versus green light and found that the DGRP lines display a large range of blue:green preference ratios. We conducted a Genome-Wide Association Study on the blue:green preferences of the DGRP lines and identified 9 single nucleotide polymorphisms (SNPs) that significantly associate with either extreme blue or extreme green preference. These SNPs affect genes involved in neuronal development and function, including Extended synaptotagmin-like protein 2, which is involved in synaptic vesicle fusion, and unc-5, which affects axon guidance and glial cell migration. This study demonstrates a remarkable range of variation in color preference within wild-derived populations and identifies candidate genes that regulate normal color vision, with implications for human genetics and the evolutionary maintenance of genetic variation.


Drosophila flight is a common quantitative behavioral trait used to evaluate organismal performance. This highly complex behavior requires a coordinated phenotypic response from many tissues and biological systems. However, the genes underlying flight performance in Drosophila are poorly understood.

Here, we sought to identify the most significant genetic modifiers of flight performance using the native Drosophila Genetics Reference Panel (DGRP) lines. We subjected approximately 100 flies of each sex from 189 DGRP lines to the flight performance assay pioneered by Seymour Benzer and further modified by the Ganetzky lab. We quantified mean landing height, dispersion of landing heights, and the proportion of flies that did not fly as distinct phenotypes.

The DGRP lines demonstrated wide variation in all measured traits, with the highest mean landing height roughly twice that of the lowest mean landing height for both sexes. These trait scores were used as inputs for separate Genome Wide Association Studies (GWAS). We found a strong positive correlation between male and female flight performance (r = 0.76, p < 0.00001) across the DGRP lines. In addition, we identified a number of loci (20 with LOD score > 8) whose association was significant across the sexes, suggesting these are likely strong candidate genes for flight ability in Drosophila. In contrast, the majority of significant loci associated with flight ability in one sex showed no association in the other sex, suggesting a complex sex-specific genetic architecture to this complex trait. In addition to intergenetic regions, candidate regions, and transcription factor binding domains, we identified a number of SNPs in candidate genes corresponding with neuronal patterning and function (Cadherin-N, Snoo, alan shepard), development (odd skipped, Dorsocross 2, bric a brac 1) and transcription factors (chameau, Sox21b). These genes and associated ontologies suggest the interdependence of various integrated biological systems that likely have a strong genetic basis for flight.

Our next step is to conduct flight performance assays in DGRP lines heterozygous for a Gal4-UAS system, allowing us to investigate specific gene functions in the Drosophila indirect flight muscle.

693 Genome Wide Association studies on nutraceutical effects of various chili peppers on Drosophila melanogaster. Nirwan Tandukar, Thangasamy Saminathan, Suresh Alaparthi, Padma Nimmakayala, Gerald Hankins, Umesh Reddy. Gus R. Douglass Institute and Department of Biology, West Virginia State University, Dunbar, WV.

Chili peppers have a plethora of health benefitting compounds like capsaicinoids, capsinoids, β-carotene, niacin, pyridoxine, etc. of which capsaicinoids are the most significant. Capsaicin, a secondary metabolite responsible for the spiciness of the peppers, has been reported to have anti-obesity, anti-hypertension, anti-diabetes, anti-oxidant and anti-inflammatory functions. In our studies, we focused mainly on the anti-obesity, antidiabetic and the antioxidant properties. Capsaicin is known to reduce adiposity by enhancing effects on energy and lipid metabolism by binding to the TRPV1 receptor. It is known to exhibit antioxidant activity by inhibiting lipid peroxidation. Therefore, the aim of this study is to evaluate health benefits of different chili peppers on Drosophila. Different horticulture groups (Habanero, Serrano, Jalapeno and Bell Pepper) that vary in content of various phytochemicals were fed to the Drosophila melanogaster Genetic Reference Panel (DGRP) lines. Changes in the body weight, triglyceride level, glucose level, negative geotaxis, and lifespan were measured after two weeks of feeding. We are currently analyzing the data using genome-wide association analysis for food intake, body weight, lifespan and negative geotaxis. Details will be presented.

694 Repeated horizontal gene transfer from bacteria to Drosophila. Kirsten I. Verster1, Jennifer Wisecaver2, Andy Gloss3, Noah K. Whitteman1 1) Integrative Biology, University of California - Berkeley, Berkeley, CA; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL; 3) Department of Biochemistry, Purdue University, Lafayette, IN.

There is debate over the extent of the role of horizontal gene transfer (HGT) in eukaryote evolution. HGT is known to be rare across Drosophilidae. Here we report evidence of three independent HGT events of a prokaryotic gene encoding a toxin into
the genomes of *Drosophila* species. We used phylogenetic analysis, genomic analysis, PCR and Sanger sequencing, gene expression studies and enzyme activity assays to characterize these HGT events. These analyses strongly indicate the horizontally transferred gene copies were not an artifact of bacterial contamination. The enzymes encoded by these horizontally transferred genes show homology at enzymatically important residues, suggesting conservation of function in highly divergent lineages. To our knowledge this is the first major HGT event reported within the *Drosophila* lineage, other than those from *Wolbachia* species.

695  **Scaptomyza flava** as a model for testing the role of gut bacteria in the evolution of herbivory.  R.P. Duncan, N.K. Whitman  Department of Integrative Biology, University of California, Berkeley, Berkeley, CA.

Several dipteran lineages convergently evolved a herbivorous diet despite major evolutionary barriers to eating plants. One barrier to herbivory is that plants produce toxic defense compounds like nicotine, caffeine, and mustard oils. While many insect herbivores have endogenous mechanisms to mitigate the effects of these toxins, there is a lot of interest in the role of gut bacteria in facilitating host plant detoxification but little evidence supporting this hypothesis. As a first step to testing the role of gut bacteria in the evolution of herbivory, we isolated bacteria from surface-sterilized larvae of the emerging model herbivore *Scaptomyza flava*. *S. flava* is a drosophilid that feeds on mustard plants and directly encounters their defense compounds (mustard oils). Through culture-dependent methods combined with gut community quantification using droplet digital PCR, we found that the bacterial community of wild *S. flava* is culturable and simple. Further, while some morphospecies fail to grow on selective medium containing the mustard oil phenethyl isothiocyanate (PEITC), others grow even when PEITC is present in high concentrations, indicating that some *S. flava* gut bacteria are resistant to high concentrations of PEITC – a result consistent with PEITC metabolism. Work is underway to test if resistant isolates can metabolize PEITC and other mustard oils in vitro.

696  **Interspecific bias in Drosophila aggression depends on genetic distance.**  Tarun Gupta, Sarah E. Howe, Marlo L. Zorman, Brent L. Lockwood  Department of Biology, University of Vermont, Burlington, VT.

Competition for mates induces intrasexual aggression in many species. Although this reproductive competition is predicted to be highest among conspecifics, heterospecifics may also compete for mates, particularly among closely related hybridizing species. However, it is not known if these interspecific reproductive interactions influence aggression behavior within vs. between species. Here we show that male *Drosophila* discriminate between conspecifics and heterospecifics in aggressive social interactions. Distantly related species pairs were more aggressive to conspecifics than heterospecifics. However, among sibling species pairs that hybridize, male aggression was asymmetric and mirrored patterns of asymmetry in pre-mating isolation. Species with females that mate indiscriminately with heterospecifics had males that were most aggressive to heterospecific males, whereas species with females that selectively mate with conspecifics had males that were most aggressive to conspecifics. To our knowledge, this is the first study to quantify aggression between *Drosophila* species and to test for a behavioral preference for aggression against conspecific vs. heterospecific rivals. Overall, our data suggest that reproductive competition plays a key role in the evolution of aggression behavior among *Drosophila* species and that male-male aggression may be a mechanism of pre-mating isolation between hybridizing species.

697  **Determining binding specificities of cell adhesion molecules from Drosophila and other related Dipterans.**  Leah Anderson, Mark Seeger  Molecular Genetics, The Ohio State University, Columbus, OH.

Neurons of both vertebrates and invertebrates exhibit a complex set of cell-to-cell interactions during successful development of the nervous system. Cell adhesion molecules (CAMs) play an important role in mediating many of these specific and stereotyped cell-cell interactions. I am investigating the binding specificities of two CAMs of the immunoglobulin superfamily from Dipteran insects: Lachesin (Lac) and Amalgam (Ama). Ama arose as a duplication of Lac early in Dipteran evolution, and both proteins still share extensive sequence similarity. In *Drosophila melanogaster*, Lac is membrane-linked and homophilically binds itself. Ama, which is a secreted molecule, has both a homophilic binding property as well as the ability to heterophilically bind another CAM, the transmembrane protein Neurotactin (Nrt). Despite the high level of amino acid sequence similarity between Ama and Lac, the two proteins are unable to bind each other, and Lac does not display any interaction with Nrt. The goal of this project is to identify the precise domain(s) of Lac and Ama that produce these differences in binding specificity. To accomplish this, chimeric constructs of the three immunoglobulin-like domains of Ama and Lac from *D. melanogaster* have been created and cloned into a vector for regulated expression in Schneider 2 (S2) cell lines. The S2 cells are then to be used for aggregation assays, which will reveal binding patterns of the chimeric proteins. Preliminary aggregation assays have revealed that the first immunoglobulin domain is responsible for the homophilic binding specificity of both Lac and Ama. Further experiments to test the secreted version of the Ama/Lac chimeras will allow identification of the domains that contribute to Nrt binding and Nrt-mediated cell adhesion. Using this approach, a thorough model can be devised for the specific interactions of Lac, Ama, and Nrt in *D. melanogaster*. In addition to studying these protein interactions, I am utilizing bioinformatic databases to locate and subsequently clone out orthologs of Ama and Lac in other Dipteran species. Testing these clones in further aggregation assays will help develop a better understanding of how the unique binding properties of Ama and Lac have changed over evolutionary time.
Organisms and the environments in which they reside are not static through time. Rather, as the organism ages, the environment fluctuates and the ecological niches utilized by different life stages changes over time, resulting in differential selection across ontogeny. In highly seasonal environments, individuals are exposed to drastically different thermal environments. This developmental variation is particularly striking in organisms with complex life cycles, wherein life history stages also exhibit distinct morphologies, physiologies, and behaviors. At the genetic level, genes that act pleiotropically across life stages, constrain evolutionary trajectories, alternatively genes may be unique to each stage, allowing for independent evolutionary trajectories in response to stage-specific selection. We aim to understand the role of genetic constraint in thermal hardiness across metamorphosis in *Drosophila melanogaster* using Drosophila Genetic Reference Panel, or the DGRP. We have estimated the genetic correlation of thermal hardiness between life stages by exposing larvae and adults to a lethal low and high temperatures and scoring survival. Additionally, we implemented genome-wide association (GWA) to estimate associations between naturally segregating variation and cold hardiness for both larvae and adults. These approaches reveal no significant correlation for thermal hardiness between life stages and that loci significantly associated with the variation in cold hardiness largely affect one life stage and not the other. These results suggest nearly complete genetic decoupling of thermal hardiness across the metamorphic boundary. Secondly, to explore stage-specific gene regulation of cold hardiness, RNA was extracted and sequenced from larvae and adults sampled from a subset of lines with extreme cold tolerance phenotypes across a time series spanning before, during, and after cold stress. Analyses of RNAseq data indicate gene regulation modules and candidate genes with specific effects on either larval or adult hardiness. Taken together, our results illustrate independent genetic mechanisms underlie stage-specific cold hardiness in *D. melanogaster*.

The compound eyes of Diptera exhibit remarkable morphological diversity often associated with behavioural adaptations that seem to play crucial roles in survival and reproductive fitness and hence in species evolution. Although we understand much about the genes and developmental mechanisms that govern eye development in insects, we lack knowledge about the genetic basis of eye phenotypic variation within populations and the evolutionary forces acting on such variation. We and others have observed substantial inter- and intra-specific variation in eye size among various wild strains of the *Drosophila melanogaster* species subgroup, but currently we lack little understanding of the genetic basis of such variation and its possible link to behavioural differences and adaptation. Here we will present work on the genetic analysis of quantitative traits associated to eye size in natural populations, such as frontal eye area, number of ommatidia and inter-ocular distance. We present data on the heritability of eye size in a population of *Drosophila melanogaster* from its ancestral range. Through an interval mapping approach, we have mapped quantitative trait loci underlying eye size, inter-ocular distance and leg size variation within *Drosophila melanogaster* and *Drosophila simulans*, using strains selected from the extremes of these species natural eye size variation. We have validated the mapped effects using reciprocal introgressions, describing both body size dependent and independent effects. We further focus more closely in fine scale introgression-based mapping of body size uncorrelated loci detected on the 3rd chromosome of both *Drosophila melanogaster* and *Drosophila simulans*, highlighting candidate regions regulating eye size specifically. Additionally, we have explored differences in eye imaginal disc development of the analysed strains, focusing on the relative sizes of the pro-neuronal vs. non-neuronal lineage in the eye primordium. We hope that our study will shed light on natural variation that is relevant for the evolution of eye morphology in *Drosophila* and help us understand new fundamental aspects of eye development in general.

During development of the *Drosophila melanogaster* embryonic central nervous system (CNS), commissural axons express Commissureless (Comm), which posttranslationally regulates the inhibitory receptor Roundabout (Robo) allowing them to cross the midline. Bioinformatic analysis of available genomic and transcriptomic data sets reveals Comm to be conserved widely in Insecta; found in some of the most basal insects, such as dragonflies and firebrats. However, there have been multiple independent losses of Comm. Within Lepidopteran sequences available, no Comm gene can be identified. Similarly, Comm is not found in more derived Hymenopterans of the suborder Apocrita (bees, wasps, ants) or in the well studied Coleopteran, *Tribolium castaneum*. Conversely, two other members of the Comm family of proteins, Comm2 and Comm3, are encoded by the *D. melanogaster* genome. During Dipteran evolution, an expansion of the Comm family occurred in derived flies, while more basal Dipterans, such as sand flies, have only a single Comm gene. Functional diversification of the Comm family members has been demonstrated by several assays. *In vivo* panneural overexpression of Comm in *D. melanogaster* has a strong gain-of-function phenotype for axons crossing the midline; while Comm2 overexpression has only a marginal gain-of-function phenotype, and Comm3 has no discernable midline phenotype when overexpressed. In S2 cells, *D. melanogaster* Comm and Comm2 prevent Robo cell surface accumulation, while Comm3 does not. Similar results are also observed when
using Comm family members from \textit{D. virilis, Musca domestica, and Megaselia abdita}. The Comm protein from \textit{Anopheles gambiense}, a basal Dipteran with a single Comm gene, has reduced ability to relocalize \textit{D. melanogaster} Robo. More ancestral Comm proteins from the Coleopteran, \textit{Onthophagus taurus}, and the Hemipteran, \textit{Oncopeltus fasciatus}, are unable to relocalize \textit{D. melanogaster} Robo. Two hypotheses could explain these results: 1) Ancestral Comm proteins do not relocalize endogenous Robo; or 2) Ancestral Comms cannot relocalize \textit{D. melanogaster} Robo, but can relocalize their endogenous Robo due to co-evolution of the two proteins. Currently, the ability of \textit{O. fasciatus} ancestral Comm to relocalize endogenous Robo is being tested.

701 Incorporation of horizontally transferred genes into the embryonic patterning network of the wasp \textit{Nasonia}. D. Pers, J. Lynch Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL.

A transcriptome profiling approach identified over 100 genes regulated along the embryonic dorsal-ventral axis of the wasp \textit{Nasonia}. Surprisingly, our previous work sought to study the wasp patterning system with enough depth and resolution to conduct a meaningful comparative GRN analysis and provide insights into the diversity of mechanisms that can be deployed in the evolution of development. However, this global comparison of fly and wasp dorsoventral GRNs has shown that a conserved patterning output, such as tissue specification in the embryonic blastoderm, can arise from GRNs that share surprisingly little similarity in terms of molecular composition.

Outside of a small core set of genes with conserved expression, the majority of the dorsoventral expressed genes uncovered are unique to \textit{Nasonia}. A particularly interesting subset of these novel dorsoventral genes is characterized by the presence of encodes multiple Ankyrin repeat domains and in many cases a clear C-terminus PRANC domain. The PRANC domain was first identified in ankyrin domain containing proteins in poxviruses, and genes of this structure have also been found in the endosymbiotic Wolbachia. These 15 \textit{Nasonia} ankyrin encoding genes have no clear orthologs outside of Chalcidoidea, the Superfamily of parasitic wasps to which \textit{Nasonia} belongs, and are in fact most similar to genes found in bacteria and viruses, suggesting acquisition via horizontal gene transfer (HGT). In order to understand the developmental and evolutionary significance of the DV expression of the PRANC-type ankyrin (PRANC-ANKs) genes in \textit{Nasonia}, we took two approaches. Furthermore, knockdown First we functionally analyzed of these genes with RNAi. Our results indicate indicates that PRANC-ANKs have been incorporated into several development processes including they have gained crucial functions in regulating expression patterns of other dorsoventral genes, morphogenetic movements, and developmental timing. Our other approach is to examine the function and expression of PRANC-ANKs in other wasp species in order to understand how these genes have been functionally integrated into developmental processes. To this end, we have developed a set of tools (in situ hybridization, RNAi, RNAseq) to establish \textit{Melittobia}, representing the next most closely related Family to that of \textit{Nasonia}, as a model system. Our initial results indicate that only a subset of the \textit{Melittobia} orthologs of \textit{Nasonia} DV PRANC-ANKs have conserved expression domains, indicating that developmental integration of these genes can occur relatively rapidly and specifically in different lineages.

702 Investigating morphological novelty in the evolution of the \textit{Drosophila} genitalia. G.R. Rice¹, W.J. Glassford², M Rebeiz¹ 1) 1 Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 2) Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

Morphological diversity is established by the gain and loss of traits. Although there are many examples of the molecular mechanisms behind trait loss, there are few studies that investigate how traits are gained. We used the rapidly evolving morphology of \textit{Drosophila} genitalia to investigate how new traits evolve. In particular, we studied both the posterior lobe, a recently evolved novelty, and penile spikes (known as the branches of the basal process) whose homology relationships are as of yet underexplored.

The phallic branches have been implicated in wounding females during mating and are found in species throughout \textit{Drosophila}. However, due to the rapid diversification of the genitalia, it is difficult to determine the homology of the phallic branches between species. Using light and SEM microscopy, it is difficult to determine which tissue-types (gonopod, aedeagus, or hypandrium) form the adult branches. We investigated whether phallic branches of different species were produced by the same tissue (homologous) or different tissues (non-homologous). We established a time course of the developing genitalia using confocal microscopy, which allowed us to capture the formation of the phallic branches early in development. We find that the ventral phallic branches of \textit{D. melanogaster} (gonopod), \textit{D. teissieri} (aedeagus), and \textit{D. ananassae} (hypandrium) actually arise from different tissue types. It is interesting to find that such similar looking features are not homologous and can form in such different tissues within the genitalia. It will be vital to determine whether non-homologous phallic spikes use similar developmental networks that are deployed in different tissues of the phallus.

Additionally, we find that a key signaling molecule: \textit{wingless} is expressed near the posterior lobe as well in aedeagus and hypandrium, two of the spike producing tissues. We have identified cis-Regulatory Modules that drive \textit{wingless} expression near all three structures. We are currently testing whether changes in these regulatory sequences account for the divergent
expression. This work provides us a window into the patterning mechanisms that may have led to the origin of these features.

703 Investigating the developmental network of the posterior lobe, a novel morphological structure. D. Shodja, W.J. Glassford, W. Johnson, M. Rebeiz Biological Sciences, University of Pittsburgh, Pittsburgh, PA.

One of the biggest challenges in studying the evolution of novel anatomical structures is deciphering how the genetic programs underlying their development initially form. Work on these morphological novelties has frequently implicated drastic changes to signaling pathways in their development and evolution. However, we generally lack an understanding of how the regulation of these pathways are altered to form new structures, and how downstream responses to signaling events evolve to shape these structures during their development. To investigate this complex phenomenon, we studied the role of Notch signaling during the evolution of a recently evolved structure, the posterior lobe. This structure is a cuticular outgrowth on the genitalia of males within the melanogaster clade. The ligand for the Notch signaling pathway, Delta (Dl), is required for posterior lobe development, and its expression has been expanded in lobe-forming species. We’ve identified a transcriptional enhancer in the Dl locus that recapitulates the endogenous expression of Dl in the posterior lobe. Comparisons of this enhancer’s activity to reporters bearing orthologous regions from non-lobed species suggests that changes have occurred upstream of Dl to expand into the lobe-forming zone. Thus, in contrast to previous examples of novelty, our results suggest that a pre-existing signaling source was expanded and recruited to generate a novel structure.

We present experiments to dissect the upstream regulators of Dl and investigate downstream targets of the Notch pathway in this novel tissue. These results highlight the nuanced view of novelty that can be obtained through the comparison of closely related species at the level of gene regulatory elements.

704 The cellular mechanisms accounting for the evolutionary adaptation of closed to open rhabdoms in compound eyes. A. Zelhof, S. Mahato, J. Nie, D. Plachetzki 1) Department of Biology, Indiana University, Bloomington, IN; 2) Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH.

An elementary question of evolutionary biology is how developmental processes are modified to produce adaptive transitions within the constraints of constructing a functional tissue. Here we address this question by investigating the cellular mechanism responsible for the transition between closed and open rhabdoms of ommatidia of apiposition compound eyes. Utilizing Drosophila and Tribolium as representatives of each rhabdom arrangement, we have identified three changes that are required for this adaptation to occur. First, EYS expression was coopted and expanded from cilia sensory neurons to include rhabdomeric photoreceptor cells. Second, EYS homologs of open systems are defined by an extension of the amino terminus thus internalizing the cleaved signal sequence. The change does not interfere with cleavage or function in ciliary sensory, but is required for targeting to the apical photoreceptor membrane. Third, a specific EYS interaction with a subset of Prominin orthologs is necessary thus defining a difference between Prominin paralogs within and between species. Altogether, these findings define a set of molecular, cellular and evolutionary constraints accounting for the transformation of the ancestral function of EYS in ciliary sensory neurons to produce a novel adaptive change in rhabdomeric photoreceptor cell arrangement.

705 Stop codon readthrough of a POU/Oct transcription factor regulates Drosophila development. Y. Zhao, S. Esfahani, X. Tang, B. Lindberg, Y. Engstroem Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, SE.

Stop codon readthrough is a mechanism commonly utilized by viruses and prokaryotes to add plasticity to the proteome without expanding the genome. Recent studies indicate that readthrough is more pervasive in eukaryotes than previously predicted. Approximately 700 genes have been proposed as stop codon readthrough in Drosophila development. The underlying mechanisms and functional importance of this phenomenon is, however, still unknown at large and remains to be deciphered.

Here we studied the impact of readthrough in the POU/Oct transcription factor drifter(dfr)/ventral veins lacking (vvl), a key factor in the expression of ecdysone biosynthesis genes in the prothoracic gland. We show that stop codon readthrough of dfr/vvl mRNA occurs at a high relative rate in the gland, resulting in a novel, extended isoform of the protein. Overexpression of the small (Dfr-S; 46 kDa) and large (Dfr-L; 74 kDa) isoforms as well as RNA interference (downregulating both isoforms) in the prothoracic gland all led to developmental arrest, but at different stages of development. This could be partly rescued by ecdysone feeding. By inducing flip-out clones in the prothoracic glands, we demonstrated that overexpression of either Dfr-L or Dfr-S diminished the other isoform, respectively. We applied CRISPR/Cas9 to generate Dfr-L frameshift mutant lines in the ORF immediately downstream of the annotated stop codon. This resulted in prolonged larval development and delayed metamorphosis. In addition, we observed compromised expression of the steroidogenic enzymes Nvd, Dib, and Sad in the prothoracic gland in the mutant larvae, which was dependent on the Dfr-L but not Dfr-S. The expression of Phm, however, was specifically dependent on the short isoform, suggesting complementary functions of the isoforms in the ecdysone biosynthesis pathway. Our findings reveal a novel regulatory mechanism, in which the relative level of stop codon
readthrough produces alternative transcription factor isoforms with different regulatory potential of downstream targets and processes.

706  **Interommatidial bristles: An exceptionally variable trait in the Dipteran tree of life.**  Kimberly Palmer¹, Joseph Neary¹, Madison Seifer¹, Kevin Begic², Tiffany Cook³, K. Aimanova, S. Gill, Markus Friedrich¹,³,⁵  ¹Dept Biological Sciences, Wayne State University, Detroit; ²University of Detroit Jesuit High School and Academy, Detroit; ³Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit; ⁴Department of Ophthalmology, Wayne State University School of Medicine, Detroit; ⁵Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit.

While the development and visual function of *Drosophila* compound eye ommatidia have been studied for decades in exceptional detail, comparatively little attention has been given to the second type of peripheral sense organs that furnishes the eye surface: the interommatidial bristles (IOBs). Comprising a socket cell, shaft cell, glial cell, and single neuron, IOBs structurally and developmentally correspond to touch receptor bristles. However, whether IOBs monitor mechanosensory information from the compound eye surface has not been addressed. Moreover, while ommatidia constitute an essential building block of the arthropod compound eye, the presence of IOBs varies widely.

To gain comparative insights into IOB function, we explored their variability in the over 100 families of the megadiverse insect order Diptera. In addition, we compared IOB variability with that of more than 350 other structural traits previously compiled for 35 dipteran families. These effort reveal that the presence of IOBs is highly variable in modern Diptera. ~60% of dipteran families lack IOBs in contrast to Drosophilidae, which are consistently IOB-positive. Further, 20% of dipteran families include species both with and without IOBs. Evidence of IOB variability exists even at the genus and species level. Ancestral state reconstruction suggests that this rampant variability resulted primarily from parallel trait loss events (~10 in 35 families alone) and possibly also cases of trait reemergence, conceivably through spatial reactivation of the deeply conserved sensory bristle development module.

Probing the large "IOB variable" families Chironomidae (>10,000 species) and Tachinidae (>8,000 species) for ecological corollaries of IOB presence, we found only tentative evidence for a correlation with temperature and yearly average sunshine. Given the exceptional variability of IOBs across Diptera and their deep conservation in drosophilid flies, we have begun to study the neuroanatomy of this bristle subtype in *Drosophila* to identify its neural target and gain a better understanding of its function and complexity.

707  **The show must go on: Maintaining a segmented body plan after the loss of a key regulatory gene.**  Alys M. Cheuttle Jarvela, Leslie Pick  Entomology, University of Maryland College Park, College Park, MD.

Gene regulatory networks precisely control gene expression in space and time and are used during development to partition embryonic territories, deploy differentiation gene batteries, and ultimately create specific cell-types and tissues. *Paired* is a *Pax3/7* transcription factor with a critical role in the *Drosophila melanogaster* segmentation gene network. Mutation or knock-down results in the loss of alternating segment primordia in not only *Drosophila* embryos, but in a number of other diverse insect species. Our work indicates that *paired*’s role in this pathway is ancient, as we see disrupted segmentation upon RNAI perturbation in a basally-branching insect, the cricket *Gryllus bimaculatus*. The segmentation network is well-resolved in *Drosophila*, allowing for detailed evolutionary comparisons of network wiring. Our phylogenetic analysis indicates that *paired* has been lost from mosquito genomes. To determine if another *Pax3/7* homolog replaced *paired* in this gene network, we first performed in situ hybridization and confirmed that *paired*’s paralog, gooseberry (*gsb*), is expressed in the correct pattern to regulate *Paired*’s target genes in the malaria vector mosquito, *Anopheles stephensi*. We also used this technique to survey the extent of regulatory gene conservation of the pair-rule network, finding that the network components are relatively unchanged in their spatiotemporal expression. One interesting difference is that sequential addition of segments is drawn-out over a longer relative developmental period in *Anopheles* than in *Drosophila*. This may reflect the dependence of segmentation on *gsb*, which is further down in the pair-rule gene network hierarchy than *prd*. Additionally, we have isolated several conserved noncoding regions around *Drosophila* *Paired*’s target gene, *engrailed* (*en*), in *Anopheles* that recapitulate the *en* expression pattern when used to drive a reporter gene in *Drosophila* embryos. Together, these data support the hypothesis that a simple substitution of *gsb* in place of *prd* could have occurred in the mosquito lineage. This type of paralog replacement has been achieved experimentally, but has never been observed in a natural gene network context. To test this hypothesis we are currently attempting to assay *gsb* function, using RNAi and CRISPR, in *Anopheles*.

708  **Identification of *Aedes* Cadherin protein function and examination of its localization by recent gene editing tools.**  J. Chen, K. Aimanova, S. Gill  Department of Cell Biology, University of California, Riverside, Riverside, CA.

In *Drosophila*, E-cadherin is a constituent of adherens junctions and so the primary role of E-cadherin is preservation of epithelial integrity. Similarly, in *Aedes aegypti* mosquitoes there is a group of E-cadherin-like proteins. One of these, the *Aedes* cadherin (AeCad), has been characterized as a receptor for *Bacillus thuringiensis* subsp. *israelensis* (Bti) Cry11A toxins. However, its localization in the mosquito guts, its function on mosquito developmentand its role on Cry11A toxicity against *Aedes* mosquitoes are not fully understood. In this study, we manipulated the cadherin gene using ZFN and TALEN. Even
though we got 2 nucleotides deletion by ZFN and 4 nucleotides deletion heterozygous by TALEN, respectively, we could never obtain the homozygous mosquito lines. Because the ZFN and TALEN have much less off-target issue, we think the cadherin gene is likely essential for *Aedes* development. In contrast, in lepidopteran insect this cadherin appears to be unessential since homozygous mutants are viable. We also examined AeCad localization by gene tagging and successfully tagged this protein with EGFP using CRIPR-Cas9-mediated homologous recombination. We also observed *Aedes* Rad51 improved the homologous recombination (HR) rate by about 1.8 fold. Confocal images showed AeCad has high expression in larval caecae and posterior midgut where Cry11A binds, and low expression in the anterior gut where the Cry11A protein does not bind. The EGFP-tagged cadherin co-localizes with the *Aedes* cadherin-specific polyclonal antibody-detected cadherin protein, suggesting it is *Aedes* cadherin receptor that has been tagged with EGFP. The EGFP-tagged cadherin proteins are only localized on the apical side of epithelium cells, distinct from that of snake skin protein, a membrane protein associated with smooth sebaceous junctions, suggesting AeCad might not function as a gap junction protein in *Aedes* midgut. But this *Aedes* cadherin is an essential gene for mosquito development.

**709  Epistatic interspecies allelic interactions generate facial developmental defects in haploid hybrid Nasonia wasps.** Lorna Cohen¹, John H. Werren², Jeremy A. Lynch¹ ¹) Biological Sciences, University of Illinois at Chicago, IL; 2) University of Rochester, Rochester, NY.

It is increasingly clear that the evolution of developmental processes often involves changes within complex networks of interacting genes. The identity of the participants and the nature of the interactions within the networks are usually obscure, but can be revealed by the phenomenon of epistasis, where the novel combination of alleles leads to a phenotype significantly different from the sum of the phenotypes of the alleles in isolation. Studying epistasis is difficult in typical diploid animal model systems, due to dominance interactions between alleles within loci, and the rapid increase in rarity of desired genotypes as the number of interacting loci increases. *Nasonia* wasps are significantly less affect by these difficulties, as all males are haploid, thus eliminating dominance interactions and increasing the frequency of desired genotypes. Viable and fertile hybrids between *Nasonia* species with morphologically distinct males can be made, and recombinant haploid F2 males are readily obtainable. With genome sequences available for the relevant species, these features make *Nasonia* a powerful system for evolutionary genetics. A common phenotype observed only in hybrid males between *N. vitripennis* and *N. giraulti* is clefting at the facial midline. Preliminary analyses have indicated that a three-way epistatic interaction network explains the vast majority of these cases. Analysis of artificially produced F1 hybrid males has shown these interactions are among recessive alleles. F2 hybrids between *N. vitripennis* and *N. longicornis* and between *N. giraulti* and *N. longicornis* indicate that temporal divergence, rather than morphological divergence best explains the origin of the negative interaction. Finally, we have introgressed one of the interacting alleles from *N. giraulti* into a *N. vitripennis* background, producing a line that consistently produces the clefting phenotype. This will be the basis for detailed analysis of the developmental basis of the phenotype, as well as mapping the causative and interacting alleles.

**710  Canonical telomeres in Photinus pyralis.** Isaac Wong, Amanda Larracuente, Christian Silva  University of Rochester, Department of Biology, Larracuente Lab, Rochester, NY.

Telomere sequences and telomerase reverse transcriptase (TERT) genes are among the most conserved elements across invertebrate genomes. In Coleoptera, a clade of species has diverged from the canonical telomere sequence of TTAGG to the sequence TCAGG. Insights into the mechanism responsible for this change could lead to a better understanding of the driving forces behind evolution and transposable elements. Here, we demonstrate that the *Photinus* genus of Coleoptera possess the canonical telomere sequence. We also provide an evolutionary analysis of the *Photinus* TERT sequence to provide insight into the complex phylogeny of Coleoptera.

**711  Sexed Adult Tissue Expression Atlas for the Drosophila genus.** H. Yang¹, J. Fear², Y. Wang², S. Mahadevaraju¹, T. Prezytycka², B. Oliver² ¹) NIDDK, National Institutes of Health, Bethesda, MD; 2) NCBI, National Institutes of Health, Bethesda, MD.

RNA-seq profiles of Drosophila have contributed to our understanding of transcriptome diversity in different sexes and tissues. Conserved expression is a valuable evidence of importance in a pathway. We are interested in understanding the conserved as well as diverse expression patterns in Drosophila genus in different sexes and tissues. In order to understand the transcriptome diversity we performed RNA-seq of not only *D. melanogaster* but 12 other species. We sequenced 664 samples of Drosophila genus in total covering 19 strains, two sexes, and eight adult tissue types (head, thorax, abdomen, digestive system, gonad, reproductive tract, and terminalia). To explore the transcriptomes of these species, high quality annotations and accurate orthologs relationships are a prerequisite. Therefore we used the RNA-seq data and built annotations for non-*melanogaster* species by optimizing nine StringTie parameters, Support Vector Machine (SVM) and recognized high quality gene models based on the *D. melanogaster* annotation. Drosophila orthologs were updated by gene synteny, expression correlation, gene structure and sequential similarity in the updated annotation. Our methods identified more than 50% increase in transcript isoforms for non-*melanogaster* species, making their gene models more realistic for gene and isoform level expression analyses. We found that 16% (1,896) one-to-one orthologs shared among the Drosophila
genus had conserved expression across different sexes, tissues, and species. These orthologs were enriched in mitochondrial translation (e.g., mitochondrial ribosomal proteins), RNA metabolic process (such as biosynthesis and splicing) and aminoglycan metabolic process. In contrast, the remaining one-to-one orthologs had dynamic changes in expression among different sexes, tissues, and species. The least conserved group of these orthologs was enriched in behavior, anatomical structure morphogenesis, and signal transduction. Our current work generated annotations for different Drosophila species, and it is a comprehensive resource for consensus and comparative transcriptome in the Drosophila genus.

712 Rpd3 controls the concentration-dependent gap genes homeostasis in Drosophila embryos. P. Das¹, U. Bhadra², M. Pal Bhadra¹ 1) Centre for Chemical Biology, Indian Institute of Chemical Technology, Hyderabad, Telangana, IN; 2) Center for Cellular and Molecular Biology, Hyderabad, Telangana, IN.

Gap genes are the cadre of earliest zygotic genes that establish distinct patterning in Drosophila embryos, including the anteroposterior axis formation. These genes operate under the synchronized gradients of the maternal genes, like Bicoid in a highly concerted fashion. The interaction of Rpd3 with the small RNA processing enzyme, Dcr-1 ushers a possibility of a new role of Rpd3 in the maintenance of chromatin architecture and integration of the expression of the gap genes-Kruppel, hunchback, giant and tailless. The Rpd3 alleles opted are dominant repressors, lethal, maternally contributed and function rapidly during development. The expressions of the gap genes were assessed in the different RNAi heteroallelic escaper mutants, particularly of Ago-1, Rpd3 and Dicer using quantitative Western blot analysis and further validated through immunolocalization studies. The loss-of-function of the two Rpd3 heteroallelic mutants showed a reduction in the gap gene expression, diminishing stripes and body size by the segmental loss. The chromatin immunoprecipitation studies have shown that the concentration for gap gene expression is maintained by a unique participation of different histone tail modifiers. Particularly, the loss-of-function of Rpd3 is found to deregulate gap genes by decreasing their attachment with the respective promoters and transcriptional machinery. This knock-down results in the alteration of the concentration of the gap gene transcript, indispensable for the anteroposterior axis formation during development. Thus, Rpd3 generates a differential stimulus to maintain the delicate balance of gap gene expression, thereby instructing the anteroposterior boundary formation.

713 Understanding diversity along Antero-posterior body axis. N.P. Singh, Bony De Kumar, Kausik Si, Robb Krumlauf 1000 E, 50th St., Stowers institute for medical research, Kansas City, MO.

Understanding the evolution of vertebrate and invertebrate developmental processes is an important goal. In vertebrates through whole genome duplications, duplicated and diverged genes evolve from invertebrate homologues and contribute to different functions in the vertebrates. We examined the conserved Hox gene locus in Drosophila and mouse to explore this question. Hoxa1 and Hoxb1 genes of mouse were knocked in the locus of at the homologous gene, labial, in Drosophila. The approach is designed in such a way that knock-in flies will have a loss of function mutation in labial gene, while the vertebrate genes, Hoxa1 or Hoxb1 will be functional and employ the regulatory elements of labial gene. We observe a complete rescue of the labial phenotype by Hoxa1 but Hoxb1 shows very weak or minimal rescue. To further understand this evolutionary divergence, we conducted ChIP-seq experiment in Drosophila embryos with Labial, Hoxa1 and Hoxb1 to evaluate their down-stream targets. We observed that genome wide binding of Hoxa1 is very similar to labial while Hoxb1 binds to a more limited set of these sites in the fly genome.

In the reverse experiment, we expressed Flag-tagged Hoxa1, Hoxb1 and Labial in mouse ES cells to map the binding sites of these proteins in the mouse genome using ChIP-seq experiments. Comparative analysis of these proteins in differentiated ES cells also suggests that labial binding is more similar to that of Hoxa1. In summary, these experiments in flies and mouse ES cells indicate that Hoxa1 has retained the evolutionary function of labial while the Hoxb1 gene has diverged and lost most of Labial function and may have acquire new functions.

714 Opposing transcriptional and post-transcriptional roles for Scalloped in Hippo-dependent fate decisions. B. Xie¹, D. Morton¹, T. Cook² ³ 1) Department of Integrative Biosciences, Oregon Health & Science University, Portland, OR 97239; 2) Center of Molecular Medicine and Genetics, Wayne State School of Medicine, Detroit, MI; 3) Department of Ophthalmology, Wayne State School of Medicine, Detroit MI.

The Hippo tumor suppressor pathway controls multiple aspects of animal development, yet how context specificity is achieved remains relatively poorly understood. Two conserved and essential effectors of the Hippo pathway are the TEAD transcription factor Scalloped (Sd) and its non-DNA binding transcriptional co-activator Yorkie (Yki). Previous studies have shown that the Hippo signaling pathway requires both sd and yki to control blue- vs green-sensitive neuronal subtype specification in post-mitotic Drosophila photoreceptors. Here we report that sd and yki play distinct functions in this process through differential regulation of the blue-sensitive opsin gene, Rh5. At the transcriptional regulatory level, we find that sd directly represses Rh5 promoter activity in Hippo-positive green photoreceptors and that yki antagonizes this repression in Hippo-negative blue photoreceptors. In addition, we demonstrate that sd promotes Rh5 protein expression in blue photoreceptors through a mechanism involving the Rh5-3UTR to. Thus, sd oppositely controls blue vs green photoreceptor fate via different cis-regulatory elements of the same gene. Combined, these studies reveal opposing transcriptional vs post-
transcriptional mechanisms by which TEAD factors control the output of the Hippo pathway to ensure robust neuronal fate decisions.

715  **Eyeless/Pax6 Promotes Eye Development from the Peripodial Membrane through Pattern Formation and Dpp Expression.** Luke Baker, Bonnie Weasner, Athena Nagel, Sarah Neuman, Arash Bashirullah, Justin Kumar 1) Department of Biology, Indiana University, Bloomington, IN; 2) Department of Pharmaceutical Sciences, University of Wisconsin, Madison, WI.

Pax6 is an extraordinarily well-conserved DNA-binding transcription factor that has been shown to regulate eye development in all seeing animals. The loss of Pax6 in humans can lead to Aniridia and severe CNS developmental defects. In Drosophila, the Pax6 homolog eyeless (ey) is expressed within the larval eye-antennal imaginal disc and has been shown to sit atop a gene regulatory network (GRN) that controls the specification and growth of the adult Drosophila compound eye. Contrary to its name, ey mutants have a wide range of phenotypes, from flies that completely lack the compound eye to those that have eyes that appear wild type. It has been suggested that the loss of ey results in the complete collapse of the downstream GRN, known as the Retinal Determination (RD) network. Here, we show that in a CRISPR/Cas9 generated ey allele (named ey<sup>ab</sup>) that lacks the eye-specific enhancer, the downstream RD members are still present. Drosophila contain a second Pax6 homolog, twin of eyeless (toy) that we propose is able to partially compensate for the loss of ey. Previous reports have indicated that toy acts as a weaker transcriptional activator than ey which might account for the inability to fully compensate for ey function. When we reduce levels of downstream RD members sine oculis (so) and eyes absent (eya), genetic targets of ey, we see an increase in the ‘eyeless’ phenotype. Together these data suggest that the varied development of the compound eye observed in ey mutants is the result of varying levels of so and eya under the control of toy. As RD members are still detected in developing discs in which mature retinal cells do not develop, it suggests the disc is still fated to become retinal tissue, however differentiation into mature photoreceptors does not occur. Expression of dpp is required at the posterior margin of the disc for the initiation of the Morphogenetic Furrow, a patterning event that induces undifferentiated cells to adopt the retinal fate. In ey<sup>ab</sup> mutants we see that eye development is unable to proceed due to the loss of dpp expression along the posterior margin. Lastly, we find knockdown of ey within the overlying peripodial tissue phenocopies ey mutants, despite continued expression within the disc proper. Overexpression of dpp solely within the peripodial epithelium rescues these phenotypes, suggesting that retinal patterning is governed by the interaction of ey and dpp within the peripodial membrane.

716  **Armadillo (β-catenin) transduces canonical Wnt signaling to specify the peripodial epithelium of the developing Drosophila eye.** D. DeSantis, Z. Zhou, S. Neal, F. Pignoni 1) Neuroscience and Physiology, Upstate Medical University, Syracuse, NY; 2) Ophthalmology, Upstate Medical University, Syracuse, NY.

The Drosophila eye disc consists of the columnar disc proper which gives rise to retina, and a squamous cell layer called the peripodial epithelium (PE). The PE is essential for normal development of the eye disc. However, while the genetic pathways determining retinal fate have been extensively studied, little is known about the specification and development of the eye disc PE. We have identified Armadillo (Arm; β-catenin) as a key factor that suppresses retina fate and promotes PE identity in the eye disc. RNAi-mediated loss of Arm in the eye disc or in the PE results in the transformation of PE into neural retina, as evidenced by the presence of markers for retinal precursors and differentiated retinal neurons on both sides of the eye disc. Conversely, overexpression of constitutively active Arm in the eye disc is sufficient to suppress retinal fate. We demonstrate that the loss of several canonical Wnt pathway components—including receptors, ligands, and nuclear co-factors—also induces PE-to-retina transformation. Consistent with regulation of Arm by phosphorylation, overexpression of the kinase Sgg, the GSK3-β homolog, results in PE-to-retina transformation, whereas depletion suppresses retinal fate. Taken together, our data suggest that the binary choice between neural retina and PE in the eye disc is dependent on transduction of the canonical Wnt pathway via Arm. Wnt signaling promotes PE identity over an underlying retinal program to specify this essential support tissue.

717  **Growth Regulatory Pathway collaborates with Axial Patterning Genes to regulate Patterning and Growth in Drosophila Eye.** N. Gogoi, M. Kango-Singh, A. Singh 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Center for Genomic Advocacy, Indiana State University, Terre Haute, IN.

In any multicellular organism, organogenesis requires axial patterning to determine Antero-Posterior (AP), Dorso-Ventral (DV), Proximo-Distal (PD) axes. Any deviation in these axes during development leads to congenital birth defects. In our model system, Drosophila melanogaster (a.k.a fruit fly), Dorso-Ventral (DV) patterning marks the first lineage restriction event. We have identified defective proventriculus (dve—a Homeobox gene), an ortholog of SATB homeobox 1 (special AT-rich sequence binding protein 1), as a new member of DV patterning genes hierarchy. We have shown that dve acts downstream of pannier (pnr, a GATA-1 transcription factor), and upstream of wingless (wg) in dorsal gene hierarchy. Loss-of-function (LOF) of dve or pnr results in dramatic dorsal eye enlargements, whereas Gain-of-function (GOF) of dve or pnr suppresses the eye specific fate. We have demonstrated that Wingless (Wg) is a downstream target of Hippo growth regulatory pathway (highly conserved) in eye. Furthermore, Wg, which acts downstream of dve, also exhibits similar eye enlargement and suppression
phenotypes (upon LOF, GOF respectively) and has been shown to play a role in growth. Here, we present that DV patterning genes interacts with Hippo signaling to regulate their common downstream target, Wg during growth and patterning of developing Drosophila eye. Our data (using GOF and LOF studies) states (1) The two pathways of DV patterning and Hippo signaling (known to be unrelated to-date) are related and interacts antagonistically of each other, (2) Activating Hippo signaling suppresses dve and pnr expressing cells, downregulates Wg and changes head, antennae specific fate to an eye, (3) Blocking cell death activity of hpo (using UAS-p35, anti-apoptotic) doesn't affects hpo ability to undergo differentiation, (4) Growth regulatory pathway regulates the expression of DV patterning genes (dve, pnr acts downstream of Hippo pathway) in the developing eye of Drosophila, and that (5) DV patterning genes regulates the expression of downstream targets of Hippo signaling. These studies present new genetic interaction between two unrelated pathways to regulate growth and patterning of an organ.

718  Yki promotes non-neural PE fate in the eye imaginal disc and is indirectly regulated by PP2A. Scott Neal, Qingxiang Zhou, Dana DeSantis, Francesca Pignoni Dept Ophthalmology, SUNY Upstate Medical University, Syracuse, NY.

In the developing Drosophila eye, the peripodial epithelium (PE) supports retina formation, fulfilling a developmental role that is analogous to that of the retinal pigmented epithelium (RPE) of the vertebrate optic vesicle. In both, the transcriptional cofactor Yorkie (Yki; YAP and TAZ in vertebrates) with its DNA-binding partner Scalloped (Sd; TEAD1-4 in vertebrates) promotes the non-neural fate, PE or RPE, over a default retinal program. Here we investigate the molecular mechanism(s) by which Yki controls this fate choice. Yki is known to promote cell proliferation and survival in Metazoa, thus we probed whether these functions of Yki are concomitant or distinct from its role in cell fate. We found that exogenous expression of the cell death inhibitor Diap1 and the cell cycle regulator CycE (known targets of Yki) rescued cell survival and proliferation in yki LOF eye discs. However, neither alone, nor the two together, rescued PE fate, thus showing this to be a distinct function of Yki, through other effectors. We also identified the STRIPAK-PP2A phosphatase complex as a Yki-dependent promoter of PE fate. Loss-of-function of STRIPAK-PP2A components induced a PE-to-Retina transformation that is suppressed by loss-of-function of the Hippo (Hpo) kinase. Since Hpo is a negative regulator of Yki that induces a PE-to-Retina transformation when overexpressed, STRIPAK-PP2A likely promotes Yki activity and PE fate through the negative regulation of Hpo.

Conclusions: In the Drosophila eye disc, as in the vertebrate optic vesicle, the binary choice between the neural retina and its support tissue, PE/RPE, is dependent on Yki. Yki function in PE fate is independent of its role in proliferation and cell survival. In addition, STRIPAK-PP2A is a positive regulator of Yki function, upstream of Hpo. Given the parallels between Yki/YAP-TAZ and Sd/TEAD functions in the eye, these mechanisms are likely also at work in vertebrate RPE.

719  Identifying Gene Regulatory Networks within the Drosophila Eye-Antennal Disc. B.M Weasner, B.P Weasner, R. Blair, J. Kumar Department of Biology, Indiana University, Bloomington, IN.

The larval eye-antennal imaginal disc gives rise to multiple adult structures including the compound eyes, ocelli, antennae, head epidermis and maxillary palps. Classical developmental studies have produced fate maps detailing the regions of the disc responsible for the development of these specific adult structures. However, with the exception of the eye disc itself, a comprehensive analysis of the individual GRNs necessary for the specification of each structure as well as how how these GRNs may interact with each other to maintain discrete regions of specified cell fate is lacking. We used cell specific nuclear isolation and RNA-Seq to determine the components of each GRN responsible for the development of all structures derived from the disc. Additionally, we examined the transcriptional profile of specific regions of the disc over the course of larval development to determine the critical time points these GRNs are required for proper cell fate specification. Finally, we are using RNAi knockdown and loss of function mutants to determine the phenotypic consequence of losing specific members of a GRN during development. Taken together these data will provide us with a better understanding of how regional cell fate is specified and maintained in the eye-antennal disc.

720  The IAP inhibitors reaper, hid, and grim prevent neoplastic transformation of regenerating tissues. Cristina D’Ancona, Faith Karanja, Adrian Halamé 1) Department of Biology, University of Virginia, Charlottesville, VA; 2) Department of Cell Biology, University of Virginia, Charlottesville, VA.

During most of larval development, Drosophila imaginal discs can regenerate following tissue damage. Damage and regeneration of imaginal discs elicits a regeneration developmental checkpoint, which delays pupation, extending the regenerative period of development. The regenerative response to damage is proportional to the amount of damage induced. More damage produces a larger regenerative response, which can be detected as a longer checkpoint delay. However, it is not clear how imaginal discs produce an appropriate amount of regenerative activity for different amounts of damage. Here, we demonstrate that the IAP inhibitors: Reaper, Head involution defective (Hid), and Grim (R HG) limit regenerative activity following damage. Reduction or loss of IAP inhibitor activity produces a strong enhancement of regeneration checkpoint delay following damage produced by X-irradiation or localized expression of eiger/TNF alpha. Despite the extended regenerative checkpoint delay, loss of RHG in regenerating tissues produces increased pupal lethality with a significant proportion of pupae failing to produce pharate adults, while surviving adults have extensive regenerative defects.
When we examine regenerating imaginal disc tissues that lack RHG function, we find substantially enhanced levels of Dilp8 and Wingless expression, consistent with increased regenerative activity. However, we also see that loss of RHG in regenerating tissues produces neoplastic tumors, which may explain the pupal lethality and wing regeneration defects we observe. This neoplastic transformation is a cell-autonomous phenotype of RHG inhibition in regenerating tissues and results from increased JNK activation in cells lacking RHG function. Therefore, the IAP inhibitors function as neoplastic tumor suppressors in regenerating tissues. Since loss of RHG does not produce tumors in undamaged tissues, it suggests that some aspect of regenerative activity promotes neoplastic transformation, and that this activity is constrained by RHG. We are currently investigating whether the tumor suppressor activity of RHG is mediated by DIAP1 in a canonical apoptotic pathway or whether RHG suppresses neoplasia through a non-apoptotic mechanism.

721  The role of the Rbf1 tumor suppressor in the differentiation and maintenance of Drosophila muscles. Maria Chechenova, Kaveh Kiani, Anton Bryantsev  Molecular & Cellular Biology Department, Kennesaw State University, Kennesaw, GA.

Retinoblastoma protein (pRb) is expressed virtually in all cell types with the main purpose of cell cycle control. A growing number of reports demonstrate that pRb is also implemented in early steps of myogenesis, but whether this function is independent from its role as cell cycle regulator remains unclear. To clarify this issue, we used a temperature-sensitive expression system to address the importance of fly pRb homolog, Rbf1, at different stages of adult muscle development. When the Rbf1 gene was downregulated in myoblasts (early knockdown), it conferred general muscle weakness: experimental flies had difficulties with the eclosion process and were flightless. Strikingly, early downregulation of Rbf1 also led to complete ablation of jump muscles whereas other large thoracic muscles (e.g. flight muscles) retained their normal localization and morphology. To test whether these phenotypes originated from impaired cell cycle control, we co-overexpressed two transcription factors, E2f1 and Dp, that antagonize Rbf1 and promote cell cycle progression. Overexpression of E2f1/Dp complexes in myoblasts, produced muscle phenotypes comparable to those observed in Rbf1 early knockdown, which suggests that Rbf1 contributes to early myogenesis by primarily repressing cell cycle progression in myoblasts.

We next determined the role of Rbf1 at later stages of myogenesis by delaying Rbf1 knockdown by several hours (late knockdown). Under these conditions, all muscles, including jump muscles, developed normally and were functional in young adults. However, after 2 weeks the experimental flies developed muscle weakness that affected their flying, jumping, and climbing abilities. This decline in muscle functionality was paralleled by a reduction in mitochondrial gene expression. Specifically, the expression of mitochondrial IV subunit COX5A in flight muscles was downregulated by 85%, while the expression of other mitochondrial components (e.g. RFeSP, SdhB, Idh, and Adk1) was down by 30-40%.

Our data demonstrate that the Rbf1 gene is involved at different stages of myogenesis. Although in early myogenesis Rbf1 functions as cell cycle regulator, later it becomes the maintenance factor, supporting muscles in the differentiation state. Further studies, performed on this model, will explore the mechanisms mediating this transition in Rbf1 functioning.


A significant amount of human to human communication is nonverbal, and depends on conveying emotion through the face. Conditions that disfigure the face can therefore be especially devastating. Three quarters of all congenital birth defects in the United States can be categorized as craniofacial abnormalities, highly visible malformations that can have deleterious effects on tissues like the eyes, ears, palate and teeth, and can leave some affected patients without the ability to alter their facial expressions. Within the developing head, their morphology is regulated by a network of transcription factors including the conserved genes Twist1, Lbx1, Msx1, Tbx1, Runx2 and Islet1. Mutations in these transcription factors lead to craniofacial defects: for example, Saethre-Chotzen syndrome in Twist1 mutants, and Cleidocranial Dysplasia in Runx2 mutants. Interestingly, the same genes that are used to pattern the human craniofacial musculature have been shown to regulate the formation of Drosophila abdominal muscles. We are using microscopy to examine the Drosophila head musculature in wild-type and mutant genetic backgrounds using a combination of fluorescent transgenes and antibodies. We have found that the identity gene transcription factors apterous, slouch and vestigial, which are required for abdominal muscle identity, are also expressed in the head mesoderm. We will use the data we are acquiring to develop a model for craniofacial abnormalities in Drosophila, where treatments can be inexpensively and efficiently screened.

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SRF and its co-activator Mrtf are known regulators of myogenesis and signaling regulation. Mrtf and SRF are especially prevalent in smooth muscle cell differentiation and are also important factors in cardiac development and repair after injury. SRF and Mrtf are working together late in muscle development to promote flight muscle structure and maturation in

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Drosophila. However, the role of SRF and Mrtf in early flight muscle development is still not well understood. Therefore, we used RNAi to disrupt SRF and Mrtf in muscle progenitors. SRF RNAi has the same phenotype when it is driven in progenitors or when it is driven in late myotubes in the flight muscles indicating a sole function in late muscle development. However, Mrtf RNAi in muscle progenitors led to lower progenitor cell numbers and subsequently no flight muscle development. Through fluorescent immunostaining of cell death markers in myoblast progenitors in the wing disc we found that there was increased cell death in the wing discs of Mrtf RNAi Drosophila. This indicates a novel role of Mrtf in myoblast survival independent of SRF function which improves our understanding of the mechanism of Mrtf function in muscle development.

724 **Analysis of novel heart defects in akirin mutants.** H. Milner, M. Hupp, S. Nowak  Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA.

Among the metazoans the heart is one of the earliest discrete organ structures to form during embryogenesis, in a process highly conserved across the phyla. Heart development is controlled by a cascade of factors beginning with the emergence of cardiac progenitors known as cardiomyoblasts. In *Drosophila melanogaster* the specification of cardiac progenitors from mesoderm, differentiation and patterning of cardioblasts, and ensuing heart formation is controlled by the recursive action of the Tinman/Nkx2-5 transcription factor, which is itself initiated by the activity of the Twist bHLH transcription factor. Previous work done in the Nowak lab has identified Akirin as a highly conserved cofactor that works with Twist to selectively regulate expression of Twist target enhancers, such as *mef2* and *tinman*. *akirin* mutants have a significant initial decrease in *tinman* expression levels as compared with wild-type embryos. *akirin* mutants further display profoundly abnormal hearts displaying defects in heart patterning, with disrupted organization and reduced numbers of Tinman-positive cardiomyoblasts. Further, live imaging assays indicate that *akirin* mutant hearts that do in fact form either display profoundly uncoordinated contractions, or completely lack contractions in Stage 17 embryos. Taken together, these data indicate that Akirin represents a new co-regulator of the cardiac developmental pathway, and is critical for heart patterning and formation.

725 **Akirin interacts with chromatin remodeling complexes to influence myogenic gene transcription.** K. Palermino-Rowland, A. Griffin, D. Hundertmark, S. Nowak 1) Master of Science in Integrative Biology Program, Kennesaw State University, Kennesaw, GA; 2) Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA.

The specification and differentiation of muscle precursor cells, or myoblasts, by the action of the Twist mesodermal regulator is a key event in the formation of the *Drosophila* larval musculature. Myoblast population dynamics are tightly controlled by gene expression moderated by Twist to determine somatic cell fates. Despite the primary importance of Twist for specifying and patterning the musculature, the identities of many molecular players involved in this process remain unknown. Recently we have discovered that Akirin, a highly conserved nuclear protein, appears to play a critical role in the regulation of Twist-dependent gene expression via interactions with the Brahma chromatin remodeling complex during mesodermal specification and muscle development. We hypothesize that Akirin serves as a cofactor to promote interactions between regulatory transcription factors and chromatin remodeling activity to impact gene expression across varying targets. Using a genetic interaction screen in *Drosophila*, we have begun to identify Akirin interacting proteins that participate in the process of muscle specification, patterning, and development. Our screening method has identified that Akirin interacts with Mi-2, the catalytic subunit of the NuRD complex, to correctly pattern the skeletal musculature. Double heterozygous mutant embryos for *akirin* and *mi-2* demonstrate a host of deranged or missshapen muscle phenotypes. Beyond Mi-2, we have also uncovered a small number of predicted gene products that appear to be involved in general transcription initiation. Through the generation of an interactome of potential partners, we will gain crucial insight into mechanism of Akirin during myoblast specification and muscle patterning.

726 **Silk phosphorylation of talin T152 is crucial for proper talin recruitment and muscle attachment in Drosophila.** Anja Katzermich, Jenny Long, Vincent Panneton, David Hipfner, Frieder Schoeck 1) Department of Biology, McGill University, Montreal, Quebec, Canada; 2) Institut de Recherches Cliniques de Montreal, Montreal, Quebec, Canada.

Talin is the major interaction protein linking integrin receptors with the actin cytoskeleton. In *Drosophila*, extended talin generates a stable link between the sarcomeric cytoskeleton and the tendon matrix at muscle attachment sites. Here we identify phosphorylation sites on *Drosophila* talin by mass spectrometry. Talin is phosphorylated in late embryogenesis when muscles differentiate, especially on T152 in the exposed loop of the F1 domain of the talin head. Localization of talin-T152A is reduced at muscle attachment sites and cannot rescue muscle attachment to the cuticle compared to wild type talin. We also identify Silk as the kinase phosphorylating talin at T152. Silk localizes to muscle attachment sites, and the absence of Silk reduces the localization of talin at muscle attachment sites causing phenotypes similar to talin-T152A. Thus, our results demonstrate that talin phosphorylation by Silk plays an important role in fine-tuning talin recruitment to integrin adhesion sites and muscle attachment.

727 **DlMx1a is required for the development of the ovarian stem cell niche in Drosophila.** Andrew Alibee, Diego Rincon-Limas, Benoit Biteau 1) Department of Biomedical Genetics, University of Rochester School of Medicine and
The *Drosophila* ovary has served as a model for pioneering studies of the interaction between stem cells and their niches, with defined cell types and signaling pathways contributing to the regulation and maintenance of both germinal and somatic stem cells in individual ovarioles. The establishment of these stem cell-niche units begins during larval stages with the formation of terminal filament-cap structures, which are crucial for the specification and development of ovarioles. While the function of these ovarian niches has been extensively studied, the genetics underlying the development of terminal filaments remains largely unknown. LIM-homeodomain proteins have essential roles during tissue patterning and cell differentiation in metazoans, from nematodes to vertebrates. For example, mammalian Lmx1a and Lmx1b are pleiotropic regulators of cell differentiation and tissue development in many organs, as illustrated by the many defects and syndromes caused by loss-of-function mutations in these genes. Here we show that *Drosophila* dLmx1a, is required specifically for ovary morphogenesis. We found that dLmx1a is expressed in early ovarian somatic lineages and that its expression is progressively restricted to terminal filaments and cap cells. Without dLmx1a, terminal filament cells are not properly specified and fail to differentiate and generate fully formed stacks. This results in the failure of the muscle sheaths to develop, a complete absence of ovaries and sterility by adulthood. We further show that dLmx1a is required specifically in terminal filament-cap cells at the time of their formation, during the larval-pupal transition. Finally, using epistasis experiments and transcriptional analysis, we demonstrate that dLmx1a functions genetically downstream of the transcription factor Bric-à-Brac, and is critical for the expression of a multitude of key components of conserved signaling pathways essential to stem cell niche development and function. Remarkably, expression of chicken Lmx1b, an ortholog of dLmx1a, in the dLmx1a lineage, is sufficient to rescue the null dLmx1a phenotype, indicating functional conservation across the animal kingdom. Our results therefore expand our understanding not only of how stem cell-niche units are established in the fly ovary, but also of the mechanisms through which LIM-HD factors contribute to tissue morphogenesis and pathology.

**728 Pri peptides temporally repress the expression on cuticle genes during Drosophila development.** H. Chanut-Delalande, M. Gallois, D. Menoret, S. Plaza, F. Payre Centre de Biologie du Développement, Toulouse, FR.

From genome wide analyses, a non-annotated class of genes emerges as containing small ORFs encoding small peptides. In Drosophila, polished-nice (pri) gene acts as small peptides in controlling epidermal differentiation. It activates the transcription factor Shavenbaby, which in turn triggers the activation of hundred genes, directly involved in trichome formation. While Svb target genes are downregulated in pri mutant embryos, we identified by transcriptomic analyses an upregulation of many genes, in a Svb independent manner. These genes are coding for cuticle proteins of various families. They display a specific epidermis expression in pri mutant embryos while they are not normally expressed during embryogenesis. We have shown that these genes are expressed in control embryos latter in development, during larval or pupal stages. Moreover, expression of Pri during larval stage represses cuticle gene expression, suggesting a specific temporal repression of their expression by Pri peptides. These results revealed a new function of Pri peptides in the temporal repression of cuticle genes to ensure a proper functional cuticle.

**729 Trithorax Group proteins cooperate with Pax6 to control proper fate identity in the Drosophila eye-antennal disc.** A.J. Ordway, G.M. Teeters, B.P. Weasner, J.P. Kumar Biology, Indiana University, Bloomington, IN.

Our research focuses on how proper fate decisions are made during development and how these decisions ensure the correct number of organs are formed. Specifically, we aim to understand how cooperation between tissue specific transcription factors and epigenetic regulators control tissue fate and organ number. We discovered that when members of the Trithorax Group (TrxG) of epigenetic regulators and Twin of Eyeless (Toy), a Pax6 family protein known for its role in eye specification, are knocked down concurrently, the antenna of the fly is duplicated. We aimed to uncover how these factors are controlling this fate decision. Specifically, we focused on one TrxG complex, the Nucleosome Remodeling Factor (NURF). We wanted to understand when and why NURF and Toy are required during development to control tissue fate. Our results suggest that the critical window for suppressing the antennal duplication is in the late second instar and that the loss of both Toy and NURF results in a fate transformation from dorsal head epidermis to antennal tissue. Surprisingly, this transformation occurs in mid-third instar. Moreover, we uncovered a potential role for Wingless (Wg) signaling in this transformation, as disrupting the Wingless (wg) pathway phenocopies the NURF-Toy double knockdown. My results demonstrate a cooperation between highly conserved epigenetic regulators and transcription factors. The concurrent loss of these factors results in patterning defects, tissue fate transformations and the duplication of an organ.

**730 Mirror Regulates Proper fate Determination in the Drosophila Eye-Antennal Disc.** G. Teeters, A.J. Ordway, J.P. Kumar Indiana University, Bloomington, IN.

Cell fate decisions and the ability to maintain these determined fates are vital to the proper development of a multicellular
organism. The determination of diverse cell fates allows for cell populations to properly respond to long range signaling molecules to facilitate organ development and patterning. Manipulations of cell fate decisions have been shown to lead to duplication of body structures such as limbs in mouse and wings in Drosophila. The gene Mirror (mir), a member of the Iroquois complex, is necessary for proper cell fate determination within the eye-antennal imaginal disc. Previous work has shown that loss of mir expression results in a duplication of this disc (Campuzano, 2000). We have found that knocking down levels of mir expression within the eye-antennal disc using RNAi driven by a ubiquitous GAL4 driver resulted in an ectopic morphogenetic furrow and duplicated antenna developing from the dorsal anterior head capsule region. We hypothesize that this phenotype is a partial duplication of the eye-antennal disc and determined this duplication is hindered by apoptosis within the transforming tissue. The partial disc duplication phenocopies the loss of Wingless signaling components, supporting the notion that the Wingless pathway plays a role in regulating Iroquois complex expression. Together these results suggest that the classical ectopic furrow phenotype, reported by Ma and Moses in 1995, may be due to a duplication of the disc and not just the result of a patterning defect. In conjunction with these studies, we recently performed an RNAi screen within the eye-antennal disc in which we knocked down members of the Trithorax Group (TrxG) of epigenetic activators and found loss of these proteins produced a phenotype similar to that of the mirr knock down. These results suggest that the TrxG proteins could play a role in the regulation of mirr and are genetically interacting with downstream targets of the Wingless signaling pathway. I am continuing my investigation by examining molecular markers for axis and firing point origins. I will also investigate the timing and regulation of the ectopic growth using time course experiments in addition to a temperature sensitive Gal4.

731 Loss of general transcription factors leads to tissue specific phenotypes. L.R. Weber, J. Zhu, G.M. Teeters, A.J. Ordway, J.P. Kumar Indiana University Bloomington, Bloomington, IN.

General transcription factors play a crucial role in the development of a given tissue, as these factors are essential for the activation of transcription. However, my experiments show a new result, that these factors play a tissue specific role. As the members of the general transcriptional factors have long been thought to play a role in activating transcription at most loci, it would be expected that knocking down any member of this group would result in the same phenotype, such as the loss of tissue. Additionally, the knockdown of members of the same complex would be even more likely to produce consistent phenotypes. Using the Drosophila eye as a model, we are studying the role of the general transcription factors during development. Specifically, I am investigating the roles of members of the SAGA, TFIID, and Mediator complexes by characterizing the resulting phenotypes after knockdown. To do this, I use RNAi lines against different members of these complexes in the early eye-antennal discs. I have unexpectedly found that knocking down members from these complexes results in a wide array of phenotypes. They primarily include antennal duplications, ectopic eyes, and reduction or loss of tissue. One knockdown in particular only allows the development of three organs, whether that is two eyes and one antenna or one eye and two antennae. The knockdown of other factors results in reduced organ size; mainly a reduced eye field, or loss of one or both eyes completely. These experiments have suggested that general transcription factors are regulating tissues either through viability, regulation of organ number, or regulating cell fate decisions. The goal of this project to determine the developmental mechanism for these complexes by determining the timing at which the members of a particular complex are functioning to properly pattern tissue. In conclusion, my experiments suggest distinct roles for members of the general transcription machinery, which result in variable phenotypes when lost in the eye-antennal disc.

732 Functional divergence between eRpl22 paralogues in Drosophila melanogaster interommatidial bristle morphogenesis. Brett Gerschman, Vassie Ware Biological Sciences, Lehigh University, Bethlehem, PA.

The Drosophila melanogaster eRpl22 ribosomal protein (Rp) family contains two structurally diverse members: eRpl22-like and eRpl22. eRpl22-like has a tissue-specific expression pattern in the testis and eye, while eRpl22 is expressed ubiquitously. eRpl22-like is subject to stage-specific post-translational modification in larval and adult stages. The developmental significance of differential expression and modification of eRpl22 paralogues is poorly understood and will be addressed through characterization of paralogue localization and phenotypic consequences of paralogue manipulation. In the midpupal retina, immunohistochemistry (IHC) analysis combined with western blot data reveals that unmodified eRpl22-like expression is restricted to one cell type. Co-localization with tissue-structure-specific F-actin staining patterns reveals that eRpl22-like is localized to the growing interommatidial hair cell. Both eRpl22 paralogues are co-expressed within the developing hair cell, however, they are present in continually shifting asymmetry. Patterns of paralogue exclusion in early bristle development shift to overlapping patterns of paralogue localization in later bristle development. eRpl22 paralogue asymmetry within the developing hair cell suggests paralogue-specific roles in cell-type-specific processes. Previous studies in the adult testis have shown that unmodified eRpl22-like acts as a ribosomal component. However, studies assessing co-localization of eRpl22 family paralogues with the core ribosomal component RpL23a, supports a ribosomal role for eRpl22 but an extra-ribosomal role for eRpl22-like in the developing eye. The role of eRpl22 paralogues was assessed in differentiated eye cells through tissue-specific knock-down (KD). Subsequent IHC analysis shows that eRpl22 depletion results in interommatidial bristle defects, tissue disorganization defects and adult-stage eye lesions. Depletion of eRpl22-like also yields mild bristle defects, but otherwise does not phenocopy eRpl22 KD. Paralogue-specific KD phenotypes reinforce
the hypothesis that eRpL22-like and eRpL22 have distinct stage-specific roles. Current investigations involve cell-type-specific paralogue manipulation in the developing eye to examine functional overlap and divergence.

733 Characterizing the regulatory role of DV patterning during axis elongation. M. Lefebvre Dept of Molecular Biology, Princeton University, Princeton, NJ.

While the regulatory role of the anterior posterior patterning system in establishing the actomyosin contractile network that is responsible for axis elongation (germband extension) during Drosophila gastrulation is well understood, the contribution of dorsal ventral (DV) patterning remains unclear. Here, we propose that DV patterning plays two distinct roles, 1) a permissive role in defining the physical limits of the germband tissue and 2) an instructive role in orienting the directionality of tissue elongation. We use MuVi SPIM light sheet microscopy, and cartographic projection analysis to visualize complex epithelial surfaces as 2D projections. We characterize cell intercalation rates, myosin expression patterns, and tissue flow patterns globally across the surface of the embryo. We find that in wildtype embryos there is a gradient of cell intercalation along the DV axis. Cells at the ventral lateral limit of the germband tissue undergo neighbor exchange with greater frequency than cells at the dorsal lateral limit, and no intercalary behavior is seen at the dorsal pole. We show that this gradient of cell intercalation is preceded by a similar DV gradient in the pattern of myosin expression. Myosin anisotropy, a known prerequisite for intercalation, is highest at the ventral limit of the germband tissue and decreases towards the dorsal midline. We propose that this DV bias in cell intercalation causes the germband tissue to curve in the direction of the dorsal midline as it elongates, presumably because the rates of elongation within the tissue match the DV gradient of intercalation. To test this model, we examined embryos with different degrees of uniform dorsalization. In embryos in which the lateral ectodermal cell fate has been extended around the circumference of the embryo, the DV gradient in myosin anisotropy is eliminated. Anisotropy is high around the entire DV circumference. In these embryos, tissue flow patterns are disrupted and the germband does not elongate onto the dorsal side of the embryo. In fully dorsalized embryos, levels of myosin anisotropy are uniformly low and again germband tissue does not elongate onto the dorsal side of the embryo. These results suggest that when the DV oriented gradient of myosin anisotropy is eliminated, the pattern of tissue elongation is disrupted.

We are interested in further characterizing the mechanism by which differential DV patterning information affects the molecular machinery responsible for cell intercalation.

734 Drosophila melanogaster imaginal disc model to identify and determine the regeneration potential of Notophthalmus viridescens-newt- genes. A.S. Mehta1, A Luz-Madrigal5, P.A. Tsonis1, A Singh1,2,3,4 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN; 5) Department of Biology, Miami University, Oxford, USA.

Notophthalmus viridescens, Red-spotted newt, possess amazing capability to regenerate its tail, limb, heart, brain, spinal cord, lens and other organs. However, the molecular-genetic mechanism driving regeneration have been hindered due to lack of genetic tools. Whereas Drosophila melanogaster imaginal disc provides an excellent opportunity to use powerful genetic tools to identify the gene regulatory pathways required for regeneration. And also as the genetic machinery between vertebrates and Drosophila is conserved, so we utilized Drosophila melanogaster to identify regenerative role of novel newt candidate genes. These genes were identified by denovo assembly of newt transcriptome. We generated the transgenic flies containing these genes. The newt candidate genes were expressed ubiquitously in Drosophila, and samples for RNA sequencing were collected at third instar larval (L3) stage. Gene ontology terms related to development, apoptosis and cell cycle were highly enriched in the group of differentially regulated Drosophila transcripts. Using transgenic approaches, we found that misexpression of these regeneration genes from newts can rescue eye mutant phenotypes and also exhibits regeneration potential in the wing model of regeneration. They regenerate new cells at site of injury by inducing cell proliferation and blocking excessive apoptosis, as revealed by Phospho histone 3 (PH-3) s and DCP-1 staining respectively. As a mechanistic insight to the observation our results demonstrated that these genes impact Hippo and Wingless signaling. Our study presents a unique regeneration potential of a gene tool kit present in newts, which allows stringent control of regeneration and will have significant bearing in the field of regenerative biology.

735 Integrins: Shaping organs and cells. C. Santa-Cruz Mateos1, G. Kannan2, I. Palacios2, MD. Martin-Bermudo1 1) Centro Andaluz de Biología del Desarrollo. CABB, CSIC, Seville, 41013, ES; 2) The Zoology Department, University of Cambridge, UK.

How can an organ control its final shape and size? The size of an organ or organism depends mainly on both the total number of cells and the size of these cells. However, while lots of attention has been paid in the last years to the role of cell proliferation on the growth of an organism or organ, the contribution of cell growth to this issue has remained largely unknown. Cell culture experiments have demonstrated that adhesion to the extracellular matrix (ECM) promotes cellular growth. However, it is still not clear whether this anchorage-dependence phenomenon of cell growth plays any role during morphogenesis. In our lab, we are trying to understand the role of the cell-ECM attachment, mediated by integrins, on the control of cell and organ growth. To this aim, we use the follicular epithelium (FE) of the Drosophila ovary as a model system.
Previous experiments have shown that reducing integrin levels in the FE results in rounded eggs. By combining classical genetic approach with the most cutting-edge techniques of live imaging, 4D image analysis, biophysics and super-resolution microscopy, we show that integrins are required for proper growth and shape of follicle cells, specially at their basal side, where cells interact with the ECM. Furthermore, laser ablation experiments reveal that integrin mutant cells display increased basal, but not apical, tension. In addition, by analysing actomyosin dynamics in vivo, we show that basal contractions are perturbed in mutant cells. Thus, integrin mutant cells are able to contract, but their pulsations start earlier and are quicker than those found in wild-type cells. Finally, we show that elimination of integrin leads to an upregulation of myosin activity. Altogether, these results led us to propose a model in which adhesion of cells to the ECM mediated by integrins is required to balance external forces that in turn is critical to sculpt a specific three-dimensional architecture.

### 736 Mechanical stress-induced cell cycle modification in epithelial morphogenesis

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Spatial and temporal regulation of cell cycle is essential for the development of multicellular organisms. Although a number of genetic signaling pathways regulating cellular proliferation and growth during development have been identified, it is not clearly understood how a transient mechanical state of cells is involved in the cell cycle regulation and how the genetic-mechanical-genetic signal transduction cycle in each cell is coordinated to generate precise patterns of tissue morphology. In Drosophila oogenesis, follicular epithelial cells undergo three rounds of endoreplication cycle (endocycle) and morphological differentiation from cuboidal to columnar or squamous cell shapes. During mid-oogenesis stages, most of the follicle cells migrate posteriorly to form tall columnar follicle cells covering the oocyte, whereas the anterior follicle cells are passively stretched and differentiated into flattened squamous cells covering the nurse cells. We found that the anterior stretched follicle cells undergo extra endocycles and that the insulin/IGF-like signaling (IIS) activity is involved in the endocycle acceleration. This suggests that the IIS activation-dependent endocycle acceleration is induced by mechanical stretching of these cells. From a directed screen to identify genes involved in the mechanotransduction process, we found that a mechanosensitive transient receptor potential (TRP) channel, NompC, is involved in the local IIS activation and the endocycle acceleration in the stretched follicle cells. Consistently, a live-cell calcium imaging analysis revealed that intercellular calcium level is endogenously higher in the stretched follicle cells than the columnar main body follicle cells and that NompC is involved in the high calcium level in stretched follicle cells. These data indicate that TRP channel activation in response to mechanical stretching stress induces calcium incorporation, thereby activating IIS pathway. This mechanism highlights how spatial and temporal mechanical state signals back to the genetic signaling to adapt cell cycle during tissue morphogenesis.

### 737 CRISPR/Cas9 Survey of Drosophila modENCODE Cell Lines

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The Drosophila Genomics Resource Center (DGRC) maintains over 100 stable cell lines and these cell lines have become an integral part of the toolkit for Drosophila research. In particular, there are 25 Drosophila melanogaster cell lines (modENCODE cell lines) reported in Cherbas et al., 2011 that have been characterized by whole-genome tiling microarray analysis of total RNA, permitting researchers to choose the most appropriate cell line for investigations into gene function and cellular biology. Furthermore, with the advent of CRISPR/Cas9 the ability to manipulate the genome of these cells has decreased the reliance on transient transfections and increased the utility of stable cell lines. Nonetheless, CRISPR/Cas9 manipulations have been limited to only a few cell types and thus there is a need to provide a systematic survey of the ability to manipulate as many different lines as possible. Therefore, here we will describe our efforts to establish a baseline/minimum set of conditions applicable to all modENCODE cell lines for CRISPR-Cas9 manipulations and reveal and note potential differences between cell lines to consider in planning experiments.

### 738 Influence of Ecdysone Receptor Signaling on Border Cell Migration Kinetics

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Migratory cells play a significant role in spatiotemporally regulated physiological processes such as normal embryonic development and wound healing; dysregulation of these cells has severe implications in birth defects and diseases such as cancer. Spatio-temporal regulation of collective cell migration involves interconnected gene networks modulating intracellular cytoskeletal rearrangements, intercellular adhesion, and cell-cell communication. We use border cell migration in Drosophila oogenesis to investigate the regulation of the timing of collective cell migration. Derived from the anterior epithelium of the egg chamber, the border cell cluster is composed of 6-8 cells that migrate together toward the posteriorly-located oocyte at stage 9 of oogenesis. Developmental timing is controlled by the steroid hormone ecdysone, which binds to a heterodimeric receptor complex and regulates transcription of its targets. My work focuses on understanding how signalling by the ecdysone receptor (EcR), a nuclear hormone receptor, affects the movement of the border cell cluster. A wild type cluster exhibits two types of movements – tumbling and translocation, hypothesized to be due to the extension of F-actin protrusions and modulation of DE-cadherin in adhesion complexes. Using a combination of live imaging video analysis and immunofluorescence analysis, preliminary data indicates that clusters expressing dominant negative EcR show multiple
phenotypes of incomplete migration, including delayed translocation with normal tumbling. The tumbling phenotype is being further studied by multi-cell labelling using Flybow, which allows us to study the cluster as a sum of moving individual cells rather than as one static group. We have also identified a number of Ecdysone target genes, including S-phase kinase-associated protein 2 (Skp2) that may mediate transcription factor turnover. Elucidating the role of EcR in cell migration kinetics will be a useful guide to understand nuclear hormone receptors and their role in development and disease.

739 Modifier screen in D. melanogaster identifies genes involved in tubulogenesis. C. Espinoza, C. Berg University of Washington, Seattle, WA.

Tubulogenesis is the underlying basis for organ formation, and errors in that process cause common birth defects such as heart malformations, spina bifida, and anencephaly. A good Drosophila model for tube formation is synthesis of the dorsal appendages (DA) of the egg; this process shares many features with vertebrate “wrapping” mechanisms, such as cell-shape modifications, neighboring cell rearrangements, and cell migration. Although the genetic basis of tube formation is limited, we know that in Drosophila, precise levels of the Imaginal disc growth factor 3 (Idgf3) gene are required for proper DA formation (both up- and down-regulation disrupts tubulogenesis), and the human homologue (CHI3L-1) is involved in cell adhesion and cell migration during tissue remodeling in fibrogenesis.

To identify genes that interact with Idgf3 during dorsal appendage (DA) formation, we carried out a modifier screen by crossing deficiencies on chromosome 3L with an Idgf3-overexpression line and analyzing laid eggs for DA defects. A primary screen uncovered 3 strongly interacting regions with an average of 46 genes per region and an additional 10 mildly interacting regions. We are screening smaller deficiencies to narrow down the regions of interaction, and RNAi lines will allow the identification of specific interacting genes. By identifying genes that enhance or suppress DA defects when crossed with an Idgf3-overexpression line, we will discover new genes in the pathway in which Idgf3 plays a role for normal tube formation.

740 Biophysical and genetic analysis of mechanical forces behind collective cell migration. A. Kabanova, E. Molina Lopez, M. D. Martin Bermudo Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Sevilla, Sevilla, ES.

Cell migration is a key process for animal development and homeostasis. Defects in cell migration result in neurological disorders, congenital heart diseases, physical and mental retardation and metastasis. Whether clusters of cells move over basement membranes, through interstitial matrices or between other cells, they employ similar mechanical forces to coordinate their movement. E-cadherin-dependent adhesion is crucial to mechanically couple cells within the cluster, whereas the actin-myosin cytoskeleton generates mechanical forces that move the cluster forward. While much effort has been done to identify the molecular players involved in collective cell migration, our understanding of the initial step of delamination of the future migratory cluster or the contribution of the surrounding substrate to cell movement is scarce. The border cells (BC) of the Drosophila ovary provide a simple genetic system for the in vivo study of the mechanisms of cell delamination, invasion and migration. The BC cluster detaches from the epithelium and migrate between the nurse cells (NCs) towards the oocyte. Throughout the migratory process, BCs create a forward migratory force by polymerizing actin, which pushes the cell towards the oocyte. However, motile BCs must push back against the non-motile NCs, which are bounded by the FC layer and the extracellular matrix that limit their movement. Cycles of dynamic actomyosin activity at the cluster periphery provide optimal levels of cortical tension that allow the cluster to achieve a compact morphology, which in turn may enable BCs to withstand forces from NCs thus promoting efficient migration. However, due to low levels of endogenous Myosin-II in NCs, it is unlikely to be the sole regulator of NC membrane pliability.

We believe that the basement membrane (BM) may contribute to the regulation of tension and pliability in the NCs. Previous study has identified laminin as a key regulator of collective cell migration and tissue stiffness. We show that reduced levels of laminin in the BM result in BC migration defects. Moreover, live imaging analysis shows that in egg chambers with reduced levels of laminins, BCs take longer to initiate their migration. In addition, we found that the majority of the BC clusters intend to migrate between the follicle epithelium and the NCs, instead of penetrating between the NCs. By combining cutting-edge quantitative live imaging approaches with novel genetic and biophysical manipulations, we hope to unravel the role of biophysical properties of BM in regulating the two aspects of the migratory behavior of BCs.

741 The extracellular protease AdamTS-B plays an important role in tracheal tube formation during embryogenesis. Abigail Thuringer, Afshan Ismat Department of Biology, University of St. Thomas, St. Paul, MN.

Proper organ formation requires certain cell types to migrate from one place in the embryo to another. Cells migrate through a dense extracellular matrix (ECM) filled with proteins and macromolecules. Many different types of proteins are used to assist cells in their migration through the ECM, including extracellular proteases that cleave or restructure parts of the dense ECM to allow cells to move through it. One family of extracellular proteases is the ADAMTS family, which is known to play an important role in cell migration. Humans encode 19 members of this protease family, while a simpler model organism, the fruit fly Drosophila melanogaster, encodes only three members. These three proteins likely do the work of the 19 proteins in humans. Drosophila AdamTS-B (CG4096), homologous to eight human ADAMTSs, is expressed in the embryonic trachea from early to late stages of tracheal development. The trachea is a highly branched network of tubular airways that go through an elaborate migration throughout embryogenesis. Embryos completely missing AdamTS-B display defects in
tracheal branching and migration and luminal discontinuity. Conversely, over-expressing AdamTS-B throughout the trachea showed increase in length of branches as well as luminal cysts. Considering the data together demonstrates the important role of AdamTS-B in proper tracheal tube formation.

742 **Macroglobulin complement-related is required for border cell migration in Drosophila melanogaster.** L. Ussher, H. Alhadyyan, R. Ward University of Kansas.

*Macroglobulin complement-related (Mcr)* encodes a core component of the septate junction, a protein complex along the lateral membrane that provides an occluding function to the epithelium. *Mcr* is a 1760 amino acid protein with α-2-
macroglobulin and LDL receptor class A domains. *Mcr* is also required for morphogenetic events throughout the life of the fly. Specifically, we identified *Mcr* in a screen for genes required for imaginal disc morphogenesis during metamorphosis, and found that *Mcr* is also required for developmental processes during embryogenesis, including dorsal closure, head involution and salivary gland organogenesis. In order to further explore the role of *Mcr* in morphogenetic processes, we are examining the requirement for *Mcr* in border cell migration during oogenesis. Border cell migration is a process whereby 4-6 cells surrounding the polar follicle cells delaminate from the anterior of a stage 9 egg chamber and migrate through the nurse cells to reach the oocyte. We are using several Gal4 lines to drive the expression of an *Mcr-RNAi* transgene to control the expression of *Mcr* in different tissues within the ovary. Reducing *Mcr* in the border cells (using Sbo-Gal4) results in only 6.6% successful migration border cells clusters by stage 10 of oogenesis. Defective migration is observed as failure to initiate migration, substantial delay in migration (incomplete by stage 10), or disassembly of the border cell cluster. Reducing *Mcr* in the nurse cells (using triple-Gal4) had little to no effect on border cell migration, suggesting that the requirement for *Mcr* in this process is in the migrating cells. To further explore this idea we are using upd-Gal4 to reduce *Mcr* specifically in the polar cells and C458-Gal4 to reduce *Mcr* in both the polar and border cells. We plan to examine cellular markers of cell signaling, adhesion and the cytoskeleton to explore possible mechanisms of *Mcr* function in border cell migration.

743 **The separation of Dorsal-Ventral layers during cellularization is orchestrated by a morphogen-regulated cell migration.** Y. Xue, A. Krishnan, R. Schweickart, J. Chahad, C. Mizutani Biology Department, Case Western Reserve University, Cleveland, OH.

During the *Drosophila* blastoderm stage, cells on the embryo surface are separated in distinct expression domains in response to morphogen gradients. Although the mechanisms of gene expression regulation by morphogens are well known, the coordination of cell fate specification and cell movements within dynamic developmental fields is still poorly understood. The cellularization stage provides an excellent model to investigate this question. Although the cellularization stage of *Drosophila* has been treated as a static field of cells for a long time, it was discovered that cells actually migrate at this stage. In this migration, cells follow stereotyped trajectories towards the dorsal midline and acquire distinct densities across the D/V axis. It has been shown that this cell density profile is disrupted in embryos without the Dorsal (Dl) gradient but it seems unlikely that this cell migration is directly regulated by this morphogen since DI is normally expressed ventrally. Since DI restricts the expression of Decapentaplegic (Dpp) to the dorsal side of the embryo, we first asked whether the disruption in cell migration caused by DI was due to an indirect effect on the Decapentaplegic (Dpp) gradient. Here we confirm this hypothesis since in the absence of the Dpp gradient, cell migration is stalled and an ectopic source of Dpp is capable of attracting cells towards its expression site. To determine how Dpp regulates this migration, we searched for genes with a distinctive D/V expression and an expression profile similar to the Dpp receptor Thickveins. This search led to the identification of two genes: frazzled (fra) and GUK-holder (gukh). We confirmed that these genes are regulated by DI and Dpp, which is consistent role in regulating cell migration during cellularization and show that mutants for these genes disrupt migration. Finally, we show that even in the presence of normal morphogen gradients the expression domains of four DV genes (*rho, ind, vnd* and *sna*) are affected in *fra* and *gukh* mutants. Together, these results show how morphogenetic activity is coordinated in a field where the position of the target cells change.

744 **kayak isoforms in Drosophila.** Carlos Alfonso Gonzalez1,2, Juan Rafael Riesgo-Escovar1 1) Departamento de Neurobiologia del Desarrollo y Neurofisiologia, Instituto de Neurobiologia, QUERETARO, JURIQUILLA, MX; 2) Maestria en Ciencias Quimico-Biologicas, Universidad Autonoma de Queretaro.

Alternative splicing is a molecular process by virtue of which a gene produces several transcripts capable of generating protein isoforms with diverse functions and localized expression patterns. Interestingly, many transcription factors are processed through alternative splicing, as is the case of the *Drosophila* gene *kayak* (the sole fly *fos* homolog). The *fos* gene family is evolutionarily conserved throughout Eukarya, variously required during development and adult life. In mammals, there are typically four *fos* genes with multiple isoforms each that exhibit partial redundancy, making functional roles difficult to assess. In *D. melanogaster* the *kayak* gene fulfills all organismal *fos* requirements with a single locus and with only six isoforms. All mutant *kayak* alleles known are embryonic lethal. The main goal of this study is to understand the different roles of *kayak* isoforms. We found that the isoforms and their expression patterns during embryonic development are conserved in other *Drosophila* species, like in *D. pseudoobscura*, suggesting conserved and specific functional roles for them. We evaluated *kayak* isoforms expression in wild-type and in mutant *kay1*, *kay2*, *kay200*, and *kay640* embryos with RT-qPCR, using isoform-
specific primers. All alleles exhibit multiple, but different, isoforms affected. The mutant alleles also display different levels of deviation from wild-type embryos. In order to quantify these mutant phenotypes, we developed a new method based on the ImageJ plug-in EggTools that allows us to accurately quantify several kayak embryonic phenotypes. We then use these two sets of data to establish relationships between them.

745 Macroglobulin complement-related is required for egg shape during Drosophila melanogaster oogenesis. H. Alhadyian, R. Ward  Molecular Biosciences Department, University of Kansas, Lawrence, KS.

Macroglobulin complement-related (Mcr) is a transmembrane protein belonging to C1r/C1s-related protein family and has been implicated in host defense against pathogens. We initially identified Mcr in a genetic screen of mutations that dominantly enhanced a malformed leg phenotype in broad mutant animals, suggesting a role for Mcr in morphogenesis. We subsequently determined that Mcr is a core component of epithelial septate junctions (SJs), which are analogous to the vertebrate tight junction in providing an essential occluding function to the epithelium. Interestingly, homozygous mutations in Mcr are embryonic lethal with defects in developmental processes including head involution and dorsal closure that occur prior to the establishment of the SJ. These data suggest a role of Mcr in morphogenesis that is independent of its role in the occluding junction. To extend these studies we are investigating the role of Mcr during morphogenetic events that occur during oogenesis. First, we investigated the expression of Mcr in oogenesis, and determined that it is expressed in the germ line stem cells and follicle cells (FCs) with the strongest expression in the polar cells. We next used cell-type and stage-specific Gal4 drivers to examine the function of Mcr in egg elongation. We find that reducing Mcr level in the FCs early in oogenesis (Traffic-jam Gal4) or by stage 8 of oogenesis (C204-Gal4) prevents completion of egg elongation. Interestingly, the ratio of length/width of Mcr knocked down eggs starts to deviate from the wild type eggs by stage 13, suggesting a requirement of Mcr later in oogenesis. We then used E-cadherin antibody to outline FC membranes and DAPI to mark nuclei. We find that Mcr knocked down FCs are multi-nucleated with irregular cell shapes. In addition, these cells lose basal actin filaments. These results suggest that Mcr is required for some aspect of FC maintenance later in oogenesis. Further analyses are being conducted to investigate the molecular and cellular mechanisms by which Mcr is involved in egg elongation.

746 What's in a wrap? Steps that link patterning and morphogenesis during dorsal appendage formation. Rachel Dani1,2, Sydney Bowker1, Celeste Berg1,2 1) Genome Sciences, University of Washington, Seattle, WA; 2) Molecular and Cellular Biology Program, University of Washington, Seattle, WA.

How do functionally distinct cell types coordinate their actions to form a cohesive tube? Tube formation is an essential developmental process across metazoa and gives rise to structures including the gut, heart, and neural tube. In particular, the wrapping process that forms the vertebrate neural tube is also conserved in creating specialized structures on D. melanogaster eggshells, dorsal appendages (DAs). Studies of DA formation have provided extensive insight into both patterning and morphogenesis during development. In oogenesis, two patches of follicle cells overlay the developing oocyte and give rise to the DAs via wrapping and crawling. Each patch is comprised of two cell types, floor cells and roof cells, which reorganize and change shape to create a pair of cellular tubes. Secretion of eggshell protein into the lumen of the tubes produces the DAs. Despite morphological and functional differences, little is known about the transcriptional landscape that distinguishes roof and floor cells, although Notch signaling is clearly involved. Furthermore, the gene expression changes that occur downstream of patterning to initiate wrapping remain largely unidentified. To examine the genetic and cellular factors that induce wrapping during DA formation, we are using the GAL4/UAS system and a magnetic-bead protocol to purify cell types in the tube-forming primordium and identify differentially expressed transcripts via RNA-sequencing. These studies yield insight into how gene expression changes specific to different cell types facilitate coordinated wrapping during tube formation.


In Drosophila, 13 rounds of synchronous nuclear division uniformly tile the surface of the blastoderm embryo with around 6000 nuclei in only two hours. Given this breakneck pace, we sought to understand how physiologically normal levels of the building blocks of DNA, dNTPs are maintained under high demand. Our recent work established that dynamic control of Ribonucleotide Reductase (RNR) activity as a key determinant of dNTP concentrations. While the necessity of dNTPs in the progression of nuclear cycles is apparent, the functional role of limiting dNTP concentrations via RNR regulation has yet to be demonstrated. Surprisingly, we find that expressing a constitutively active RNR enzyme in the early embryo not only increases dNTP concentrations 20 fold, but also causes specific defects in morphogenesis. For instance, embryos with excessively high levels of dNTPs fail at germband retraction. While this resembles defects observed in the well-studied patterning mutants, blastoderm patterning in embryos with deregulated RNR activity is normal. Our live imaging studies reveal that these embryos show defects in early gastrulation: in contrast to the wild-type embryos, where the first invagination is dictated by the expression of the dorsoventral patterning genes, embryos with increased levels of dNTPs have ectopic epithelial invaginations which interfere with normal gastrulation movements and cause lethality. Our working model for these morphogenetic defects is that deregulated RNR activity destroys the spatiotemporal coherence of the early cleavage cycles,
which in turn results in defects in nuclear packing. When combined with normal patterning of mechanical forces, these defects thwart the normal progress of gastrulation. We will present direct tests of this model by live imaging of blastoderm dynamics and computational modeling of three-dimensional epithelial morphogenesis. These results provide a new view into the dynamics of the earliest events in Drosophila embryogenesis.

748  Spindle Orientation Drives Tissue Regularity in an Elongating Epithelium.  T.M. Finegan1,4, D. Na2, A.V. Skeeters3, N.S. Dawney2, A.G. Fletcher1, P.W. Oakes3, D.T. Bergstrahl1,2,3,4  1) Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK; 2) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY, USA; 3) Department of Physics & Astronomy, University of Rochester, Rochester, NY, USA; 4) Department of Biology, University of Rochester, Rochester, NY, USA; 5) School of Mathematics and Statistics and Bateson Centre, University of Sheffield, Sheffield, UK.

We investigated the relationship between proliferation and tissue topology in an epithelial tissue undergoing elongation. We found that cell division is not required for elongation of the early Drosophila follicular epithelium, but does drive the tissue towards optimal geometric packing. As the tissue ages, cells pack in an increasingly regular manner. To increase tissue regularity, cell divisions are oriented in the planar axis, with a bias towards the direction of tissue expansion. Planar division orientation is governed by apico-cortical tension, which aligns with tissue expansion but not with interphase cell shape elongation. Hertwig's Rule, which holds that cell elongation determines division orientation, is therefore broken in this tissue. We tested whether this observation could be explained by anisotropic activity of the conserved Pins/Mud spindle-orienting machinery, which controls division orientation in the apical-basal axis. We found that Pins/Mud does not participate in planar division orientation. Rather, we found that this epithelium is under anisotropic tension and that this is translated into planar division orientation in a manner dependent on Canoe/Afadin, which links actomyosin to adherens junctions. These findings demonstrate that division orientation in different axes - apical-basal and planar - is controlled by distinct, independent mechanisms in a proliferating epithelium.

749  The FERM protein Yurt is required for the stability of adherens junctions and couples DE-cadherin to actomyosin.  M. Pellikko1, J. Silver1, T. Zuluetu1,2, R. Fernandez-Gonzalez1,2, U. Tepass1  1) Dept Cell & Systems, University of Toronto, Toronto, ON, CA; 2) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, CA.

Yurt is a multifunctional regulator of epithelial organization. Yurt (i) acts as a basolateral polarity regulator together with members of the Yurt/Coracle group, (ii) it binds to and negatively regulates the apical determinant Crumbs, and (iii) it associates with Neuroglian at septate junctions and regulates epithelial barrier function. As a basolateral polarity protein, Yurt acts in parallel to Coracle with no known interaction partners. Here, we show that Yurt genetically interacts with myosin II and DE-cadherin (DEcad) to support epithelial integrity in the Drosophila embryo. We found that a pool of Yurt localized to the adherens junctions (AJs) and biochemically interacts with myosin II, DEcad and α-catenin. Already pre-gastrulation embryos depleting of Yurt show a marked reduction of DEcad levels suggesting that Yurt is required to form or stabilize AJs independent of myosin II engagement. Reduced levels of DEcad and a disruption of myosin II are seen during gastrulation with overt fragmentation of AJs and polarity defects becoming apparent during stage 11, as we reported previously. These results suggest that Yurt has a general role in AJ stability and the coupling of the cadherin-catenin complex to myosin II. Moreover, the striking polarity defects observed in yurt null mutant embryos may result from a loss of AJ stability combined with a loss of Yurt acting as a basolateral polarity protein in antagonism to apical polarity regulators.

To further explore the interactions between Yurt, DEcad, and myosin we examined Yurt function in dorsal closure and wound healing. Dorsal closure fails in yurt zygotic mutants. We show that Yurt is essential for the recruitment of DEcad to the leading edge-aminiosisera interface, and the assembly of the leading edge multicellular myosin II cable. In wound healing, we found that Yurt can be recruited de novo to the site of myosin II cable assembly. Our findings support a model whereby an apico-lateral pool of Yurt links the cadherin-catenin complex and the actomyosin cytoskeleton to support AJ stability, representing a novel, fourth role of Yurt in support of epithelial organization.

750  Cytonemes mediate formation of a morphogen gradient of FGF during branching morphogenesis of Drosophila trachea.  Lijuan Du, Sougata Roy  Cell Biology and Molecular Genetics, University of Maryland, College Park, College Park, MD.

Concentration gradients of a limited number of conserved signaling proteins create diverse forms and shapes during animal development. Drosophila Fibroblast Growth Factor, Branchless (Bnl) is one of the essential paracrine signals that induces tracheal branching morphogenesis. This study aims to understand how Bnl gradient is formed during its movement from the signal source to the recipient tracheal branch and how the Bnl gradient creates diverse patterns of the branches during different developmental stages. With an endogenously expressed GFP tagged Bnl, which was generated by employing CRISPR/Cas9 based genome editing, a Bnl gradient was visualized for the first time. The Bnl gradient was found to change its shape during each embryonic and larval stages of tracheal development. The Bnl gradient asymmetrically conformed to the shape of the recipient tracheal branch, rather than its source tissue. The recipient tissue-specific shape of the gradient is created by FGF transport via specialized actin-based signaling filopodia or cytonemes. Different regions of the recipient
tracheal epithelium extend a variable number of cytonemes that can contain its receptor and contact the bnl-source to receive Bnl. We found that differential uptake of Bnl generates a concentration gradient along the recipient epithelium. Bnl gradient in the recipient branch induces threshold dependent activation of differential gene activity in distinct zones of the epithelium. These genes, in turn, regulate the formation of Bnl-specific cytonemes in the specific expression zones. Therefore, a Bnl gradient is formed and self-sustained by controlling the mechanism of its cytoneme-dependent transport.

751 Protolytic cleavage of Bnl is necessary for its long-range dispersion and signaling. Alex Sohr1, Lijuan Du1, Rufoan Wang1, Li Lin2, Thomas Kornberg2, Sougata Roy1 1) University of Maryland, College Park, MD; 2) University of California, San Francisco, CA.

Branchless (Bnl), a Drosophila Fibroblast Growth Factor, is an essential signal that induces branching morphogenesis of the Drosophila tracheal epithelium. How Bnl secretion and dispersion is controlled during tracheal morphogenesis is unknown. This research uncovers a proteolytic control of Bnl dispersion during the development of the 3rd instar larval Air Sack Primordium (ASP), a precursor of the adult air sac. A group of wing disc cells produces Bnl, and a local gradient of its dispersion is considered to control the directional growth of the ASP toward the disc bnl-source. By tagging GFP at different locations in Bnl and expressing the various tagged versions in the disc bnl-source, we characterized several functional forms of Bnl:GFP that disperse over long distances during ASP morphogenesis. Analysis of the dispersion patterns of various tagged versions of Bnl revealed that the molecule is proteolytically cleaved before its delivery to the recipient ASP tracheal cells. With simultaneous dual HA and GFP tags at various locations flanking the putative cleavage sites in the protein and by mutagenesis of the double-tagged constructs, we identified the cleavage site in the Bnl backbone. To confirm Bnl cleavage and analyze the functional role of the cleavage, two endogenously expressed knock-in Bnl:HA-GFP fusion products were generated using CRISPR/Cas9-based genome editing. One of these contained the mutations at the specific cleavage site. The uncleaved mutant Bnl:HA-GFP could not disperse over long distances in comparison to the control Bnl:HA-GFP. Defective signal dispersion of the mutant Bnl was associated with an abnormally stunted ASP growth. These findings show that proteolytic cleavage of Bnl is necessary for its long-range dispersion and ASP morphogenesis.

752 The Notch signaling pathway specifies cardiac cell subtypes by utilizing distinct permissive and instructive mechanisms to regulate the expression of different pericardial genes. J. M. Dalloul1,2,3, M. Panta2,3, A. J. Kump2,3, K. Schwab2,3, S. M. Ahmad2,3 1) Terre Haute South Vigo High School, Terre Haute, IN; 2) Department of Biology, Indiana State University, Terre Haute, IN; 3) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN.

The development of a complex organ involves the specification and differentiation of diverse cell types constituting that organ. The Drosophila heart is comprised of two major cell types: contractile cardiac cells (CCs) that constitute an inner tube and pericardial cells (PCs) that form a sheath surrounding the CCs. Our previous work showed that binding sites of Suppressor of Hairless [Su(H)], an integral transcription factor in the Notch signaling pathway, were enriched in the enhancers of genes specifically expressed in the PCs. Furthermore, by using cis- and trans- assays with enhancer-reporter constructs for a PC-specific gene, Holes in muscle (Him), we demonstrated that Notch signaling activates Him expression in PCs in a permissive manner: in the absence of Notch signaling, Su(H) forms a repressor complex with co-repressors and binds to the Him enhancer, repressing its transcription; upon alleviation of this repression by Notch signaling, Him transcription is activated. Here, once again using relevant enhancer-reporter constructs, we provide preliminary data showing that Notch signaling activates the expression of Zn finger homeodomain 1 (Zfh1), another PC-specific gene, in a distinctly different, instructive manner: mere alleviation of repression by preventing the binding of the Su(H) repressor complex to the Zfh1 enhancer is not sufficient to activate transcription in PCs. Our results suggest that, in the case of Zfh1, upon Notch signaling, the Notch intracellular domain must bind with Su(H) to change the Su(H) complex bound on the Zfh1 enhancer from a repressor to an activator complex, and that this activator complex is necessary for bringing about Zfh1 transcription. Collectively, these data show how the same feature, enrichment of Su(H) binding sites in the enhancers of PC-specific genes, can be utilized by two distinct mechanisms, one permissive, the other instructive, to contribute to the same overall goal: the specification and differentiation of pericardial cell types by activation of the pericardial gene program.

753 Drosophila fibulin is required for proper somatic and visceral muscle development during embryogenesis. Anna Doane-Ramkhallawon, Anmol Suri, Afshan Ismat Department Biology, University of St. Thomas, Saint Paul.

Drosophila fibulin (fbl) (CG31999) is an extracellular matrix (ECM) gene and is associated with cellular adhesion and elastic fiber connectivity. In Drosophila, fbl mRNA is highly expressed in both the visceral and somatic mesoderm during embryogenesis. The visceral mesoderm is composed of an underlying layer of trunk visceral mesoderm (TVM) on which a second cell type, the caudal visceral mesoderm (CVM), migrates. The somatic muscles form a stereotypical pattern of multinucleate muscle fibers in each abdominal segment of the embryo. In this study, we show that knock-down of fbl in muscle cells resulted in a discontinuous band of TVM cells, as well as huge gaps in the TVM. Initial observations in somatic muscles showed a decrease of muscle attachment and fiber thickness, differences in organization, and shape of somatic
muscles when fbl was knocked down and over-expressed. Over-expression of fbl in both the TVM and CVM resulted in mis-migrating CVM cells. Interestingly, over-expression of fbl in the somatic muscles also resulted in gut morphology defects. Our findings provide evidence that Fbl may be acting both cell autonomously and non-cell autonomously for proper organ formation.

754 Downstream targets of the Forkhead domain transcription factor Jumeau mediate cardiac progenitor cell specification and division. A. J. Kump1,2, M. Panta1,2, Y. Chen3, X. Wang3, S. M. Ahmad1,2 1) Biology, Indiana State University, Terre Haute, IN; 2) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN; 3) National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

While at least eight Forkhead (Fkh/Fox) transcription factors (TFs) are required for proper cardiac development in mammals and mutations in four Fkh genes have been linked to human congenital heart defects, relatively little is known about the molecular mechanisms or the downstream target genes by which these Fkh TF-mediated cardiogenic functions are brought about. Our prior work has shown that the Drosophila Fkh gene jumeau (jumu) mediates both cardiac progenitor cell specification by regulating the expression of Heartless and Frizzled, the receptors of the FGF and Wnt signaling pathways respectively, and cardiac progenitor cell division by regulating the activity of the Polo kinase. However, the significant enrichment of Fkh TF binding sites in the enhancers of cardiac genes suggested that jumu might be utilizing additional downstream target genes to regulate these two cardiogenic processes. Using RNA-sequencing to compare genome-wide transcriptional expression profiles of flow cytometry-purified mesodermal cells from wild-type and jumu loss-of-function embryos, we detected 1,272 putative jumu targets, i.e. genes exhibiting significant differential expression in jumu mutants compared to wild-type. Our ongoing phenotypic analysis of a prioritized subset of these downstream targets with amorphic and hypomorphic mutations shows that jumu does indeed transcriptionally activate at least eight additional genes mediating both asymmetric and symmetric cardiac progenitor cell divisions — Inner centromere protein, barren, nebbish, Cyclin-dependent kinase subunit 30A, Structural maintenance of chromosomes 2, scraps, Kinesin-like protein at 61F, and glue — and yet another gene, tumbleweed, required for the cytokinesis of cardiac progenitor cells. Intriguingly, Cyclin-dependent kinase subunit 30A, one of the jumu-regulated genes mediating cardiac progenitor cell division, also appears to play a role in cardiac progenitor specification. We are using more detailed phenotypic analysis, genetic interaction assays, epistasis tests, and rescue assays between these jumu targets themselves and also between the jumu targets and genes previously known to be involved in cardiac progenitor specification and cell division to determine their roles and positions in these cardiogenic pathways.

755 The role of Forkhead domain transcription factors and their downstream targets in mediating proper positioning of cardiac cells. M. Panta1,2, A. J. Kump1,2, Y. Chen3, X. Wang3, N. Jeffries3, S. M. Ahmad1,2 1) Indiana State University, Terre Haute, IN; 2) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN; 3) National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

The development of a complex organ requires the specification of appropriate numbers of each of its constituent cell types as well as the correct positioning of these cell types within the organ. Our previous work on Drosophila embryonic heart development had shown that the Forkhead (Fkh/Fox) domain transcription factors Checkpoint suppressor homologue (CHES-1-like) and Jumeau (jumu) determine the correct number of different cardiac cell types by regulating the division of cardiac progenitor cells through a Polo-dependent pathway. Here we show that CHES-1-like and jumu are also required for the correct positioning of these cardiac cell types: null mutations in either gene result in the misalignment and incorrect location of both cardiac and pericardial cells within individual hemisegments. Since defective cardiac progenitor cell divisions in CHES-1-like and jumu loss-of-function mutants frequently result in individual hemisegments having different numbers of cardiac cells than their partners across the dorsal midline, we first examined this asymmetry as a possible steric cause of incorrect positioning. Our analysis revealed that steric constraints imposed by the differing number of heart cells in contralateral hemisegments cannot explain all of the observed defects in cardiac cell positioning; statistically significant increases in the number of positioning defects are also observed in Fkh mutants compared with wild-type embryos when only members of contralateral hemisegment pairs having the same number of each cardiac cell type are compared. In order to find downstream targets utilized by CHES-1-like and jumu to bring about correct positioning, we next compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and embryos lacking functional copies of CHES-1-like, jumu, or both Fkh genes. We detected 2,131 genes exhibiting significant differential expression in single or double Fkh mutants compared to wild-type. Our preliminary phenotypic analysis of a prioritized subset of these downstream targets suggests that the Fkh transcription factors bring about the correct positioning of cardiac cell types by restricting the expression of G protein gamma 1 (Gγ1): CHES-1-like and jumu function in a mutually redundant manner to repress Gy1 expression levels, while ectopic overexpression of Gy1 in the mesoderm phenocopies cardiac cell positioning defects observed in CHES-1-like and jumu loss-of-function mutants.

756 Ribbon determines cell growth during tubulogenesis by transcriptional regulation of the translational machinery. R. Loganathan1, D.M. Johnson1, M.B. Wells1, B.E. Kerman1, P.L. Bradley1, J.S. Lee2, M. Slattery2, D.J. Andrew1 1) Cell Biology, Johns Hopkins University, Baltimore, MD; 2) Biomedical Sciences, University of Minnesota Medical School, Duluth,
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stereotypic shape and size of the rhabdomeric/stalk membra
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Molecular Cell Biology and Genetics, Dresden, DE.
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contrast to the smooth leading edges of wild type embryos. Our results support the hypothesis that SJ proteins play critical
mutants is a significant reduction (~55% WT) in cell volume, with a notable effect on cytoplasmic but not nuclear size. To
determine how Rib controls cell size, we performed a SG-specific ChIP-Seq assay to identify potential transcriptional targets of
Rib. ChIP-Seq results revealed that Rib bound 494 target genes in the SG. DAVID analysis to find enriched functional clusters
based on gene ontology assignments revealed Rib targets to include a range of morphogenetic genes, but the major target
class of Rib bound genes was the ribosome. Indeed, Rib bound ~86% of ribosomal protein genes in the ChIP-Seq analysis. Rib
also bound genes encoding several translation factors and the HSP70 chaperones that function during protein
synthesis. Analysis of a subset of ribosomal protein genes by in situ hybridization and RT-qPCR revealed that Rib is required
for their full levels of expression. Imaging analysis revealed abnormal nucleolar morphology in rib mutant SGS, suggesting
compromised ribosome biogenesis. In addition, increased accumulation of P-body foci in the rib mutant SGS suggest an
abundance of untranslated mRNA accumulation. Taken together, these results suggest that Rib drives cell growth by
transcriptional regulation of the translational machinery, presumably to accommodate the demand for increased protein
synthesis necessary for the high level secretory function of the SG. Interestingly, Rib localizes to the TC-rich sequences
embedded at the transcription start site of the ribosomal protein genes suggesting further that it might function in
transcription initiation at these loci.

757 Septate junction proteins play a critical role during dorsal closure. C.A. Rice, R.E. Ward Department of Molecular
Biosciences, University of Kansas, Lawrence, KS.
Animal bodies are separated into distinct compartments by epithelial tissues, which require the formation of barriers to the
flow of water and other small molecules for effective separation. In invertebrates such as Drosophila melanogaster, the
occluding function of epithelial tissues is provided by protein complexes known as septate junctions (SJs). These complexes
form during embryogenesis, providing an occluding function by stage 15 and forming an ultrastructurally mature junction by
stage 16. Despite the late formation of mature SJs, embryos carrying homozygous mutations in individual SJ genes have been
found to fail during earlier stages of development. These embryos frequently exhibit defects in head involution and dorsal
closure, suggesting a morphogenetic role for SJ proteins beyond their known role in mature SJs. To gain a mechanistic
understanding of this additional role, we are using live imaging and fixed tissue analysis to examine how SJ proteins
contribute to the process of dorsal closure, when the cells of the lateral epidermis come together over the amnioserosa to
close a dorsal hole following germ band retraction. Cuticles of embryos carrying homozygous mutations in the SJ genes
Macroglobulin complement-related (Mcr) and coracle have shown that these embryos exhibit frequent defects in dorsal closure,
and live imaging of Mcr embryos demonstrates that these embryos fail to completely close more than 50% of the time. One
consistent phenotype in Mcr mutant embryos undergoing dorsal closure is the scalloped appearance of leading edges, in
contrast to the smooth leading edges of wild type embryos. Our results support the hypothesis that SJ proteins play critical
roles in morphogenetic processes such as dorsal closure. These roles may include supporting a continuous actin-myosin
cable along the leading edge and regulating transmission of tensile forces across tissues during dorsal closure. We are using
antibody staining and FRET analysis of epithelial tension in fixed tissues to explore possible mechanisms by which these
proteins may be acting.

758 A different perspective on the role of crumbs in rhabdomere morphogenesis: linking redox balance and
sphingolipid metabolism in eye development. S. Hebbar, K. Schuhmann, A. Shevchenko, E. Knust Max Planck Institute of
Molecular Cell Biology and Genetics, Dresden, DE.
The evolutionary conserved gene, crumbs (crb), has roles in formation and maintenance of functional epithelia. This dual
role is best exemplified in the Drosophila retina, where crb is involved in morphogenesis (Izaddoost et al., 2002, Pellikka et al.,
2002, Johnson et al., 2002) and maintenance of retinal photoreceptor cells (PRCs) (Johnson et al., 2002). Polarized PRCs are
characterized by a prominent apical domain, called the rhabdomere, a microvilli-enriched photosensitive organelle. Crumbs
localizes to a portion of the apical membrane, called stalk, between the rhabdomere and the adherens junctions. Perturbations in crb expression causes abnormal rhabdomere formation and size of the stalk.
We are interested in understanding how this transmembrane protein regulates morphogenesis of PRCs in forming the
stereotypic shape and size of the rhabdomeric/stalk membrane domain. Given the membrane abnormalities in crb mutants,
we conducted a lipidomics-based screen to assay the lipidome of different mutants. A specific increase in sphingolipid
hydroxylation was observed in crb alleles exhibiting defects in PRC morphogenesis. This was associated with increased
expression of fatty-acid-2-hydroxylase (fa2H), the gene product of which catalyzes the synthesis of 2-hydroxysphingolipids.
Genetically modulating fa2H expression influences the severity of the crb mutant phenotype.
It was previously demonstrated that *crb* mutant tissue has increased oxidative stress (Chartier et al., 2012). Our data suggest that increased oxidative stress, specifically in developing PRCs, is the link between loss of *crb* and dysregulated lipid metabolism. We provide a new perspective on rhabdome morphogenesis, in that we propose a crucial role for *crumbs* in regulating the intracellular redox balance. Loss of this regulation, as in *crb* mutants, impacts on the shape and size of the apical membrane and hence PRC morphogenesis.

**Functional domains of the ADAMTS-like protein Lonely heart and its role on cardiac matrix formation.**  
Y. Post, B. Rotstein, M. Reinhardt, A. Buhr, J. J. Heinisch, H. Meyer, A. Paululat  
Osnabrueck University, Osnabrück, Lower Saxony, DE.

The *Drosophila* heart constitutes a tubular organ formed by contractile cardiomyocytes and pericardial cells. After embryogenesis a subset of pericardial cells differentiate into nephrocytes. Importantly, these nephrocytes are not connected to the heart tube by direct cell-cell contacts, but via a meshwork formed by an extracellular matrix (ECM). To ensure that the heart's integrity is maintained during ongoing heartbeats, and to withstand the resulting mechanical forces, a strong supportive tissue is of crucial relevance. The required biomechanical properties of the cardiac matrix are established by incorporation of several typical, evolutionary conserved ECM proteins, like Integrins or Laminins, but also by the recruitment of proteins specific to the cardiac ECM.

Here we focus on a unique combination of an ADAMTS-like adapter protein called Lonely heart (Loh) and its interaction partner, the Collagen IV-like protein Pericardin (Prc). Loh is crucial for the recruitment and incorporation of Pericardin into the cardiac ECM and thereby essential to provide the tissue with a balanced amount of flexibility and stiffness, allowing it to fulfill its function throughout the animal's life.

By creating several deletion constructs of Loh, we were able to study the protein's interactions with the cell surface and with Prc in a highly detailed manner. Our results show that two thrombospondin type 1 repeats (TSR1) are key players to anchor Loh to the cell surface as well as to bind and recruit Prc to the ECM. In summary, we are able to shine new light onto how Loh functions in shaping the cardiac matrix and provide new insight into the function of the so far poorly understood thrombospondin type 1 repeats.

**Imaginal Disc Growth Factor (IDGF) mutant phenotype worsens under CO2 stress.**  
Anne E. Sustar, Celeste A. Berg  
Dept of Genome Sciences, Univ of Washington, Seattle, WA.

*Imaginal Disc Growth Factors (IDGFs)* are secreted small proteins that accumulate in fly hemolymph and are thought to play a role in cell growth, detoxification, and innate immunity (Kawamura et al., 1999; Broz et al., 2017). One human homolog, CHI3L-1, is upregulated in inflamed tissue in several diseases and during tumor metastasis. Previously our lab found that precise levels of *IDGFs* are critical for proper tube formation in the developing dorsal appendages of the *Drosophila* egg. We reasoned that the mechanism by which *IDGFs* affect cell shape, cell adhesion, and cell migration in the *Drosophila* egg chamber may help to reveal their role in human disease.

Flies have six *IDGFs*. We generated null mutants for individual and multiple *IDGFs*, including a fly strain that is mutant for all six *IDGFs*. We scored the morphology of the dorsal appendages. We observed various defects such as ectopic branching, multiple appendages, and pooling of eggshell chorion at the base of the appendages. The penetrance of these defects, however, was highly variable from experiment to experiment.

In investigating the underlying cause of the variation, we tested whether the flies are sensitive to the CO2 that we were using for anesthetization. As a proof of concept, we treated control flies (*w1118*), flies mutant for individual *IDGF* genes (*IDGF1Δ*, *IDGF2Δ*, *IDGF3Δ*, *IDGF4Δ*, *IDGF5Δ*, or *IDGF6Δ*), and sextuple mutants (*IDGF1-6Δ*) with a 60-second pulse of 100% CO2 twice per day. We scored the resulting eggshell phenotypes. While control eggs were unaffected by the treatment, the dorsal appendage phenotypes worsened after CO2 treatment for all six individual *IDGF* mutants, with the most dramatic defects occurring in *IDGF6Δ* flies. Strikingly, the phenotype of the *IDGF* sextuple mutant became 10x worse, from a 7% severe phenotype to a 66% severe phenotype.

Interestingly, the eggshell phenotypes do not deteriorate with multiple acute exposures. Also, in contrast to the strong effect of acute exposure, chronic low-level CO2 treatment (20% CO2 over one week) does not produce the same profound effect on the eggshell phenotype.

To explore this surprising observation, we are asking if the patterning of key regulatory genes is affected, if cell migration is affected, or possibly both processes are disrupted. Our long-term goal is to define the molecular pathway that ties together the *IDGF* pathway with CO2 exposure. This work reveals underlying mechanisms for how environmental stresses affect tube formation.

**Using Oxford Nanopore Sequencing to Investigate the Y Chromosomes of the Malaria Mosquitoes.**  
Austin Compton, Jake Tu  
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Mosquito-borne infectious diseases are a global health and economic threat. An increase in insecticide resistance has jeopardized current mosquito control measures. Novel genetics-based control strategies are needed to limit the spread of harmful mosquito-borne pathogens. We are interested in manipulating mosquito sex determination pathways to introduce a male bias into a mosquito population because males do not bite. In this way, we can potentially reduce the number of egg-laying and disease-transmitting females and ultimately suppress mosquito populations. Sex determination relies on a male initiation factor which is found on the Y chromosome in the malaria mosquitoes of the genus Anopheles. One of the major challenges of studying genes in these regions is the abundance of repetitive DNA. Long stretches of DNA repeats restraints the quality of assemblies due to a lack of anchoring. Thus, we used Oxford Nanopore MinION single-strand sequencing technologies to produce long reads towards generating Y chromosome assemblies in mosquitoes.


Transcription of protein-coding genes in eukaryotes is controlled by the RNA polymerase II system. A key component in the initiation of transcription in this system is the core promoter element (CPE) sequences, including the TATA box and Initiator (INR), which are thought to be involved in the recruitment of RNA polymerase II. In this study, we investigate the sequence composition of the TATA box and INR at experimentally defined promoter regions in Drosophila melanogaster and Homo sapiens. Using computational and statistical methods, including modified position weight matrix (PWM)-based methods in our MARZ algorithm, we examine the significance of individual nucleotides and potential interdependencies between nucleotides within these sequences. The results will improve our ability to predict the location and potential regulatory contribution of the CPEs at promoters.

763 The role of protein-protein interaction motifs in coordinating the DNA binding and regulatory specificity of Hox proteins. W. Glassford1, O. Storms1, R. Mann1,2,3,4

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Transcription factors perform a key role in regulating gene expression, but our ability to interpret regulatory DNA and predict regulatory function by identifying transcription factor binding sites is lacking. One contributing factor is that each transcription factor has the potential to interact with cofactors, sometimes in structurally distinct ways, adding to the ability of transcription factor complexes to bind distinct DNA sequences. Perhaps the best illustration of this idea is the Hox family of proteins, which pattern the anterior/posterior axis in all metazoans. When bound to the cofactor Extradenticle (Exd), Hox proteins exhibit latent differences in DNA-binding specificity necessary to carry out their unique activities. Protein-protein interaction (PPI) motifs mediate the interaction between Hox proteins and their cofactors; the physical interaction between Hox and Exd has been particularly-well studied and is mediated by a canonical PPI motif present in all Hox proteins. Some Hox proteins contain additional motifs, however, which may promote alternate Hox-Exd-DNA conformations. This presents a potential mechanism for implementing the multiple roles of Hox genes, such as paralogue-specific binding and enhancing or repressing gene activity. To test this hypothesis, I first determined differences in DNA-binding affinity between mutant Hox-Exd complexes by utilizing SELEX-seq (Systematic Evolution of Ligands by Exponential Enrichment). In parallel, I tagged and mutate Exd-interaction motifs in the endogenous loci of select Hox genes in order to perform motif-specific ChiP-seq (Chromatin Immuno-Precipitation) and RNA-seq. These experiments identify differences in DNA-binding specificity effected by specific Hox-Exd PPI motifs, and generate a genome-wide collection of in vivo Hox binding sites whose binding and transcriptional activity is motif-specific or motif-redundant.


Cells use histones to organize and compact chromatin into chromosomes. During S phase, cells coordinate synthesize large amounts of five histone proteins to rapidly package newly replicated DNA into chromatin, a process essential for proper organization of the genome. At the onset of S phase in Drosophila and other animals, histone production is initiated by activation of a set of multi-copy genes termed the replication dependent (RD) histone genes. In D. melanogaster, the histone genes are present at a single locus, with 100 copies of a 5 KB sequence containing one copy each of the 5 RD histone genes. The RD histone mRNAs differ from all other cellular mRNAs. They are not polyadenylated but end instead in a conserved stem loop. Associated with RD histone genes is a nuclear body called the Histone Locus Body or HLB, which contains factors required for histone gene transcription and formation of the 3' end of histone mRNA. A critical HLB component is a scaffolding protein Mxc (multi-sex combs) present only in the HLB. Histone expression is activated by phosphorylation of Mxc by cyclin E/cdk2. We do not know exactly how the HLB affects histone mRNA production, what all of the components are, or how the components are targeted to and organized into a structure that specifically associates with RD histone genes. We are using several concurrent approaches to address these questions. We are using high-resolution microscopy to determine the localization of several known HLB factors. We find that each of these factors have distinct
locations within the HLB, forming subdomains of the HLB. Using a fly expressing GFP-MXC we have partially purified HLBS in order to comprehensively determine the factors that comprise the HLB. In order to determine how HLBS receive cell cycle input, we are analyzing HLBS from actively cycling cells, G1 arrested cells in embryos, as well as cells that reenter the cell cycle. Using imaging and isolation of HLBS we should be able to determine if and how HLBS change structurally during cell cycle progression, particularly at the onset of S phase. Lastly, using information obtained from these approaches we will test the effects of perturbing HLB structure on histone mRNA transcription and processing.

765 A CTD of RNA polymerase II composed solely of consensus heptads is sufficient for Drosophila development. Feiyue Lu\textsuperscript{1,2}, Bede Portz\textsuperscript{1}, David Gilmour\textsuperscript{1} 1) BMB Department, Center for Eukaryotic Gene Regulation; 2) The Huck Institutes of Life Sciences, Penn State University, University Park, PA.

The CTD is a repetitive, intrinsically disordered region at the C-terminus of the largest subunit of RNA polymerase II (Pol II). It serves as a docking site for a myriad of factors involved in various co-transcriptional events. The CTD of yeast is comprised primarily of repeating heptads Y,S,P,T,S,P,S; whereas the CTDs of higher eukaryotes have significantly more repeats, with many repeats divergent from the consensus ones at one or multiple positions. This increase in length and sequence complexity is thought to be essential for the evolution of complex patterns of gene expression in higher eukaryotes. However, the functional significance of the non-consensus heptads towards development remains largely untested. Here we show that regions that are comprised solely of non-consensus heptads are less important than a region that contains consensus heptads. In addition, the human CTD, which is composed of a different assemblage of non-consensus heptads than Drosophila, is able to support the development of Drosophila to adulthood. Remarkably, a viable homozygous fly line can be made by replacing the entire naturally occurring Drosophila CTD with 29 consensus heptads. Our results argue against the hypothesis that the non-consensus heptads in the CTD are required to mediate complex patterns of gene expression. We propose that the non-consensus heptads control the overall biophysical properties of the CTD and do not provide unique binding sites for factors involved in regulating gene expression.

766 An optogenetic approach to investigate Zelda function in the early embryo. S. McDaniel, M. Harrison Biomolecular Chemistry, University of Wisconsin, Madison, WI.

All metazoans begin life relying on RNAs and proteins deposited into the oocyte by the mother. During this time the zygotic genome is transcriptionally quiescent. For development to proceed, control must be transferred from the mother to the zygote during a process known as the maternal-to-zygotic transition (MZT). It remains unclear how a genome is rapidly transformed from a transcriptionally inactive state to a transcriptionally active one. In Drosophila, the genome is gradually activated with widespread activation occurring at the 14\textsuperscript{th} nuclear division. Zelda is an essential transcription factor required for zygotic genome activation. As early as the 8\textsuperscript{th} nuclear cycle, Zelda is already bound to the cis-regulatory regions of hundreds of genes that will be activated throughout the MZT, including most of the genes that will not be activated until an hour later at nuclear cycle 14. Because many of the protein products of the earliest expressed genes are required for proper gene expression at cycle 14, it has been challenging to separate the functional requirement for Zelda in activating widespread transcription at this later time point from its role in activating transcription early. To determine whether Zelda is essential for activating transcription throughout the MZT, we developed a strategy to specifically and rapidly inactivate Zelda at precise stages by tagging the endogenous Zelda with the optogenetic CRY2 tag. Building on recently published work on the transcription factor Bicoid, we demonstrated that CRY2 enabled inactivation of Zelda within seconds upon exposure to blue light. To elucidate the role of Zelda throughout early embryonic development, we inactivated Zelda beginning at either nuclear cycle 10 or cycle 13 and maintained this inactivation until cycle 14. Inactivating Zelda at either time during the MZT was lethal to the embryo, which failed to progress through gastrulation. To determine the effects on transcription, we will isolate and sequence mRNA from single embryos in which Zelda was inactivated at specific time points spanning the MZT. Through precise temporal control of transcription factor activity, we will define the specific requirements for Zelda in driving gene expression at multiple time points spanning the MZT and thus help to determine how this essential factor reprograms the genome to allow for the development of a new organism.

767 Post-Translational Modification of Vestigial Is Required for Proper Fate Specification in Wings and Embryonic Muscle. V.L. Pimmett\textsuperscript{1}, H. Deng\textsuperscript{2}, J. Haskins\textsuperscript{1}, R.J. Mercier\textsuperscript{1}, P. LaPointe\textsuperscript{1}, A.J. Simmonds\textsuperscript{1} 1) Cell Biology, University of Alberta, Edmonton, Alberta, CA; 2) Howard Hughes Medical Institute, Dept. of Physiology, UT Southwestern Medical Center, Dallas TX USA.

Changes in expression of the human Vestigial-like (VGLL) proteins is associated with increased tumor invasion, increased cell proliferation and poorer patient outcome in a variety of childhood and adult cancers. Using Drosophila we are investigating mechanisms of post-translational regulation of the human VGLL family homologue Vestigial (Vg). Through in vitro and in vivo experiments, we have shown that phosphorylation of Vg on serine 215 by p38B MAPK is necessary for proper fate determination during development of the embryonic somatic musculature as well as the adult wing. Vg phosphorylation is modulated by the Hippo pathway effector Scalloped (Sd), a homologue of the TEAD family proteins in humans that is also associated with several types of cancers. Vg requires the presence of Sd to function in its role as a transcriptional co-activator,
and this interaction is potentially part of a regulatory balance driving fate determination via the Hippo pathway. Alternative interactions of Vg and a second Vestigial family protein, Tondu-domain-containing Growth Inhibitor (Tgi), require the same binding domain of Sd to exert their regulatory influence. How the post-translational modification status of Vg affects this equilibrium is as yet unclear, but may be a new path forward in examining cancer progression via the downstream Hippo pathway effectors.

768 The gene tfia-s-2 encodes a testis-specific homolog of a TFIIA subunit. Helen Shapiro-Albert, Kelly Budge, Mark Hiller Goucher College, Baltimore, MD.

The General Transcription Factor TFIIA functions during the initial stages of RNA polymerase II transcriptional activation. The TFIIA complex is made up of α, β, and γ subunits, and Drosophila melanogaster may express two testis-specific homologs of the γ-subunit of TFIIA, γ-2a and γ-2b. Testis-specific γ-2a or γ-2b expressed in vitro will associate with the widely expressed α and β subunits, indicating that they may assemble into TFIIA-like complexes. The widely-expressed version of TFIIA physically associates with several subunits of TFIIID. Association with the TFIIID complex can occur with the TATA-Binding Protein (TBP) or some of the fourteen, unique TBP-associated factors (TAFs). There exists several testis-specific TAF homologs that are collectively known as tTAFs. A loss of function mutation in any of the tTAFs leads to a disruption in typical transcription of genes that are normally expressed during the primary spermatocyte phase of spermatogenesis and a complete block in cellular differentiation. There are no known tfia-s-2 mutants. We are investigating the possibility that the testis-specific tfia-s-2 gene products work with the tTAFs to activate testis-specific transcription.

769 Systematic Screening for Transcriptional Regulators of Adult Myogenesis in Drosophila by RNAi. Tommy Soudachanh1, Sandy Oas1, Tyler Mendes2, Anton Byrantshev3, Richard Cripps1 1) Biology Department, University of New Mexico, Albuquerque, NM; 2) Cellular, Molecular, and Biochemical Science Program, Ohio State University, Columbus, OH; 3) Department of Molecular and Cellular Biology, University of Kennesaw, Kennesaw, GA.

The muscles of Drosophila show similar structure and pathway of development to the muscles of humans, and allows Drosophila to model human muscle disorders. However, little is known about many of the genes and their potential role in muscle development. Thus, the focus of this project is to efficiently screen for potential transcriptional regulators of muscle development and identify associated disorder phenotypes. Genes were selected based on having a potential role in transcription, which were silenced using RNA inhibition (RNAi). The 1151-gal4 driver was utilized to induce RNAi in developing adult muscles. Combined with the driver, the flies harbored enhancers of either Flightin-LacZ or TpnC41C-LacZ. The resulting transgenic flies were collected and assayed to measure the effects of this genetic loss. All 101 genes in the latest set have been assayed for a change in enhancer activity of less than 70% or greater than 130%, in Flightin and TpnC41C; the genes selected for immunostaining exhibited semi-lethality or lethality, and indicated a loss or gain of LacZ. The immunostaining would reveal several factors: muscle morphology, and expansion or loss of enhancer activity associated with specific flight and jump muscle genes. The resulting data was classified into three categories, severe loss, moderate loss, or no change in muscle development. Genes with a severe loss indicates a potential issue with myoblasts fusion, and genes with a moderate loss indicates issues with adult muscle formation and maturation. Therefore, the results demonstrate that the screen is efficient in identifying potential transcriptional regulators and muscle disorder phenotypes.

770 Pointed is necessary and sufficient for establishing the posterior end of the follicular epithelium. C. Stevens, R. Caur, N. Yakoby Rutgers, The State University of New Jersey, Camden, NJ.

The anterior-posterior axis during Drosophila oogenesis is regulated by a small number of cell signaling pathways. The Janus-kinase/Signal Transducers and Activators of Transcription (JAK/STAT) is activated in both posterior and anterior ends of the follicular epithelium. Previously shown, JAK/STAT activation is required for the expression of decapentaplegic (dpp), the bone morphogenetic protein (BMP) signaling ligand, which consequently activates this pathway in the anterior follicular epithelium. In the posterior, JAK/STAT works in concert with the epidermal growth factor receptor (EGFR) to express the ETS-transcription factor pointed (pnt). Pnt was shown to control the dorsal midline width, which sets the distance between the two dorsolateral domains of the respiratory dorsal appendages primordia. Here we show that Pnt is necessary for determining the posterior fate of the follicular epithelium. In addition, our results indicate that Pnt is sufficient to repress anterior fate formation, as seen by the loss of BMP signaling. This complex signaling and transcriptional network provide insight into the establishment of the anterior-posterior axis of the fly.

771 modERN project update. Alec Victorsen1, Lijia Ma1, Kevin White1, Michelle Kudron2, Valerie Reink2, Bob Waterston1 1) University of Chicago, Chicago, IL; 2) Yale University, New Haven, CT; 3) University of Washington, Seattle, WA.

In order to better understand gene regulation, the modERN project aims to map binding sites for every Transcription Factors (TFs) in D. melanogaster and C. elegans. To date, we have generated ChIP-seq data for roughly 60% of TFs in both organisms using recombined BACs and fosmids which express TF-GFP fusion proteins at near endogenous levels. While this approach will work for almost all TFs, we have begun to use CRISPR in C. elegans to create genomic TF-GFP gene fusions
as an alternative tagging approach for TFs that are incompatible with the BAC/fosmid method. modERN ChIP-seq data is currently available at encodeproject.org.


The Drosophila even skipped (eve) locus spans about 16kb, flanked by two insulators, homie and nhomie. In order to investigate homie and nhomie function, we created a transgenic "pseudo-locus" that contains the entire eve locus, plus the 3' flanking TER94 locus through its the 3' exon. In this pseudo-locus, eve and TER94 coding regions are replaced by lox2 and GFP, respectively. When homie is removed, TER94-GFP is repressed. Without homie, the eve PRE (Polycomb response element), which resides just upstream of homie, stimulates spreading of H3K27me3 over TER94-GFP, which represses its expression. Simultaneously, eve-lox2 shows weakened early stripes (1, 4, 5, and 6), as well as weakened tissue-specific expression, all of which are driven by enhancers between homie and the eve coding region. These data suggested that homie is protecting the eve 3' enhancers from a flanking repressive influence.

We now find that this enhancer inactivation is caused by transcription read-through from a promoter in the 5' element end, which flanks the eve pseudo-locus. Both homie and nhomie are capable of preventing transcription read-through to protect the enhancers from inactivation. In contrast to the orientation-dependence of homie and nhomie's pairing activity, which can promote both long-range enhancer-promoter interactions in cis and transvection, transcription blocking (like classical enhancer blocking) is orientation-independent. Moreover, other known insulators, such as gypsy, Fab7, and Fab8, are also able to inhibit transcription read-through. Thus, read-through transcription blocking is a common function of insulators, and represents another important activity that functionally isolates chromosomal compartments.

773 Notch activity elicits changes in Su(H) nuclear dynamics. Maria Gomez-Lamarca1, Julia Falo SanJuan1, Robert Stojnic1, Sohaib Abdul Rehman1, Leila Muresan1, Zoe Pillidge1, Kevin O'Holleran1, Francois Payre1, Rhett Koval1, Sarah Bray1 1) PDN, Univ Cambridge, Cambridge, GB; 2) University of Cincinnati, Department of Molecular Genetics, Biochemistry and Microbiology, Cincinnati, OH; 3) Centre de Biologie du Développement, University of Toulouse, 31062 Toulouse, France.

A key feature of Notch signaling is that it directs immediate changes in transcription via the DNA-binding factor Suppressor of Hairless (Su(H)). How Notch can generate both a sensitive transcriptional response and the ability to activate the transcription factor CSL at target loci, even in the absence of any amplification step, is not well understood. To address this, we have performed real-time analysis of Su(H) dynamics, including single molecule tracking in vivo. In Notch-ON nuclei, we found that Su(H) displays highly dynamic behaviour, with only a small proportion of molecules bound to DNA at any one time. In Notch-ON nuclei, CSL becomes highly enriched at target loci, while the molecules have a longer dwell-time. We find that this change in CSL dynamics relies on two different mechanisms that together provide a paradigm for how Notch activity is transduced at target loci.

774 Dynamic localization of Zelda in syncytial mitoses. S. Huang, K. Xu, M. Warshafsky, C. Rushlow Department of Biology, New York University, New York, NY.

Zelda (ZLD) is a pioneer transcription factor that plays a key role in zygotic genome activation. ZLD lowers the nucleosome barrier at enhancers of early expressed genes to lend accessibility of other transcription factors, including the patterning morphogens Bicoid (BCD) and Dorsal (DL), which effectively potentiates their activity (Xu et al., 2014; Sun et al., 2015; Schulz et al., 2015). Although ZLD protein is present in nuclei as early as mitotic cycle 2, earlier than BCD and DL, the dynamics of nuclear localization during mitosis as the nuclear membrane breaks down and as it reforms is unclear. To study ZLD localization dynamics, we used two approaches: live imaging of a sfGFP-ZLD fusion protein generated by CRISPR, and immunocytochemistry with anti-ZLD and anti-Lamin (a nuclear envelope marker) antibodies. Our results show that ZLD exits the nucleus at prometaphase when the nuclear envelope starts to break down, and re-enters the nucleus in early telophase as the nuclear envelope reforms. Strikingly, using in situ hybridization to detect transcripts of the ZLD target gene, holo, we found that transcripts can be detected as early as telophase, suggesting that as soon as ZLD enters the nucleus after anaphase, it can function as a transcriptional activator. We also examined the dynamics of BCD and DL localization, and they have the same dynamics during mitosis as ZLD. We are currently assessing whether BCD and DL target genes are first transcribed in telophase, and whether this is affected in the absence of ZLD. Any observed delay in their activation after telophase would indicate that even though the proteins re-enter simultaneously, ZLD must function first for timely activation.


775 A dominant modifier genetic screen for factors that interact with CDK8-Cyclin C identifies components of the Dpp signaling pathway in Drosophila. Xiao Li1, Mengmeng Liu1, Jian-Quan Ni2, Jun-yuan Ji1 1) Molecular and Cellular
Dysregulation of CDK8 (Cyclin-Dependent Kinase 8) and its regulatory partner CycC (Cyclin C) have been linked to a variety of human cancers. Because gain of CDK8 activity can drive tumorigenesis in melanoma and colorectal cancer patients, there are considerable interests in developing CDK8 inhibitors to target the oncogenic functions of CDK8 for cancer treatment. However, for this pharmaceutical approach to be successful, it is essential to understand the regulatory network of CDK8-CycC in both normal development and tumorigenesis. To identify upstream regulators or downstream effectors of CDK8-CycC, we performed a dominant modifier genetic screen based on the vein patterning defects caused by specific overexpression or depletion of CDK8-CycC. Further mapping of these enhancers and suppressors led us to identify the genetic interactions between CDK8-CycC and the components of the Dpp signaling pathway. Our analyses based on in vivo reporters of Mad-dependent gene expression suggest that CDK8-CycC positively regulate Mad-dependent transcription. Further analyses of other subunits of the Mediator complex led us to identify six additional Mediator subunits that are required for Mad-dependent gene expression in wing imaginal discs. The genetic interactions between Mediator subunits and the Dpp signaling pathways in patterning Drosophila wing veins provide a biological context to dissect the molecular mechanisms underpinning Mad-dependent transcription in nucleus.

776 Identifying Gcm/Repo transcription co-regulators in glial cell development.  
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The glial cells missing (gcm) gene was identified as a “master regulator” of glial cell fate in the fruit fly Drosophila. However, gcm is also expressed in and required for the development of larval macrophages and tendon cells, and lamina neurons in the adult CNS. Thus, Gcm protein activates the transcription of different sets of genes in different developmental contexts. How Gcm regulates these different outcomes is not known. Our goal is to identify co-regulators that act with Gcm to promote the transcriptional activation of Gcm target genes specifically in glial cells, or prevent their activation in the other tissues in which Gcm is expressed. Previously we characterized cis-regulatory elements of the glial-specific Gcm target gene reversed polarity (repo) and we defined a minimal cis-regulatory element that recapitulates the endogenous repo expression dependent on a single Gcm binding site. We performed yeast one-hybrid screens using this cis-regulatory element and identified several trans-acting factors that are potential Gcm co-regulators. We are taking genetic and biochemical approaches to test whether these candidate co-regulators act with Gcm to regulate Gcm-dependent gene expression.

777 Novel roles for Retinoblastoma proteins: linking cell cycle and cellular growth.  
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Retinoblastoma (RB) tumor suppressor proteins function as transcriptional corepressors to regulate eukaryotic cell cycle gene expression. Additional roles for these proteins in physiology and development are less well understood; we recently reported on RB protein regulation of cell polarity genes in the fly. Genes for RB family proteins are deeply conserved, and most invertebrates have a single RB gene, but expansion of the family is found in Drosophila, which possesses two conserved paralogs (Rbf1 and Rbf2), and mammals, with three (RB, p107, p130). We investigated the functional significance of Drosophila RB protein functional diversification with genomic approaches; our ChIP-seq studies of these two proteins showed Rbf2 binding to a diverse set of genes not occupied by Rbf1, and in new functional studies, we find that Rbf2 regulates a large set of genes important for cellular growth control, including ribosomal protein and nuclear-encoded mitochondrial genes. Interestingly, cell cycle genes appear to be largely regulated by Rbf1, although Rbf2 may play an antagonistic role in reducing the extent of Rbf1 repression. By diversification of the target specificity of RB proteins, this regulatory system provides a nexus to coordinate cellular growth control with cell cycle, with special significance in the context of reproduction. Intriguing initial work indicates that these aspects may be a feature of the multi-component RB system in mammals as well, impacting development and disease.

778 Polymization-driven transcriptional regulation and dysregulation in the developing fly eye: a view from the human ETS repressor TEL/ETV6.  
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Mammalian TEL/ETV6 and its Drosophila homolog, Yan, are evolutionarily conserved repressive ETS family transcription factors (TFs) with the distinct ability to homotypically polymerize. Polymer formation is mediated by a N-terminal Sterile Alpha Motif (SAM), which has been shown to be essential for TEL and Yan’s ability to regulate gene expression during development. TEL regulates blood development in vertebrates and in humans is frequently the target of chromosomal translocations that fuse it’s SAM to the DNA binding domain of another TF, such as RUNX1, leading to hematologic malignancies, including acute myelogenous leukemia (AML). Cell culture models have shown the importance of SAM-SAM interactions to the transforming activity of TEL oncogenic fusion proteins. However, the relationships between SAM-SAM affinity and polymer length to target gene selection and regulation are not well...
understood in either the normal or oncogenic context. Specifically, it is not known how polymerization shapes the formation and function of specific TEL complexes at target genes. To answer these questions, we have engineered a set of biochemically characterized human TEL SAMs with a range of different affinities for use in Drosophila. By using the developing eye as a model system, we can assess the consequences of overexpression of these mutants with respect to gene expression, cell fate specification, and overall tissue patterning. Our system also allows us to uncouple the contribution of polymer length and protein-protein affinity to TEL-mediated dysregulation by restricting TEL to dimers and then independently modulating SAM-SAM affinity. Preliminary results indicate a positive correlation between the SAM-SAM interaction strength and the severity with which TEL overexpression disrupts the normally well-organized eye. Further, even at wild-type affinity, dimers are insufficient to recapitulate full-length TEL activity, suggesting that higher order polymerization is required for TEL-mediated dysregulation. Future work will explore the molecular mechanism for polymerization-dependent TEL-mediated regulation and dysregulation of gene expression.

779 Understanding the role of Capicua in genome-wide regulation in the early fly embryo. S.E. Keenan1,2, S.A. Blythe4, E.F. Wieschaus2,4, S.Y. Shvartsman1,2,3 1) Chemical and Biological Engineering, Princeton University, Princeton, NJ; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 3) Department of Molecular Biology, Princeton University, Princeton, NJ; 4) Department of Molecular Biosciences, Northwestern University, Evanston, IL.

Capicua (Cic) is an HMG-Box transcription factor and a downstream effector of the Torso signaling pathway in Drosophila melanogaster. It plays a major role in terminal structure formation during embryonic development. Cic regulates genes such as tailless and huckebein to repress their expression throughout the embryo. Once the rapidly dividing nuclei move to the periphery of the embryo, the Torso receptor is activated by a controlled release of ligand at the anterior and posterior poles. This results in loss of repression of Cic at the poles. Subsequent expression of tailless and huckebein in these regions begins the formation of head and tail structures of the future larva. While this role of Cic is well understood, Cic has additional targets that are activated independently of the Torso pathway in distinct regions of the embryo. What regulatory function does Cic perform at these additional targets, and how do mechanisms for de-repressing these targets compare with Torso-mediated activation of terminal gene expression at the poles?

In this study, we use ChIP-seq to identify all gene targets of Cic. Preliminary data identifies 231 peaks of Cic binding throughout the Drosophila genome, corresponding to 142 unique gene targets. Some genes have multiple enhancers that are bound by Cic. We then classify the expression of these 142 genes based on location (anterior/posterior, dorsal, ventral, or uniform expression) or by time of activation (before cellularization or after cellularization). In this way, we predict different mechanisms by which Cic functions within these different regions. Furthermore, we cross-reference Cic binding sites to those of other important morphogens/transcription factors such as Bicoid or Dorsal. We additionally classify these gene overlaps by location and time of expression within the embryo and examine any patterns that emerge. Further analysis of Cic binding sequences may inform us about the nature of weak/strong binding of Cic to DNA and its role in repression at different areas of the embryo. We are currently using genetic manipulation to remove Cic and top gene targets of Cic from the embryo. Examining morphological changes in these embryos may explain the downstream role of particular genes and how they interact with Cic.

780 Temporal features of transcriptional enhancer activity dynamically specify segment polarity in Drosophila. P. Batut, B. Fukaya, B. Lim, M. Levine Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

The establishment of form and pattern during development has long been a major focus in biology. The patterning of the anterior-posterior axis in Drosophila constitutes a classical paradigm in the field, and is one of the best-characterized examples in the animal kingdom. In this system, gradients of maternally deposited factors provide a rudimentary coordinate system that is decoded by a zygotic transcriptional regulatory cascade. The activation of gap and pair-rule genes in increasingly restricted spatial domains culminates in the establishment of parasegment boundaries by segment-polarity genes. The central tenet of current textbook models is that gap factors form local gradients and act as morphogens to pattern the expression of downstream pair-rule genes in partially overlapping, precisely offset stripes. Ultimately, the offset of even-skipped and hairy stripes specifies the expression domain of the segment polarity gene engrailed.

In a challenge to this prevailing local-morphogen model, we provide evidence that the temporal dynamics of pair-rule gene expression play a crucial role in establishing segment polarity. Using CRISPR-based genome engineering and quantitative imaging of transcription in live embryos, we show that eve and h are initially transcribed in very broad and fully overlapping domains, which are only gradually refined into distinct stripes. Importantly, these complex expression dynamics are encoded by individual stripe enhancers. The eve and h stripe 2 enhancers mediate drastically different kinetics of refinement of their respective expression domains, leading to their progressive resolution over time. We provide evidence that direct regulation by other pair-rule genes might be responsible for the complex transcriptional dynamics of individual stripe enhancers. We propose that a self-organizing system of mutual interactions between pair-rule genes dynamically specifies segment polarity.
in the *Drosophila* embryo. In this system and others, temporal aspects of gene expression may constitute a more fundamental aspect of spatial patterning than is currently appreciated.

782 Capturing the MicroFoam Structure of Regulatory Modules via Maximal Homology Alignment.  A.J. Erives  Department of Biology, University of Iowa, Iowa City, IA.

Real genomes change more so by replication slippage than by simple base substitution particularly in non-protein coding sequences. When transformed into a gapped multiple sequence alignment, this tandem micro-homology is aligned with artificial gap characters resulting in orphaned homology. Replication slippage is further exacerbated by unconstrained microsatellite repeats leading to dynamic instability. The complexly-patterned DNA sequence resulting from this natural process is particularly enriched in the spacer sequences intervening clustered transcription factor binding sites, and somewhat less so in the protein-coding sequences encoding random coils interspersed between secondary-structure. Thus, spacer elements in regulatory and protein coding regions become enriched in complexly-patterned repeats leading to a private (lineage-specific) “microfoam” structure. Microfoam sequence is dynamically evolving and reflects a mixed history of tandem repeat instability, cryptic micro-gene conversion, and molecular parallelism related to their adjacency to functional elements. Here we describe maximal homology alignment to capture and reveal the extent to which functional genetic sequence of the *vnd* gene of *Drosophila* is embedded in microfoam structure.

783 Visualization of transvection suggests the occurrence of transcription hubs in living *Drosophila* embryos.  Tyler Heist¹, Bornyi Lim¹, Michael Levine¹², Takashi Fukaya¹ 1) Lewis Sigler Institute, Princeton University, Princeton, NJ; 2) Department of Molecular Biology, Princeton University, Princeton, NJ.

The activation of gene expression by transcriptional enhancers remains a central mystery in molecular biology. The traditional view is that enhancers loop to the promoters of genes they regulate in a targeted fashion. However, recent Hi-C studies raise the possibility that enhancer-promoter communication occurs within the context of chromosomal loop domains. We employ quantitative imaging methods to investigate the role of such domains in the regulation of gene expression in living *Drosophila* embryos. We devised a strategy to visualize the process of transvection, whereby enhancers located on one homolog activate transcription on the other homolog. A shared enhancer was found to produce coordinated transcription from linked reporter genes in *cis* and in *trans*. Transvection depends on sustained physical association of homologous chromosomes, which is stabilized by various pairs of insulator DNAs. Further, we provide evidence for promoter competition between two reporter genes (regulated by a shared enhancer) across homologs, implying the partitioning of shared Pol II complexes. These observations are consistent with the occurrence of transcription “hubs”, which trap the transcriptional machineries mediating gene expression.

784 Investigating the evolutionary conservation of insulator sequences in *Drosophila*.  L. Manoj¹, T. Hellmig¹, M. Fujioka², J. Jaynes², H. Mistry¹ 1) Dept. Biology, Widener University, Chester, PA; 2) Dept. Biochemistry & Mol. Biology, Thomas Jefferson University, Philadelphia, PA.

Temporal and spatial control of gene expression regulates normal development in multicellular organisms. Chromatin is subdivided and organized into discrete domains via the action of specialized DNA sequences known as boundary or insulator elements. The even-skipped (eve) locus of *D. melanogaster* has been studied to identify regulatory sequences that dictate eve expression in specific tissues and at precise developmental time points. At the 3’ end of the eve locus is a Polycomb response element (PRE) flanked by an insulator (homeo), which functionally isolates eve regulatory DNA from the essential housekeeping gene *TER94*. To test whether the PRE and insulator activities have been conserved during evolution, we looked at other *Drosophila* species. In *D. erecta*, which is closely related to *D. melanogaster*, eve is linked to *TER94*. In the more distantly related species, *D. pseudoobscura* and *D. virilis*, eve is adjacent to CG30421. Using comparative sequence analysis, we have identified and isolated DNA fragments homologous to the *D. melanogaster* PRE-insulator region from these 3 species. The *D. melanogaster* PRE-insulator fragment is capable of facilitating enhancer-promoter interactions in *trans* (transcription) by homologous pairing. Using this assay, we will test whether pairing function is conserved. We cloned each fragment into both a reporter transgene and an enhancer transgene. These transgenes were injected to generate transgenic stocks via phiC31-mediated recombination in *D. melanogaster*. We will first test whether there is transvection- mediating activity within the PRE-insulator-homologous fragment from a single species. We will then test interactions among the fragments from different species. Based on prior studies, we expect to observe different intensities of reporter expression depending on the extent of functional conservation. Future analyses will involve detailed dissection of functional sequences, and the identification of proteins that mediate pairing activity. Thus, our comparative approach using these 4 species will give us an understanding of how the homolog pairing function of PREs and insulators has changed during evolution, and the mechanistic basis of those changes.

785 *Cis*-regulatory requirements for wing-specific expression of the *apterous* gene.  M. Müller, D. Bieli, G. Born, M. Sickmann, M. Affolter  Biozentrum, University of Basel, Switzerland.
The selector gene *apterous* (*ap*) plays a key role during the development of the *Drosophila* wing as it governs the establishment of the dorso-ventral (D-V) compartment boundary. The D-V compartment boundary is known to serve as an important signaling center that is essential for the growth of the wing. The role of *Ap* and its downstream effectors in this process have been studied extensively. Our focus has been on the wing-disc-specific transcriptional regulatory patterns of the *ap* gene. We have previously analyzed the cis-regulatory requirements for wing-specific *ap* expression. Three discrete cis-regulatory modules (CRM) were identified: two enhancer elements located within a ~25 kb interval upstream of a third CRM, a promoter-proximal Polycomb-response element (PRE). We are currently investigating the cis-regulatory requirements of these CRMs in more detail by introducing small deletions in the context of the endogenous *ap* locus.

**Enhancer decommissioning during Drosophila wing development.** Matthew Niederhuber\textsuperscript{1,2,3,4}, Daniel McKay\textsuperscript{1,2,4} 1) Department of Genetics, UNC; 2) Department of Biology, UNC; 3) Curriculum in Genetics and Molecular Biology, UNC; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina, Chapel Hill, NC.

Dynamic changes in chromatin accessibility play a central role in regulating gene expression during development. Transcription factors are typically occluded from binding their target sequence motifs within DNA regulatory elements such as enhancers by the presence of nucleosomes. For a gene to be competent to respond to transcription factor input, corresponding enhancers must be sufficiently depleted of nucleosomes or “opened.” Because nucleosome-depleted DNA is permissive to transcription factor binding, the reverse process of “closing” or “decommissioning” enhancers is equally important in preventing the inappropriate activation of genes at specific times or locations during development. Recent work in the McKay lab has found that during *Drosophila* wing development, temporal changes in chromatin accessibility are coordinated by the steroid hormone ecdysone. We have found that the ecdysone-induced transcription factor Eip93F is both necessary and sufficient to decommission enhancers. This raises important questions about how mechanistically Eip93F regulates decommissioning and what other factors are involved in this process. Here we present our ongoing work to identify the proteins and the mechanisms involved in ecdysone dependent enhancer decommissioning.

**Proper coordination of gene expression and chromatin accessibility during wing metamorphosis requires the Broad-Complex.** S.L. Nyström\textsuperscript{1,2,3,4}, C.M. Uyehara\textsuperscript{1,2,3,4}, D.J. McKay\textsuperscript{2,4} 1) Curriculum in Genetics & Molecular Biology; 2) Department of Biology; 3) Department of Genetics; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Organismal development requires proper temporal and spatial coordination of gene expression by transcription factors. A central point of control over transcription factor binding to their target sites is regulation of chromatin accessibility, where accessible binding sites are often permissive for transcription factor binding, while inaccessible sites are often a barrier to transcription factor binding. Therefore, dynamic regulation of chromatin accessibility plays a critical role in execution of the myriad gene expression programs required for proper development of cells and tissues. We have previously shown that the chromatin accessibility landscape of developing appendages changes progressively over time, but the molecular mechanisms underlying this dynamic regulation are unclear. The appendages are not in physical contact during development, and yet the chromatin accessibility landscapes change coordinately. Therefore, we hypothesized that a secreted signal may be involved. In *Drosophila* and other insects, pulses of the steroid hormone ecdysone initiate transitions between developmental stages in part by inducing expression of a suite of stage-specific primary response genes (“early genes”). Early genes encode transcription factors that have been implicated in both transcriptional activation and repression at ecdysone-responsive loci in target tissues; however, the full extent to which early genes affect transcription in response to ecdysone is unknown, as the majority of studies to date have examined effects at a small number of loci. We hypothesize that these ecdysone-induced transcription factors play a global role in regulation of temporal gene expression in-part by coordinating changes in chromatin accessibility. Here we show that the early gene, encoded by the Broad-Complex (br), provides temporal specificity to the wing transcriptional program by regulating chromatin accessibility at temporally dynamic sites between larval and pupal stages of wing development.

**Enhancer communication: Individual enhancers with non-overlapping expression patterns can modulate each other’s activity.** João Raimundo, Tyler Heist, Michal Levo, Thomas Gregor, Michael Levine Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, United States.

The developmental complexity of multicellular organisms is commonly associated with a cumulative intricacy in gene regulation. New and more complex patterns of gene expression can evolve by regulating a gene with a multiple set of enhancers. Although recent studies have acknowledged the importance of having different individual enhancers regulating the same gene, it is still unclear whether these enhancers function in a completely modular fashion or if, alternatively, they can influence each other’s activity. To address this question we use quantitative live imaging methods to analyze the transcriptional dynamics of *giant*, a *Drosophila* gap gene regulated by multiple enhancers. We investigate if the deletion of individual enhancers can affect the activity of other enhancers with non-overlapping expression patterns. *giant* becomes expressed in broad anterior and posterior domains during the early blastoderm stage of development that
later refine into two anterior stripes and one posterior broad stripe. The precise temporal and spatial dynamics of giant's intricate expression pattern is essential to correctly specify the segmentation of Drosophila embryo. The expression of giant is regulated by a set of four enhancers that span up to 10 kb upstream from giant's promoter. gt-10 enhancer, located -10kb from the promoter, is activated mainly by bicoid and specifies the anterior giant domain. The gt-3 enhancer, located -3 kb, is activated by caudal, repressed by Krüppel and regulates the expression of the posterior giant domain. The 7 kb distance between the gt-10 and gt-3 enhancers and the fact that they activate non-overlapping expression patterns under the regulation of a distinct group of transcription would suggest that their activity would be completely independent from each other.

In the present study, we show that deleting the giant anterior enhancer decreases the expression level of the posterior giant domain. Additionally, deleting the posterior enhancer increases the expression levels of the anterior giant domain and increases its activity in the central regions of the embryo during early nuclear cycles. These results suggest that individual enhancers with non-overlapping domains can influence each other activity, either through negative or positive synergy. We propose that individual enhancers are not completely modular but, instead, can communicate with each other when constrained inside the same transcription hub.

789 Dissection of the regulatory logic that underlies color vision. J. Rister¹, J. Bunker¹, D. Dewett¹, C. Poupault¹, D. Vasiliauskas² 1) UMass Boston, Boston, MA; 2) Paris-Saclay Institut des Neurosciences, Paris, France.

Color vision requires the establishment of different photoreceptor types that express specific color-sensing Rhodopsins. The growth regulator Melted and the tumor suppressor Warts play a major role in this process by controlling the mutually exclusive expression of blue-sensitive Rh5 or green-sensitive Rh6 in distinct photoreceptor subsets. Melted is expressed in one subset and promotes Rh5 expression, while warts is expressed in the other subset and promotes Rh6 expression. Melted is activated by the transcription factor Orthodenticle (Otd) and receives positive feedback from the co-activator Yorkie that acts in a complex with the transcription factor Scalloped (Sd).

Our goal is to compare the cis-regulatory logic that underlies the co-expression of melted and Rh5 in the same photoreceptor subset. We previously analyzed the proximal Rh5 promoter and identified an intronic melted enhancer that are both sufficient to drive a reporter specifically in the Rh5 subset. Similar to the proximal Rh5 promoter, the melted enhancer depends on Otd and contains two Otd motifs that are required for activation. Moreover, the melted enhancer also contains two Sd motifs that are necessary for reporter activation. Mutating a single Sd motif and a single Otd motif causes a loss of melted reporter expression, suggesting that the binding of both factors is required for the activation of melted.

Although both genes depend on Otd and Sd motifs (and the same activators), their arrangement is very compact in the proximal Rh5 promoter (within melted enhancer (within ~1kb). Another striking difference is that Rh5 contains a Rhodopsin Core Sequence (RC5) motif (TAATTAGCTC) that is required for subset-specific expression, while melted has three partial motifs that closely resemble the 5' RCS (TAATG/Q50) and another partial motif that matches the 3' RCS (TAGATTC).

Taken together, there are different solutions for terminal differentiation gene expression in blue-sensitive photoreceptors: melted and Rh5 depend on the same transcription factors and rely on similar motifs, but their arrangement is very different.

790 Caudal counter-represses Hunchback to regulate even-skipped stripe 2 expression in Drosophila embryos. Ben Vincent, Max Staller, Francheska Lopez-Rivera, Meghan Bragdon, Zeba Wunderlich, Javier Estrada, Angela DePace Department of Systems Biology, Harvard Medical School, Boston, MA.

Hunchback is a bifunctional transcription factor that can activate and repress gene expression in Drosophila development. We investigated the regulatory DNA sequence features that control Hunchback function by perturbing enhancers for one of its target genes, even-skipped. While Hunchback directly represses the eve stripe 3+7 enhancer, we found that in the eve stripe 2+7 enhancer, Hunchback repression is prevented by Caudal binding—this relationship is called counter-repression. We found evidence that this relationship is conserved by comparing predicted binding sites for Hunchback and Caudal across orthologous eve stripe 2 enhancers. These results alter the textbook view of eve stripe 2 regulation wherein Hb is depicted as a direct activator. Instead, to generate stripe 2, Hunchback repression must be counteracted by Caudal binding. These results demonstrate that correcting the current model of eve stripe 2 regulation reveals new aspects of its evolution.

791 Characterizing the regulatory network of Salvador-Warts-Hippo pathway. L. Wang¹, N. Baker² 1) Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, TW; 2) Albert Einstein College of Medicine, Bronx, NY.

Coordination of growth and patterning is essential during development to reach the right size and shape. Drosophila wing development is one of the best tissues to study such coordination. Their growth, proliferation and differentiation are regulated by several conserved signaling pathways. The Salvador-Warts-Hippo (SWH) pathway has been recognized as significant regulators for growth control. Defects in SWH pathway lead to dramatic tissue outgrowth. To further investigate the regulatory mechanisms by which SWH regulates wing growth, we analyzed the cis-regulatory elements of the expanded
(ex) gene, which is one of the best characterized SWH pathway targets. Our findings have identified separate cis-elements responsible for regulations by distinct signaling pathways, such as Notch pathway. In addition, this study will provide insights to better understand the integration of different pathways in growth control.


Zygotic genome activation is driven by the Drosophila specific transcription factor, Zielo. In zelda mutants, embryo wide defects are seen in the domains of early patterning genes. To further investigate this phenomenon, we focus on the neuroectoderm patterning gene, short gastrulation (sog). sog is expressed in a bilateral stripe beginning at nuclear cycle 11, and opposes the activity of the dorsally expressed gene dpp. Transcriptional activation of sog requires intermediate levels of the Dorsal morphogen. To evaluate Zielo's input to sog activation, we used quantitative FISH to measure sog expression in both wildtype and zelda mutant embryos. We find not only is the domain narrowed across the D/V axis in mutant embryos, but also the defined edge on the dorsal side of the lateral stripe is lost. To understand this Zelda-dependent effect, we turned to live imaging using the MS2-MCP system to detect nascent transcripts in real time. We created two transgenic MS2 reporter constructs driven by the sog shadow enhancer, with one enhancer lacking Zelda binding motifs. Surprisingly, the rate of transcription was unaffected as assessed by MCP foci intensity, however, the timing of transcriptional activation was significantly delayed in a Dorsal concentration dependent manner. By integrating the rate of transcription in a position dependent manner, we accurately recover both the spatial domains and intensity profiles of the wildtype and zelda mutant sog expression pattern. These data demonstrate that our transgenes are accurately reporting on our observed phenotypes, and suggest that Zielo is capable of boosting transcription not by increasing the rate of steady-state transcription but by coordinating the timing of patterning across wide transcriptional domains.

793 Determination of EGFR Signaling Output by Opposing Gradients of BMP and JAK/STAT Activity. S. De Vito, M. Fregoso Lomas, J.F. Boiclair Lachance, L. Nilson 1 1) Department of Biology, McGill University, Montreal, Quebec, CA; 2) Ben May Department for Cancer Research, University of Chicago, Chicago, Illinois, USA.

Localized activation of EGFR signalling determines the anterior-posterior (AP) and dorsal-ventral (DV) axes of the ovarian follicular epithelium. In early stages, EGFR signalling is restricted to the posterior and patterns the AP axis by inducing expression of T-box transcription factors Midline and H15 (Mid/H15). In later stages, EGFR signalling shifts to the anterior where it patterns the DV axis by inducing expression of the homeobox transcription factor Mirror (Mirr). We recently showed that these differential EGFR signalling outcomes depend on spatially localized input from other signalling pathways. At the posterior, localized production of Unpaired, which activates the JAK/STAT signalling pathway, cooperates with EGFR signalling to induce expression of Mid and H15; JAK/STAT signalling in these cells also independently represses Mirr. In the anterior, localized Dpp cooperates with EGFR signalling to induce expression of Mirr; Dpp signalling in these cells also independently represses Mid/H15. Moreover, we showed that expression of Mid/H15 and Mirr is regulated via their mutual repression. Together these data define a regulatory network in which the choice between these two alternative EGFR targets is defined by input from Dpp and Upd and stabilized by their mutual repression.

Our data suggest that expression of mid/H15 and mirr is regulated at the level of transcription, but whether these multiple regulatory inputs are integrated at the level of the mid/H15 and mirr regulatory elements remains unclear. Here we identify genomic regions from the mid/H15 and mirr loci that reproduce their endogenous expression pattern in the follicular epithelium, suggesting that they contain cis-regulatory modules (CRMs) that control the patterning of this tissue. Focusing first on posterior outcomes, we show that, like endogenous mid/H15, the putative mid/H15 CRM is positively regulated by JAK/STAT signalling. Conversely, the putative mirr CRM, like endogenous mirr, is negatively regulated by JAK/STAT signalling. The identification of these elements will allow us to test whether they interact directly with the effector transcription factors of the regulatory inputs we have defined genetically. Such a direct interaction would define a simple regulatory circuit in which positional information from multiple localized extracellular signals is integrated directly by the mid/H15 and mirr CRMs to generate a bistable choice between alternative EGFR signalling outcomes.

794 The Holes in Muscles Gene is a contributor to myogenesis and muscle development. S. McKitrick Biology, University of New Mexico, Albuquerque, NM.

Understanding the regulatory mechanisms involved in myogenesis and the control of these developmental systems is of crucial importance when attempting to comprehend the processes which drive developmentally derived diseases. We generated a null mutant of the Him (Holes in Muscles) gene to better understand its contribution to myogenesis. Him is a myogenic repressor gene that was previously shown to inhibit Myocyte enhancer factor-2 (MEF2) activity, and is expressed in myoblasts but not differentiating myotubes. Through this inhibition of MEF2 Him additionally is predicted to acts as a block on cell differentiation and proliferation. Using a line of CRISPR-Cas9 (III) flies and a Him sgRNA targeting plasmid, we successfully obtained a knockout mutant caused by a frameshift mutation. The Him mutant is able to persist in the homozygous state but shows distinct differences in muscles morphology leading to a reduced ability or inability to fly, as well as a lessened ability to jump. Using fluorescent staining of muscle sections we have observed that the jump muscle has a
distinctly different phenotype than observed in yw control flies. Ongoing research is being currently performed to further classify this mutation. This data helps to provide insight into the mechanisms of cell development and the role Him plays as a possible regulatory agent of cell differentiation.

795 The m^A pathway facilitates sex determination and neuronal function in Drosophila. I. Kan^1, J Vedanayagam^1, C Lin^1, V Despic^1, B Joseph^1, E Lal^1, D Pati^2, Y Huang^3, S Kondo^4, N Pang^1 1) Sloan-Kettering Institute, New York, NY; 2) Weill Medical College, WMC, New York, NY; 3) Florida State University, FSU, Florida, FL; 4) National Institute of Genetics, NIG, Shizuoka, Japan.

N^6-methyladenosine (m^A) has recently emerged as a widespread and conserved RNA modification that modulates messenger RNA (mRNA) processing and activity. The m^A writers, erasers and readers comprise a system for epitranscriptomic regulation, akin to chromatin-based epigenetic systems. The existence of differential transcript methylation indicates target specificity, and supports regulatory roles for m^A. Diverse functional consequences of m^A and/or m^A factors have been reported, including by promoting mRNA splicing, affecting RNA structure, facilitating mRNA degradation, promoting expression and/or canonical translation, and promotion of cap-independent translation.

We used miCLIP sequencing to map m^A modifications during Drosophila embryogenesis, and characterize Drosophila components of the m^A "writer" methyltransferase complex [METTL3, METTL14, FL(2)D and Nito], and validate m^A-binding properties of the YTH domain-containing proteins, YTHDC1 and YTHDF. We also used CRISPR to generate a panel of mutants in the m^A pathway. While m^A factors with additional roles in splicing are lethal, m^A-specific mutants are viable but present certain developmental and behavioral defects. While our mutants in dedicated writer and reader machinery are not required to accumulate Sxl protein in the ovary, maternal loss of m^A pathway components collaborates with reduction of Sxl or Sxl-regulating factors to induce female lethality. Notably, we found that m^A facilitates the master female determinant Sxl, since multiple m^A components enhance female lethality in Sxl sensitized backgrounds.

We are currently examining the basis of neural-related defects in m^A mutants, which are common to both sexes. To do so, we are hope to integrate genomic data (including gene expression data from mutants and CLIP-seq data of the readers) with genetic analysis of mutants to understand specific neural circuits and transcripts that are affected by m^A regulation.

796 Tools to Regulate Gene Expression in Drosophila. T. Jacobsen, G. Ying, P. Archer, C. Beisel, G. Reeves Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.

Tools for regulating gene expression have been widely developed for single-celled organisms, such as bacteria and yeast. However, these genetic tools are currently limited or have yet to be established in more complex organisms. Here we describe self-cleaving hammerhead ribozymes; catalytic RNA constructs that can undergo a phosphodiester cleavage reaction, resulting in mRNA cleavage and degradation, thus reducing gene expression. To implement fine-tuning capabilities for this genetic tool, various "competing sequences" can be cloned upstream of the ribozyme. Depending on thermodynamics, these "competing sequences" can interact with a major stem of the ribozyme through Watson-Crick base pairing, thus allowing for various conformational changes of the ribozyme's secondary structure. Since the cleavage activity of the ribozyme is largely dependent on the kinetics of the cleavage reaction, as well as the thermodynamics of the "competing sequences", the level of gene expression can be predicted and tuned using various sequences.

Multiple ribozyme constructs were cloned either upstream or downstream of gfp, which was under the control of a constitutive CMV promoter. These constructs were transiently transfected into HEK293T cells. The addition of various "competing sequences" resulted in variable expression of GFP, but also appeared to interfere with its translational activity. Future work involves assessing these tools in vivo in Drosophila.

797 Translational regulation during embryogenesis by the Bin3 RNA methyltransferase. Ryan Palumbo, Steven Hanes Biochemistry and Molecular Biology, Upstate Medical University, Syracuse, NY.

Non-coding RNAs (ncRNAs) are critical effectors of gene regulation, from transcription to translation. The 7SK ncRNA is present in humans (332 nt) and Drosophila melanogaster (444 nt). 7SK acts as a scaffold, and forms a snRNP with the proteins MePCE, Larp7, and HEXIM. This snRNP sequesters Positive Transcription Elongation Factor b (P-TEFb), preventing it from phosphorylating the carboxy-terminal domain (CTD) of RNA Polymerase II (RNAPII), which in turn inhibits transcription elongation.

Our lab originally identified the Drosophila ortholog of MePCE, Bin3, in a screen for proteins that interact with the developmental morphogen Bicoid (Bcd). Bcd is both a transcriptional activator, and direct translational repressor of caudal (cad) mRNA. We have shown that during early embryogenesis, Bin3, Bcd, and 7SK form a novel RNP that represses translation of cad. Bin3 is required for stabilization of 7SK, and for the affinity of Bcd for cad mRNA. Loss of bin3 eliminates 7SK RNA, and disrupts proper head development due to loss of translational repression of cad at the anterior of the embryo. Thus, Bin3 plays dual roles in transcription and translation, forming different RNPs to carry out these functions.

We suspect that Bin3 has additional targets beyond 7SK and cad. To this end, we have used CRISPR to FLAG-tag endogenous bin3, with the goal of identifying ncRNAs and mRNAs that interact with Bin3. We are particularly interested in
whether Bin3 is required for the stabilization of ncRNAs in addition to 7SK, and if stability is dependent on the methyltransferase function of Bin3. We are also interested in determining whether Bin3 is required for the translational regulation of miRNAs in addition to cad. For this purpose, we are generating new bin3 deficiencies for use in translating ribosome affinity purification (TRAP), to determine whether miRNAs that co-immunoprecipitate with Bin3, are also translationally regulated by Bin3. Identification of new ncRNA and mRNA targets will give insight into the role of Bin3 during development.

798 miRNA regulation of dacapo expression in the Drosophila embryo. C.I. Swanson, Emily Custer, James Petley Biology, Arcadia University, Glenside, PA.

Cyclin-dependent kinase inhibitors (CKIs) play important roles in embryonic development by directly inhibiting the cell cycle machinery to promote cell cycle exit and limit tissue growth. The Drosophila CKI Dacapo (Dap) contributes to normal cell cycle exit in multiple developmental contexts, including the embryonic epidermis, nervous system, and eye. Because of its function as a potent cell cycle inhibitor, altered Dap expression can significantly disrupt normal development. For example, in the embryonic epidermis, premature Dap expression can induce early cell cycle exit, while the absence of Dap expression leads to delayed cell cycle exit and inappropriate cell division. Thus Dap expression must be carefully controlled to ensure normal tissue growth and differentiation. Indeed, multiple mechanisms have been shown to contribute to the regulation of Dap expression, including transcriptional regulation via a complex cis-regulatory region, miRNA-mediated translational inhibition, and regulated proteolysis via interaction with the CRL4Cas ubiquitin ligase. We have further examined the role of miRNA-mediated regulation of Dap expression in the embryonic epidermis. We have found that a dap transgene expressed under the control of the endogenous cis-regulatory region but lacking the endogenous 3'UTR is expressed prematurely in the embryonic epidermis. In addition, miRNA "sensor" transgenes that include the dap 3'UTR are repressed in comparison with controls. Finally, embryos lacking expression of miRNAs known to bind the dap 3'UTR exhibit premature dap expression, altered expression of cell cycle markers, and reduced survival. Future experiments will continue to explore the functional significance of miRNA-mediated repression of Dap expression in the embryonic epidermis.

799 miR-31b regulates adult muscle development in Drosophila melanogaster. D.Lloyd. Wilson, T. Dohn, R. Cripps Biology, University of New Mexico, Albuquerque, NM.

Micro-RNAs (miRNAs) are post-transcriptional regulators essential to muscle development and homeostasis. These small, non-coding RNA molecules prevent maturation of protein-coding genes by inhibiting transcription or mediating mRNA degradation in a sequence-specific manner. Previous research has linked miRNAs to both muscle development and maintenance, however the roles of many miRNA are unknown. We screened four miRNAs previously suggested to be important in myogenesis for adult muscle functional defects. Further studies then focused on miR-31b due to significant developmental defects during adult muscle formation. We used the UAS/Gal4 system in Drosophila to over-express and under-express miR-31b in the adult muscle. Functional tests measuring flight and jump ability showed decreased muscle function in both over-expression and under-expression adults as determined by weak or absent flying and reduced jump distance at 10 days after eclosion. Over- and under-expression individuals were cryosectioned at 16h, 24h, 48h, and 96h after puparium formation (APF) to observe muscle development and organization. Sections were fluorescent antibody stained and displayed significant developmental defects in muscle fiber organization, including visible holes in the indirect flight muscle (IFM) myofibrils. Computer analysis using multiple miRNA target prediction databases revealed potential target genes regulated by miR-31b during muscle development. Strong gene targets such as sbd, ewg, and CG16947 were identified by being present on at least three miRNA target prediction databases. This list of target genes was further refined by the analysis of expression patterns reported in literature. Quantitative PCR (qPCR) will confirm these potential molecular interactions, illuminating the role that miR-31b plays in influencing proper muscle development and function in Drosophila melanogaster.

800 Temperature regulated silencing of genomic repeats. E. Wood, J. Artus, J. Brask, M. Kalliomaa, C. Keago, V. Krawiec, D. Petrus, E. Reiss, S. Tracy, A. Arsham Biology, Bemidji State University, Brookyn Park, MN.

Eukaryotic genomes protect themselves against invasive mobile DNA using a wide range of mechanisms both before and after genomic insertion. Approximately half of the sequence of the human genome is thought to be made up of the remnants of invasive repetitive DNA deposited over the course of evolution. These mobile elements and their movements within and across genomes increasingly appear to play a driving role in evolution, but germline transposition events are typically deleterious for an individual organism and are silenced by targeted heterochromatin formation. We use a variagated white+ reporter construct containing roughly 10kb of bacterial lacO repeats to investigate the silencing of repetitive genomic sequences in Drosophila melanogaster. P-element-mediated transposition inserted the reporter within the nesd gene on chromosome 2L in a gene-rich region characterized by high mRNA expression, euchromatic histone marks, and low repeat density. Despite being located in a genomic region predisposed to gene expression, w+ transgene expression in the adult eye of flies reared at 25°C is predominantly silenced in a repeat-dependent fashion, concomitant with heterochromatin protein 1a binding. Surprisingly, rearing larvae at 18°C suppresses variegation in the adult eye and leads to w+ expression approaching that of wild type flies. Temperature-dependent regulation of variegation persists throughout larval and early pupal
Identifying chromatin modifiers that regulate stochastic spineless expression. L. Yuan, K. Viets, R. Johnston

Proper development depends on many regulatory pathways that generate cell fates in a reproducible manner. Certain developmental pathways utilize stochastic mechanisms to further diversify cell types. While much is known about reproducible developmental pathways, stochastic cell fate specification is less well understood. In the fly, stochastic expression of the transcription factor Spineless (Ss) in 67% of R7 photoreceptors activates expression of the photopigment Rhodopsin 4 (Rh4) and represses (Rh3). The ss locus contains two putative Polycomb Response Elements (PREs), and we found that deletion of these elements caused an increased in ss expression frequency. In addition, modENCODE data predicted that ss is marked with H3K27me, indicative of chromatin repression. Since chromatin state regulates stochastic ss expression, we performed an RNAi screen against chromatin modifiers, including components of the Trithorax (Trx) and Polycomb Group (PcG) complexes. We identified two genes, menin1 (mnn1) and ash2 (ash2), which are required for wild type ss expression. Knocking down mnn1 or ash2 significantly decreases ss frequency, suggesting that Mnn1 and Ash2 are activators of ss. Mnn1 and Ash2 are Trx group proteins and may contribute to H3K4 methyltransferase activity at the ss locus, potentially counteracting the spread of repressive heterochromatin to determine stochastic ss expression.

Predicting microRNA targeting efficacy in Drosophila. V. Agarwal1,2,3,4, A. Subtelny1,2,6, P. Thiru1, I. Ulitsky3, D. Bartel1,2 1) Whitehead Institute for Biomedical Research and Howard Hughes Medical Institute, Cambridge, Massachusetts 02142, USA; 2) Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA; 3) Computational and Systems Biology Program, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA; 4) Present address: Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA; 5) Department of Biological Regulation, Weizmann Institute of Science, Rehovot, 7610001, Israel; 6) Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts 02139, USA.

Important for understanding the regulatory roles of miRNAs is the ability to predict the mRNA targets most responsive to each miRNA. Here, we acquired datasets needed for the quantitative study of microRNA targeting in Drosophila. Analyses of these data expanded the types of sites known to be effective in flies, expanded the mRNA regions with detectable targeting to include 5′ UTRs, and identified features of site context that correlate with targeting efficacy. Updated evolutionary analyses evaluated the probability of conserved targeting for each predicted site and indicated that more than a third of the Drosophila genes are preferentially conserved targets of miRNAs. Based on these results, a quantitative model was developed to predict targeting efficacy in insects. This model performed better than existing models and will drive the next version of TargetScanFly (v7.0; targetscan.org), thereby providing a valuable resource for placing miRNAs into gene-regulatory networks of this important experimental organism.

Expanding the Drosophila melanogaster pigmentation gene regulatory network to include post-transcriptional regulation by microRNAs. A. M. Lamb1, J. A. Kennell1, P. J. Wittkopp1,2 1) Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI; 2) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 3) Biology, Vassar College, Poughkeepsie, NY.

Pigmentation within the genus Drosophila is a fruitful model system of development and evolution. Decades of intense study of Drosophila pigmentation have provided an increasingly well-characterized developmental genetic network rich with many empirically identified cis-regulatory sequences and transcriptional regulatory connections, many of which are at the center of case studies of regulatory evolution. While great effort has gone into the identification of transcriptional regulators of pigmentation genes, very little is known about the role of post-transcriptional regulation in this model system. We conducted a screen of 172 microRNAs to identify those which may be involved in the regulation of Drosophila pigmentation. Here we present the results of this screen, as well as follow-up experiments on a subset of identified microRNAs that affect pigmentation. This set of experiments indicates a previously unappreciated role of post-transcriptional regulation in the development of Drosophila pigmentation. Expanding the pigmentation regulatory network to include microRNAs may allow Drosophila pigmentation to serve as a useful model system of the development and evolution of post-transcriptional regulatory networks.

Evolutionarily conserved transcription factor binding sites and S2 cell occupancy as context-specific gene regulatory network priors. I. Berger1, J. Fear1, Y. Wang2, H. Lee1, T. Przytycka2, B. Oliver1 1) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 2) Computational Biology Branch, National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD.
We use gene regulatory networks (GRNs) to examine the effects of genetic (e.g. RNAi) and/or environmental (e.g. drug treatment) perturbations of gene expression. Although Schneider 2 (S2) cells are a popular cell line for D. melanogaster, we do not have a reliable S2 cell specific GRN. We can build GRNs with Network Rewiring using Expression (NetREX) from expression profiles and a prior, which is a set of weights that quantifies the expected relationships between transcription factors and the genes they regulate. Our previous work has shown that the quality of the prior directly influences the resulting GRN. We therefore strive to obtain a precise S2 cell prior network model by incorporating context independent sequence data to map transcription factor binding sites (TFBS) in D. melanogaster and evolutionary conservation of those sites elsewhere in the genus. Position weight matrices for over 300 transcription factors were used to map putative motif binding sites. Because compiling a reliable list of TFBS is impeded by the tendency of these sequence motifs to be very short and therefore more likely to occur by chance, we devised a metric to indicate likelihood that a transcription factor/gene relationship exists by weighting conserved motifs. We added context specific information through use of all publically available S2 cell transcription factor ChIP-Seq data from modENCODE and the Sequence Read Archive (SRA). Here the presence of a transcription factor's ChiP-Seq “peaks” in a gene suggests a relationship. We used a standardized workflow to download and post-process peak calls, including lifting over to the current FlyBase release, intersecting gene information, and removing previously published phantom peaks. We used the presence of a peak as a binary indicator of the existence of a transcription factor/gene relationship. The integration of putative binding sites and S2 cell specific occupancy into our NetREX model is crucial for building predictive, context specific GRNs. We have developed a generic transcription factor network model based on motifs, conservation, and occupancy that will allow more accurate inference of the S2 cell regulatory network.

805 Exploring the universal mechanism of feedback regulation of gene activity in Drosophila. D. Kitamura, M. Nakamura, T. Igaki Laboratory of Genetics, Graduate School of Biostudie, Kyoto University, Kyoto, Japan.

The balance between gene expression activity and its product level needs to be strictly regulated. For instance, in the tryptophan operon of E.coli or Hes7 expression in mammals, negative feedback regulation of gene activity is achieved by its own product. However, whether and how gene products universally regulate their gene activities still remains unknown. Here, we found a possible universal mechanism of negative feedback regulation of gene activity in Drosophila. Ectopic overexpression of a ribosomal protein RpS20 in a part of the wing disc strongly repressed the endogenous RpS20 gene expression activity. Similarly, ectopic overexpression of RpS3 resulted in strong suppression of the endogenous RpS3 gene activity. Importantly, overexpression of RpS3 did not suppress the expression of endogenous RpS20 gene, suggesting the existence of a specific feedback regulation of gene activity by its product. To understand the mechanism underlying this feedback regulation, we conducted a genetic screen in Drosophila wing discs for genes required for the negative feedback regulation of the RpS20 gene caused by overproduced RpS20. As a result, we isolated an EMS-induced mutant line defective in the feedback regulation activity. We are currently analyzing the mutant phenotypes and trying to identify its responsible gene, which will be discussed.

806 Determining the function of the transcription factor Zelda in driving neural stem-cell fate. Elizabeth Larson1, Danielle Hamm1, Hideyuki Komori2, Cheng-Yu Lee2, Melissa Harrison1 1) Biomolecular Chemistry, University of Wisconsin at Madison, Madison, WI; 2) Cell and Developmental Biology and Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Stem cells are unique in that through asymmetric divisions they are able to both self-renew and generate differentiated progeny. Maintaining the precise balance between self-renewal and differentiation is necessary for proper development and when misregulated can lead to tumor formation. Transcription factor regulatory networks are essential for controlling stem-cell fate and differentiation following asymmetric division. The mechanism by which transcription factors control this balance remains unclear. We and others have demonstrated that Zelda (ZLD), a zinc finger transcription factor, governs cell fate in the early Drosophila embryo by activating the zygotic genome to initiate embryonic development. ZLD activity must be precisely regulated, as too much or too little is lethal to the embryo. ZLD is also expressed in the developing central nervous system. We showed that in the larval brain ZLD expression is limited to the neural stem cells (neuroblasts) and that overexpression of ZLD leads to increased neuroblast formation. These data suggest that ZLD drives self-renewal and contributes towards stem-cell maintenance in neuroblasts. Misexpression of ZLD in the partially differentiated progeny of the neuroblasts reprogrammed these cells back to a stem-cell like fate. Thus, similar to the early embryo, ZLD activity must be precisely regulated during neuronal differentiation such that it can be rapidly inactivated following asymmetric division. Data suggest that Brain tumor (Brat) may regulate ZLD levels both in the early embryo and in the neural stem cell lineage. Thus, common regulatory mechanisms may control the dramatic changes in cell fate that occur during these two stages of development. To advance our understanding of how transcriptional and post-transcriptional mechanisms are coordinated to precisely regulate changes in cell fate, we are investigating the role of ZLD and the regulation of ZLD activity both in the early embryo and larval neural stem cells.

807 Cooperative recruitment of Yan to paired high affinity ETS sites organizes repression to confer specificity and robustness to cardiac cell fate specification. J.-F. B. Lachance, J.L. Webber, I. Rebay Ben May Department for Cancer Research, University of Chicago, Chicago, IL.
Cis regulatory elements (CREs) are defined by unique combinations of transcription factor binding sites. Emerging evidence suggests that the number, affinity and organization of sites play important roles in regulating enhancer output and ultimately gene expression. Here, we investigate how the cis-regulatory logic of a tissue-specific CRE responsible for even-skipped (eve) induction during cardiogenesis organizes the competing inputs of two ETS members, the activator Pointed (Pnt) and the repressor Yan. Using a combination of reporter gene assays and CRISPR-Cas9 gene editing, we show that Yan and Pnt have distinct preferences for affinity of sites. Not only does Yan prefer high affinity sites, but a tandem pair of such sites is necessary and sufficient for Yan to tune Eve expression levels in newly specified cardioblasts and to block ectopic Eve induction and cell fate specification in surrounding progenitors. Mechanistically, the cooperative Yan recruitment promoted by this conserved high affinity ETS pair not only biases Yan-Pnt competition at the specific CRE, but also organizes Yan repressive complexes in 3D across the eve locus. Taken together our results uncover a novel mechanism by which differential interpretation of CRE syntax by a competing repressor-activator pair can confer both specificity and robustness to developmental transitions.

808 Deciphering the role of cis-regulatory elements in stochastic gene expression. J. Lu Biology, Johns Hopkins University, Baltimore, MD.

Stochastic mechanisms play a crucial role in cell fate specification. The fly retina provides an excellent model for studying the mechanism underlying stochastic gene regulation. The Drosophila retina consists of 800 ommatidia, and each ommatidium comprises eight photoreceptor cells, named R1-R8. The R7 photoreceptor randomly adopts one of two cell fates by expressing one of two Rhodopsin proteins: Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). The transcription factor Spineless (Ss) determines the expression of these two Rhodopsins. The presence of Ss induces the expression of Rh4, whereas the absence of Ss induces the expression of Rh3. Ss is expressed in a random subset of R7 cells, providing a simple binary output ideal for studying stochastic gene expression. To understand the mechanism underlying stochastic ss regulation, we have generated a series of CRISPR deletions across the ss locus to screen for cis-regulatory elements that govern its expression. From our analysis so far, we have found that deletion of a promoter region, an intronic element, and an upstream element cause a complete loss of ss expression. Furthermore, deletion of two upstream repressive Polycomb Response Elements (PREs) leads to derepression of ss. These results suggest that a complex cis-regulatory logic incorporating multiple activating and repressing DNA elements determine stochastic expression of the ss gene.

809 cis-regulatory architecture of an EGFR organizing center in Drosophila melanogaster distal leg. R. Voutev1, S. Newcomb2, A. Jory1, R. Delker1, M. Slattery1, R. Mann1 1) Department of Biochemistry and Molecular Biophysics and Department of Systems Biology, Columbia University, New York, NY, USA; 2) Department of Biological Sciences, Columbia University, New York, NY, USA; 3) Institute for Stem Cell Biology and Regenerative Medicine, National Center for Biological Sciences, Bangalore, India; 4) Department of Biomedical Sciences, University of Minnesota. Duluth, MN, USA.

cis-regulatory modules (CRMs) that control the expression or activity of signaling molecules coalesce the information that a cell receives and have the potential to trigger responses that modify the fates of entire tissues. Signaling hubs that pattern developing organs are called organizing centers. We elucidated the mechanism that governs the establishment of an Epidermal Growth Factor Receptor (EGFR) organizing center (EOC) in Drosophila melanogaster leg imaginal discs. We find that EGFR activation in leg discs of third instar larvae occurs by sequential activation of the EGFr ligand Vein (Vn) and the EGF ligand-processing protease Rhomboid (Rho). Through differentially regulated CRMs. Each CRM integrates in a distinct manner the inputs from the Wingless (Wg) and the Decapentaplegic (Dpp) signaling pathways as well as from the leg selector transcription factors Distal-less (Dll) and Sp1 (in the case of the vn enhancer). Elimination of individual vn and rho EOC enhancers vnE and rhoE, respectively abolishes the expression of these genes in the center of leg discs. A vnE rhoE double deficiency, but not the single deletions, exhibits loss of expression of EGFr downstream genes and loss of distal leg fates, suggesting an absolute requirement of the two CRMs for EOC activity. In addition, the cis-regulatory logic of vnE and rhoE transceeds the leg EOC developmental program because we identified genomic regions with similar features, based on genome-wide binding and sequence data, and faithfully predicted co-regulated CRMs in the fruit fly distal leg disc. The combinatorial input of Wg, Dpp, Dll and Sp1 on vnE and rhoE reveals a combinatorial mechanism for coordinating gene expression that might be applicable to many other multi-cellular systems.

810 Profiling the Effects of Endogenously Expressed Fat Intracellular Domain Mutants on the Wing Disc Transcriptome. D. Potter1, Y. Qu1, M. Wilson1,2,3, H. McNeill1,2,3 1) Division of Biology and Biomedical Science, Washington University in St. Louis, St. Louis, USA; 2) The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada; 3) Department of Molecular Genetics, University of Toronto, Toronto, Canada; 4) Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Canada; 5) Heart and Stroke Richard Lewar Centre of Excellence in Cardiovascular Research, Toronto, Canada.

The large Drosophila atypical cadherin Fat (Ft) is a key regulator of tissue growth and planar cell polarity (PCP). Ft is genetically upstream of the Hippo signaling pathway, dysregulation of which is shown to be the cause of overgrowth in Ft mutants. Despite overgrowth, larvae show developmental delay which is proposed to be a consequence of the recently
described Ft intracellular domain (Ft-ICD) role in the regulation of mitochondrial function. In Ft mutant wings, tissue polarity orthogonal to the cellular apical-basal axis is seen to be disrupted; a disruption manifested as aberrant orientation of hair and cuticle membrane ridges and altered wing vein spacing. Extensive genetic and biochemical analysis has helped to illuminate downstream targets and regulators of Ft both in a Hippo and PCP context. With a steadily increasing number of interacting proteins and post-translational modifications described, comprehensive interpretation of the molecular mechanisms of Ft is complex and incomplete. Further, whilst multiple regions are conserved in human the homologue Fat4, Ft-ICD harbors no predictable structural domains. Multiple structure-function analyses have helped to define regions that give rise to particular growth and PCP phenotypes in particular tissues, but no study to date has evaluated Ft mutants from a transcriptomics perspective. Using RNA-Seq of CRISPR generated Ft-ICD mutants, both deletions and truncations, we looked to further understand the cellular response to Ft-ICD mutation. We present a comprehensive analysis of the wing disc transcriptome in response to 9 distinct Ft-ICD mutants in an endogenous context. We summarize changes across the transcriptome, expanding on current annotations of phenotypic regions with clustered GO term analysis and reveal novel gene expression changes and splicing events in response to Ft-ICD mutations.


Olfaction relies on the proper expression of olfactory receptor (OR) genes in olfactory receptor neurons (ORN) and the correct transmission of signals to the brain. Individual ORNs make two important choices: they chose to express one OR gene from a large family of ORs and project their axons to a particular target region in the brain. We focus on the Drosophila olfactory system because of its relative simplicity. ORNs in Drosophila are housed in sensory hairs called sensilla located in two olfactory organs, the antenna and the maxillary palp. Each sensillum contains 1-4 ORNs. It has been shown that after initial patterning sensilla subtype diversification and the expression of particular OR genes depends on a regulatory code that is governed largely by transcription factors. Our studies focus on the function of a transcription factor gene family, Iroquois complex (IroC), in ORN specification. The Iro family includes three highly conserved genes, namely araucan (ara), caupolican (caup), and mirror (mirr). Previously, we have shown that these transcription factors regulate the co-expression of two rhodopsin genes in the fly retina. It is also known that these genes are important for the proper targeting of photoreceptor axons. In the present study, we show that IroC genes are expressed in ORNs. In the maxillary palp iroC is expressed in the pb2 subset of ORNs, whereas in the adult antenna expression appears more distributed. We perform lineage-tracing experiments to define the iroC-positive ORN subset. Initial loss-of-function and gain-of-function experiments show that Iro proteins are involved in the regulation of OR gene expression. To define iroC target genes in the olfactory system we use a genome-wide transcriptome analysis approach. We have performed RNASeq analysis on antenna and maxillary palps using triple mutant flies and define differentially expressed genes. In addition, to investigate these genes individually we have generated single mutants for each iroC gene using CRISPR/Cas technology and are in the process of performing RNASeq analysis for these mutants. We will present our results of these transcriptome studies and the role of IroC genes in OR gene regulation.


Current inducible expression systems preclude accurate spatiotemporal control of gene expression and involve steroid hormones, antibiotics, heavy metals, or heat shock, which can induce toxicity or pleiotropic effects. What if transgene expression could be rapidly activated or immediately reversed with a switch triggered by light? Here, we present the generation and implementation of a genetically encoded light-dependent expression system in Drosophila based on the fast photoactivation of Light Oxygen Voltage domain (LOV2) from Avena sativa phototropin 1. In response to blue light, LOV2 domain undergoes a conformational change that exposes a NLS allowing translocation of the system to the nucleus triggering a cascade of signal transduction events. Our hypothesis is that this tool will serve as a high-resolution device to sculpt gene expression in Drosophila with agile on-off control and with extraordinary precision and resolution.

813 Step-by-step evolution of Bicoid's target specificity. Pinar Onal1, Rhea Datta1, Qinwen Liu2, Julia Rogers4, Urs Schmidt-Ott3, Martha Bulyk2, Joe Thornton1, Stephen Small1 1) Biology, New York University, New York, NY; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL; 3) Department of Human Genetics, University of Chicago, Chicago, IL; 4) Department of Genetics, Harvard Medical School, Boston, MA; 5) Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL.

Understanding how animal body plans evolve is a central goal of biological research. Genetic mapping strategies using closely related species have identified mutations in cis-regulatory elements that cause evolutionary changes such as pigmentation and trichome patterning in Drosophila, coat color pattern in mice or branching size in maize. But major changes in body plans among distant taxa originated in the deep evolutionary past and may have occurred by different mechanisms, including the appearance of novel genes and protein neo-functionalization. Here we combine ancestral protein
reconstruction with biochemical and in vivo gene replacement assays to explicitly test how the homeodomain (HD) protein Bicoid (Bcd), a product of a recent gene duplication, evolves as an instructive morphogen that patterns the anterior body plans of Drosophila melanogaster and other flies. Our results suggest a time interval during which this evolution occurred, and identify a single historical substitution in the ancestral HD that is sufficient to shift its DNA-binding preferences detected using Protein Binding Microarray (PBM). When tested in a gene replacement assay in Drosophila, however, Bcd protein carrying ancestral HD with this single substitution fulfills only a subset of Bcd’s regulatory functions. Adding several other substitutions in DNA-binding helix of the HD does not affect the ancestral HD’s in vitro binding preferences, but gradually increases its in vivo activities. Furthermore, we observed that distinct sequence changes in the HD result in similar phenotypes. Together, these results highlight stepwise modification of a protein as a powerful mechanism for driving body plan evolution and suggest that Bcd HD has a modular structure, which might be detrimental in the rapid evolution and divergence of target specificity of Bcd HD and HDS in general.

814 Drosophila third instar larval testes transcriptome study by single cell RNA sequencing (scRNA-Seq). S. Mahadevaraju1, J. Fear1, M. Akeju2, B. Oliver1, E. Matunis2 1) NIDDK, NIH, Bethesda, MD; 2) Johns Hopkins University School of Medicine, Baltimore, MD.

The Drosophila testis consists of several different cell types, including somatic cells (mesodermal origin) and germ cells (primordial germ cell origin). Although each cell is genetically identical, they adopt distinct cell fate through regulating specific genes they express. We are interested in understanding expression profile of different cell types at a single cell level that allowing us to identify the different cell populations that comprise the testis. We adapted the 10X genomics platform to capture individual cells and performed single cell transcriptome analysis of late third instar larval testis cells. We captured and sequenced 483 cells from dissociated testes and identified seven distinct cell clusters using Seurat. To determine if these clusters represent distinct cell populations in testes, we examined each cluster for genes known to be expressed in a distinct cell type. We found that each distinct cluster is comprised of transcripts known to be specific to a particular testis cell population, with some clusters appearing to be made up of more than one specific cell type. For example, we found gbb (expressed in Cyst Stem cells) expressed in cluster 2 and sox100B with emc (expressed in pigment cells) only expressed in cluster 5. Importantly, different cell types were not only clustered based on spatially defined patterns of expression in testes, but also based on the temporal pattern of differentiation within a lineage. For example, we found both phf7 and tej (spermatogonia and early spermatocytes) in the early germ cell cluster 3, but found sunz (spermatids) in a distinct late germ cell cluster 4. Finding seminal genes expressed in specific cell clusters suggest that scRNA-Seq is a sensitive method to distinguish different cell populations based on their expression profile. We validated scRNA-Seq expression profiles by comparing to targeted DamID (TaDa), a cell type specific expression profiling method. DamID uses a DNA adenine methyltransferase fused to a Pol II to methylate adenines of actively transcribed genes. We drove expression of this Pol II Dam fusion protein using a Gal4 system in a cell type specific manner, in somatic stem cells and early somatic cells. Comparisons between the genes identified by TaDa and the transcripts identified in cluster 2 by scRNA-Seq found that there were 164 genes captured by both the methods supporting the conclusion that we have identified distinct cell populations by scRNA-Seq. Our study identifies distinct cell types in late third instar larval testes, including both spatially distinct cell types as well as specific cell type lineages and these results show concordance with an independent cell type specific sequencing technique.

815 Positional information dependent acquisition of unique chromatin accessibility states. S.A. Blythe1, E.F. Wieschaus2 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Molecular Biology, Princeton University, Princeton, NJ.

Embryos initiate pattern formation with a largely, but not entirely, homogeneous chromatin landscape common to all cells of the blastoderm stage. Cell-type specific patterns of chromatin architecture emerge as cells acquire unique fates over the course of development. However, it is unclear which mechanisms are responsible for establishing cell-type specific chromatin signatures, and to what extent these patterns are deterministic for the acquisition of specialized cell functions.

In order to determine when such unique patterns begin to emerge in developmental time, we have performed single-embryo ATAC-seq across nuclear cycle 14 (NC14) comparing wild type embryos with those deficient for all maternal patterning inputs. Unlike wild type embryos, quintuple bicoid oskar capicua torsolike Toll (RM9) mutant embryos develop with an apparent single uniform cell identity that carries a molecular signature of posterior endodermal progenitors. We confirm that most heterogeneity in chromatin accessibility at the beginning of NC14 (at 12 minutes) is attributable to the activity of Bicoid. Approximately 20% of ATAC peaks undergo dynamic changes in chromatin accessibility over the next hour of developmental time, evenly divided between regions that gain and those that lose accessibility. These dynamic peaks are significantly over-represented for putative enhancer elements that gain accessibility over the course of NC14, and are typically not bound by known pioneer factors GAGA and Zelda. Within this set of dynamic peaks, 25% of these changes depend on positional
information, and can be attributed to influences from at least one of the maternal patterning systems. We conclude that by the onset of gastrulation, spatially restricted patterns of chromatin accessibility have begun to be established throughout the embryo in response to positional cues by mechanisms distinct from those operating at the maternal-to-zygotic transition.

816 Condensin II and Drosophila telomere clustering dynamics. V. Rana1, H. Wallace2, G. Bosco1 1) Department of Molecular and Systems Biology, Dartmouth College, Hanover, NH; 2) Department of Genetics, Harvard Medical School, Boston, MA.

Telomeric DNA protects chromosomes from loss of genetic information and is important for replication of chromosome ends. Telomeres themselves form a protective structural configuration and associate with capping proteins preventing telomeres from being recognized as double-strand breaks. Across multiple organisms, chromatin organization and telomere positioning appear to be non-random. In Drosophila, telomeres organize into distinct clusters in the nucleus during interphase. The mechanism by which telomeres cluster and the biological significance of clustering is currently unknown. We hypothesize known chromosome pairing proteins are involved in the position or clustering of telomeres. Condensin II is a well-established homologue anti-pairing factor modulating pairing and clustering of peri-centric heterochromatin. We explore telomere clustering across Drosophila cell types and find that condensin II can affect telomere clustering and organization during interphase.

817 Histone abundance changes chromatin accessibility in the early Drosophila embryo. H. Wilky, S. Chari, L. Govindan, A. Amodeo Lewis-Sigler Institute for Integrative Genomics, Princeton, NJ.

In Drosophila, the early embryo undergoes 13 rapid, synchronous divisions prior to a developmental transition known as the midblastula transition (MBT). These divisions are driven by mRNAs and proteins deposited by the mother before fertilization. During the early divisions the zygote is largely transcriptionally inactive. After 13 divisions there is an elongated 14th cell cycle before gastrulation and initiation of bulk zygotic transcription. Concurrently the chromatin landscape is reorganized with increased accessibility of promoter and enhancer regions. We investigate if the abundance of the deposited histone pool can change chromatin accessibility and transcription leading up to the MBT. Using a mutant that increases the amount of histones deposited into the embryo we found changes in global chromatin accessibility at promoter regions as well as downregulation in a small number of zygotically transcribed genes. Interestingly, in addition to altering these phenotypes the increased histone levels result in an extra nuclear cycle before gastrulation in a subset of mutant embryos. Further examination will elucidate the relationship between histone levels and chromatin accessibility as well as how histone levels influence cell cycle remodeling and transcription.

818 The chromatin remodeling protein Kismet regulates synaptic pruning by controlling steroid hormone receptor expression. Nina Latcheva1,2, Jennifer Viveiros1, Daniel Marenda1,2,3 1) Biology, Drexel University, Philadelphia, PA; 2) Molecular Cell Biology and Genetics, Drexel University College of Medicine, Philadelphia, PA; 3) Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA.

Epigenetic changes in gene expression are the interface between external and internal developmental cues. The proteins involved in these changes include chromatin readers, which recognize different histone tail modifications and help coordinate the appropriate chromatin rearrangements. A subset of chromatin readers are chromodomain proteins that are thought to recognize methylated histone tails, although their direct influence on gene expression remains largely unknown. In Drosophila melanogaster, one such chromatin reader is encoded by the kismet (kis) gene, which is homologous to the mammalian Chromodomain Helicase DNA binding (CHD) protein family, including CHD7, an ATP-dependent chromatin remodeling protein. Haploinsufficiency of CHD7 leads to CHARGE syndrome, a neurodevelopmental disorder that affects approximately 1 in 10,000 individuals worldwide. Decreased Kismet function has been shown to lead to defects in axonal guidance, synaptic pruning in mature neurons, and immediate recall memory. Here, we show that axon pruning defects are present in both kis mutants and flies expressing RNAi knockdown of kismet. Further, these pruning defects are due to a decrease in gene expression of the steroid hormone receptor Ecdysone Receptor isoform B1 (EcR-B1) in the mushroom body (MB) Kenyon cells. EcR-B1 has been well documented in initiating development of axonal rearrangements in the Drosophila MBs. Supplementing exogenous EcR-B1 rescues pruning defects in both kis mutant and knockdown flies. Additionally, the pruning defects associated with decreased kismet persist into adulthood and can be rescued by forced expression of EcR-B1. In 3rd instar larval brains, endogenous kismet is enriched at cis-regulatory enhancer sites and the transcriptional start site (TSS) of the EcR-B1 locus. Kismet was further shown to activate transcription from a specific EcR enhancer site 16.1 kb upstream of the TSS. Finally, the immediate recall memory defect in kismet knockdown animals was rescued by exogenous expression of EcR-B1 in the MBs. We therefore demonstrate that the defects associated with decreased kismet function in the Drosophila CNS are due to the misregulation of the kismet target gene, EcR-B1. This study provides greater insight into the epigenetic regulation of neuronal gene expression and is crucial to further understanding of neurodevelopmental events that lead to CHARGE syndrome.
819  **HDAC inhibitors rescue aberrant phenotypes associated with loss of chromatin reader Kismet.**  J. Viveiros¹, N. Latcheva²,³, D. Marendà¹,²,³  ¹ Biology, Drexel University, Philadelphia, PA; ² Molecular Cell Biology and Genetics, Drexel University College of Medicine, Philadelphia, PA; ³ Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA.

Histone modifications mediate epigenetic changes in gene expression by integrating external and internal developmental stimuli. The crosstalk of the proteins involved in these modifications has been the site of intense recent study. The *Drosophila* chromodomain containing protein Kismet, an ortholog of Chromodomain Helicase DNA Binding protein 7 (CHD7), is an epigenetic reader associated with the activation of transcription. Reduced expression of this epigenetic coordinator results in defects in neuroplasticity and memory. The interaction between epigenetic readers and proteins that mediate histone modifications is not well understood. Preliminary data in our lab indicates that defects associated with loss of kismet can be rescued by inhibiting histone deacetylases (HDACs). This class of enzymes acts to remove acetyl modifications from histone tails and to strengthen histone-DNA interactions. We have used a variety of behavioral assays to assess the therapeutic potential of seven specific and general HDAC inhibitors in our model of CHARGE Syndrome, a neurodevelopmental disorder caused by haploinsufficiency of the CHD7 gene. External morphology and locomotor function assays determined that suberoylanilide hydroxamic acid (SAHA) and suberyl bis-hydroxamic acid (SBHA) treatments were the most effective. Further assessment of synaptic morphology and transmission showed that SAHA and SBHA were able to mitigate the defects associated with reduced kismet. These findings provide a promising avenue for novel therapeutic treatment of CHARGE syndrome.

820  **Using photoswitchable proteins to assess chromatin stability.**  J.F. Ray, K.A. Maggert  Cellular and Molecular Medicine, University of Arizona, Tucson, AZ.

Nucleosome modifications along the genome influence local heterochromatin state. For example, methylation and acetylation patterns on the N-terminal amino acid tails of four core histone proteins forming the nucleosome octamer determine the strength of heterochromatin silencing. Varied examples of epigenetics rely on transgenerational effects and heritability of expression states between cell (mitotic) or organismal (meiotic) generations. Most of the current focus is on transgenerational inheritance of expression states determined by methylation of lysine-4 and lysine-9 sites of histone H3. Testing the nature of histone-based heritability using a protein fusion of the native *Drosophila melanogaster* histone H3 sequence with photoswitchable protein mEos2 provides an opportunity to monitor chromosome-scale histone stability, recycling, and turnover, to monitor chromosome movement in live nuclei, and to critically test histone inheritance through cell division. Photoswitching will provide a snapshot of histone behavior and elucidate the contribution of expression state modification to transgenerational effects. Recent progress in creation, validation, and analysis will be presented.

821  **Identification and characterization of inseparabile, a mutation that gives rise to compound chromosomes.**  F. Buglio  University of Arizona, Tucson, AZ.

It is hard to overstate the importance of the C(1)RM chromosome, the first compound- or attached-X. It's discovery by Lily V. Morgan (1928) allowed half-tetrad analyses of meiotic recombination products in animals, providing clear evidence of the details of strand selection during exchange. Seventy years later, Mel Green astutely reasoned that C(1)RM was not generated spontaneously, but was likely due to a mutation in the original Morgan strains, and Green and Piergentili later found and named this mutation "inseparabile" (ins) (2000). Green and Piergentili showed that the ins phenotype manifests as a high frequency of C(1)RM chromosomes from homozygous mutant males, although they did not identify the mutated locus at the time. We now report that inseparabile is an amorphic allele of the ortholog of the *S. cerevisiae* mutagen-sensitive gene MMS19. MMS19 is a component of the cytoplasmic iron assembly complex, and mutation is expected to deplete many cytoplasmic and nuclear proteins of FeS clusters in their active sites – including many with roles in DNA repair. Hence, the actual biochemical defect is likely a pleiotropic effect mediated through reduced activity of multiple iron-containing DNA metabolism genes. We will report our analysis of the ins allele of MMS19, and our investigations into the particular features of the X chromosome that renders it susceptible to compound attachment. Our analysis of ins-derived C(1)RM chromosomes further suggests that the accepted mechanism of C(1)RM formation is incorrect, and instead our data support an origin from centromere misdivision (Darlington and others, ca. 1940) followed by a chromatid-type Bridge-Fusion-Breakage cycle (B. McClintock, 1938).

822  **Driving Gene Expression in the Heterochromatic Environment of the Fourth Chromosome of *D. melanogaster.***  J. Cantrell, E. Gracheva, S.C.R. Elgin  Biology, Washington University in St. Louis, St. Louis, MO.

Genomes of higher eukaryotes can be divided into two fundamental and dynamic subtypes: euchromatin and heterochromatin. In general, genes that are active in a euchromatic environment are silenced when transposed to heterochromatin. However, heterochromatin is not devoid of actively functioning genes. The goal here is to identify regulatory elements that drive transcription of heterochromatin genes. The fourth chromosome of *Drosophila melanogaster* represents an excellent model for this study: ~80 genes within this heterochromatic domain are expressed. Insertion of an *hsp70-white* transgene (which results in a uniform red eye phenotype when present in euchromatin) into a heterochromatic
region on the fourth chromosome results in sporadic silencing, or Position Effect Variegation (PEV). We created a construct where we replaced the hsp70 promoter with the 5’ upstream regulatory region of a highly-expressed fourth chromosome gene, Rad23, including an adjacent 1360 element. Insertion of the Rad23-white transgene into the same location switched the hsp70-white PEV phenotype to a uniform full red eye, suggesting that the Rad23 fragment is sufficient to drive strong expression of the euchromatic white reporter. A series of experiments with reporter constructs containing fragments of varying lengths of the Rad23 promoter region identified the minimal length of the Rad23 promoter fragment needed to drive full white expression as 100 bp. Since the 1360 element is a known target for silencing, we designed a construct to bring the 1360 element closer to the transcription start site of the Rad23-white transgene. However, closer proximity of the 1360 element had no effect on the Rad23-white reporter (red eyes). An additional construct where a 100 bp Rad23 promoter fragment replaces the corresponding portion of the hsp70 promoter in the hsp70-white transgene resulted in the loss of PEV, but low-level expression (light orange eyes). We are currently investigating additional promoter elements of the 3’ noncoding regulatory region of the Rad23 gene.

823 Repeat-induced Silencing in Drosophila melanogaster. Sarah C.R. Elgin, Sukruth Shashikumar, Gary Huang, Sophia Bieser, Michael Grupe, Tingting Gu, Elena Gracheva Dept Biology, Washington University in St Louis, St Louis, MO.

Genomes of higher eukaryotes can be divided into two fundamental and dynamic subtypes, euchromatin and heterochromatin, which differ in patterns of histone modification and associated proteins. Heterochromatin is enriched in repetitive DNA, both tandem arrays and interspersed transposable elements (TEs); these TEs are effectively silenced by this packaging. Genes that are generally active in a euchromatic environment are silenced when transposed to heterochromatin, demonstrating Position Effect Variegation (PEV). Expansion of a triplet repeat in mammals will drive local silencing through heterochromatin formation. To characterize such heterochromatin formation using Drosophila, we built a transgenic construct with 310 copies of the triplet GAA (DNA fragment originating from a Friedreich’s Ataxia patient) inserted upstream of an hsp70-white reporter. The GAA310-hsp70-white transgene was incorporated into the fly genome at the base of Chr. 2L within the active euchromatic nesd gene but close to a heterochromatic block (site 1198; 2L:20,094,249). At this location, hsp70-white yields a red-eye phenotype, while GAA310-hsp70-white results in silencing of hsp70-white (PEV phenotype). Genetic crosses with null mutants for HP1α, SU(VAR)39, and Sin3A result in loss of GAA310-hsp70-white reporter silencing, suggesting local heterochromatin formation. Loss of G9a or egg function also leads to a loss of silencing, emphasizing a role for H3K9 methylation. Mobilization of the P[GAA310-hsp70-white] element results in 4% recovery of PEV lines; mapping indicates insertion into repetitive domains, including the base of chromosome arm 2L, telomeres 2R and 3R, and the Y chromosome, but not the fourth chromosome. Insertion of a lacO126 fragment at the 1198 site similarly induces silencing (PEV) of hsp70-white, but while this silencing is sensitive to mutations in the HP1α gene and mutations that perturb histone deacetylation, there is no sensitivity to mutations in any of the known H3K9 histone methyltransferases. This line also exhibits unusual temperature sensitivity, losing silencing when grown at 18°C. Thus while the mechanism for silencing these repeat clusters shows hallmarks of heterochromatin formation in both cases, differences are also observed and are being explored.

824 Epigenetic changes but no derepression of transposable elements or global loss of gene regulation in aging D. melanogaster germline and reproductive tissue. A. Erwin, J. Blumenstiel Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS.

Epigenetic changes have been implicated as playing an important role in the aging process in cells of the soma across model organisms. Specifically, genome-wide heterochromatin redistribution during aging has been linked to the derepression of transposable elements and an overall loss of gene regulation. Whether or not epigenetic factors are perturbed in reproductive and germline tissues is of particular interest because some epigenetic factors are known to transmit across generations. Since ovaries are highly heterogeneous, consisting of a mixture of somatic tissues, germline-stem cells and many different stages of oogenesis, we focused our analysis on stage 14 egg chambers along with 0-1 hour embryos. This allowed us to minimize variation of cell type composition and to enrich for age-effects in the germline. Using mRNA sequencing as a proxy for epigenetic change, we asked whether transposable elements were de-repressed, if genes in or near heterochromatin boundaries were aberrantly expressed, and if genes were globally misregulated. Similar to previous results in the soma, we report that there is evidence of some heterochromatic change with age in the late stage egg chambers and embryos between young and old mothers. Conversely, we do not find global TE derepression or global gene misregulation with age, although some TE and strain-specific age effects occur.

825 Epigenetic effects of transposable elements in 3D nuclear space. Grace Y.C. Lee1,2, Giacomo Cavalli3, Gary Karpen1,2 1) Lawrence Berkeley National Lab, Berkeley, CA; 2) University of California, Berkeley, Berkeley, CA; 3) Institute of Human Genetics, CNRS, Montpellier, France.

Pericentromeric and peritelomeric heterochromatin are enriched for epigenetic marks H3K9me2/3 and heterochromatin proteins (particularly HP1α), and are widely observed to coalesce into a common 3D domain in the nucleus, likely due to liquid fusion properties associated with biological phase separation. This heterochromatin domain is thought to have a high concentration of proteins necessary for heterochromatin assembly and function, while depleted for proteins essential for
gene expression. This spatial organization of heterochromatin significantly influences gene and genome functions, as evidenced by the silencing of euchromatic genes abnormally juxtaposed with heterochromatin (position effect variegation, PEV). Interestingly, spreading of heterochromatic marks from epigenetically silenced transposable elements (TEs) can also generate enrichments for repressive heterochromatic marks in flanking euchromatic regions. We analyzed previously published Hi-C data to investigate if TEs with enrichment of repressive heterochromatic marks in euchromatic regions spatially interact with constitutive heterochromatin. We observe that euchromatic TEs associated with spreading of repressive heterochromatic marks into flanking sequences are significantly more likely to be involved in 3D associations with pericentromeric regions, compared to TEs without heterochromatic mark spreading. This spatial interaction between euchromatic TEs and heterochromatin is also associated with more extensive spreading of heterochromatic marks around TEs. Because of tight homolog pairing in somatic cells, spatial interactions between heterozygous TEs and pericentromeric heterochromatin can potentially drag the homologous chromosome to the same heterochromatin domain, resulting in trans spreading of heterochromatic marks and dominant PEV, a hypothesis we are actively testing.

826 Sequence and organization of Drosophila melanogaster centromeres. J Palladino1, C-H Chang2, A Chavan1, S Cordi1, N Martins3, C-C Chen1, C-T Wu3, A Larracuente2, B Mellone1,4 1) Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT; 2) Department of Biology, University of Rochester, Rochester, NY; 3) Department of Genetics, Harvard Medical School, Boston, MA; 4) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

Centromeres mediate accurate chromosome segregation by specifying the site of kinetochore attachment during mitosis and meiosis. Centromere identity is thought to be mediated not by centromeric DNA, but by the presence of the centromere-specific histone H3 variant, CENP-A, within centromeric chromatin. However, increasing evidence suggests that intrinsic features of centromeric DNA sequences may guide centromere assembly and inheritance. Yet, to date, the centromeres of complex eukaryotes have been refractory to sequencing and assembly due to their highly repetitive nature and the challenges associated with the assembly of short-reads. We report our efforts in assembling and validating the sequences of Drosophila melanogaster centromeres. Using long-read whole-genome sequencing assembly, we identify candidate contigs with a sequence organization similar to that of the previously reported X-chromosome derived minichromosome, Dp1187. CENP-A Chip-seq experiments in embryos reveal that these contigs are highly enriched in CENP-A, consistent with their centromere location. FISH with oligopaints combined with immunofluorescence for CENP-A confirms these CENP-A-enriched contigs make up the centromeres. High-resolution optical mapping of centromeres with extended chromatin fibers IF-FISH uncovers the size and organization of centromeric chromatin. Our work provides a glimpse into animal centromere complexity and provides a framework for the full assembly of centromeres of other species.

827 Functions of the Drosophila melanogaster HP1 homolog HP1B. M. Momeni, N. Riddle University of Alabama at Birmingham, Birmingham, AL.

Chromatin is a complex of DNA and proteins that aids in the control of gene expression. The Heterochromatin Protein 1 (HP1) family is a highly conserved protein family that carries out a variety of functions, including gene regulation and the maintenance of chromatin structure. Drosophila melanogaster has three major homologs of the HP1 proteins: HP1a, HP1B, and HP1C. HP1a is well characterized, and it has been found to be required for heterochromatin formation. However, HP1B and HP1C – which are mostly bound to euchromatic regions in the genome – are less well understood. HP1B is the Drosophila HP1 protein that most closely resembles the three human HP1 paralogs. Analysis of a Drosophila mutant lacking HP1B revealed that animals deficient in this chromatin protein showed a variety of phenotypic changes. In particular, loss of HP1B negatively impacted fertility and animal activity. Additionally, HP1B knockout mutants exhibited altered feeding behavior and higher body fat content. Furthermore, gene expression analysis indicated that their overall metabolism had been altered. Here, we characterize Drosophila genetically engineered to overexpress HP1B. We examine larval crawling to assess the early locomotor abilities of the animals carrying the transgene, exercise response to illustrate locomotor abilities at various adult time points, and body composition using quantitative magnetic resonance. Data from these assays illustrate the impact of HP1b overexpression on chromatin structure, animal health, and behavior.

828 Reassessing the fundamental mechanisms of transgenerational epigenetic inheritance. Nicholas Panayi, Keith Panayi University of Arizona.

Transgenerational epigenetic inheritance is conventionally defined as the heritability of gene expression patterns through mitosis and/or meiosis, a consequence of regulatory information encoded on chromosomes but separate from the DNA sequence. According to the prevailing paradigm, this information determines the expression patterns of genes, and is faithfully maintained during DNA replication, ensuring the same transcriptional state of the gene in clonally related cells. Epigenetic inheritance in Drosophila is most commonly monitored by analyzing position effect variegation (PEV), the consequence of which is observable as the varied deposition of pigment in the Drosophila eye. Genes undergoing PEV are affected by heterochromatin in cis. Thus, a prediction of current epigenetic models is that even identical genes can behave differently based on differences in their local chromatin structure, akin to the genomic imprinting observed in mammals. We have designed an experimental approach that will critically test whether gene-regulatory information can be carried at
specific chromosomal loci, and whether that information is stable in interphase and inherited through mitosis. We are creating a genotype with two distinct reporter genes embedded in allelic loci of heterochromatin. If epigenetic modifications act locally, differences in reporter expression will reflect distinctive allelic chromatin modifications. Alternatively, if the level of variegation is determined by nucleus wide changes in heterochromatin stability, the expression of both reporter genes will co- vary. By challenging the existing model for transgenerational epigenetic inheritance, we will better understand “epigenetic” modes of disease progression; providing higher resolution to a natural biological phenomenon that has proven elusive.

829  Functional genomics study of satellite DNAs in Drosophila melanogaster.  X. Wei¹, D. Eickbush², A. Larracuente²  1) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 2) Department of Biology, University of Rochester, Rochester, NY.

Satellite DNAs (satDNAs) are tandemly repeated DNAs typically found at centromeres, telomeres, and on sex chromosomes. Although typically thought as transcriptionally inert sequences without any function that only spread in genomes and populations due to selfish replication, it has been shown recently that satDNAs play important roles in the formation and maintenance of heterochromatin, chromosome recognition and genome stability. Many satDNAs are transcribed, and misregulation of their expression can result in genome instability. However, the regulation and transcription profiles of satDNAs are largely unknown. One of the best known satDNAs in Drosophila is Responder (Rsp)—a repeat that is the target of the selfish male meiotic drive system called Segregation Distorter (SD). During spermatogenesis, SD induces dysfunction of drive-sensitive spermatids bearing many Rsp repeats but not drive-insensitive spermatids bearing few or no Rsp repeats. The mechanism by which Rsp is involved in this process is still unknown. By analyzing RNA-seq data, we find that Rsp is transcribed into long noncoding RNAs (lncRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs). Furthermore, we detect Rsp expression in adult testes by fluorescence in situ hybridization. Currently, we are examining the potential role of Rsp in regulating genome architecture. In summary, using a combination of genomic, cytological and molecular approaches, we characterize the expression pattern of Rsp, which will shed light on the underlying mechanisms by which Rsp is involved in the SD system. Such mechanisms may also provide valuable information regarding the functional pattern of satDNAs.

830  Germline stem cell maintenance factor Stonewall regulates transposons and testis-specific clusters in ovaries.  Daniel Zinshetym, Daniel Barbash  Cornell University, Ithaca, NY.

Germline stem cells (GSCs) are the progenitor cells for the entire population of an organism's germline. In Drosophila, these cells reside in a well-defined cellular niche that is required for both their maintenance (self-renewal) and differentiation (asymmetric division resulting in a daughter cell that differs from the GSC). Dozens of genes have been implicated in these processes and most of them are required for production of viable gametes. The critical function of these genes suggests that they should be highly conserved across Drosophila taxa. However, population genetic analyses have shown that many of them have undergone adaptive evolution in some lineages along the Drosophila phylogeny. One possible cause of this evolutionary signature is the critical role that many of these genes play in regulating genomic parasites, particularly transposons. These genes may be locked in a dynamic arms-race with rapidly evolving selfish elements.

The stem-cell maintenance factor Stonewall (Stwl) is a particularly intriguing candidate, as it has undergone adaptive evolution and has been implicated in heterochromatin maintenance. We performed RNA-seq on stwl mutant ovaries and testes to assay the transcript abundance of transposable elements in the absence of a functional Stwl. We find that mutant stwl ovaries (but not testes) show significant de-repression of many transposon families. Surprisingly, heterochromatic genes do not appear to be preferentially misregulated relative to euchromatic genes. These data suggest that Stwl may be required for regulation of transposons via a mechanism other than general heterochromatin maintenance. We find that some of the most abundantly mis-expressed genes in stwl mutant ovaries are in large clusters of testis-specific genes. Such genomic regions have been shown in previous studies to be regulated by nuclear architecture, suggesting that Stwl is implicated in spatial organization of the genome. We have developed antibodies against Stwl and are currently working to identify its genomic targets by ChIP-Seq.

831  Zinc-finger containing protein CLAMP promotes gypsy chromatin insulator function.  I. Bag, R. Dale, E. Lei  Nuclear Organization and Gene Expression Section, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Chromatin insulators are DNA-protein complexes that establish higher order DNA domains to influence transcriptional regulation. They can block the interaction between enhancers and promoters to prevent inappropriate communication between adjacent cis-regulatory elements or act as a barrier to protect genes from nearby condensed chromatin that can silence expression. In Drosophila, the well-characterized gypsy insulator complex harbors three core components: Su(Hw), CP190 and Mod(mdg4)672. Here, we identify for the first time a novel role for Chromatin-linked adaptor for MSL proteins (CLAMP) in promoting gypsy insulator function. Previously, CLAMP was identified as a dosage compensation factor that helps
recruit the MSL complex to the X-chromosome in males. Interestingly, recent work has shown that CLAMP is a component of the late boundary complex and can also activate transcription on autosome.

To investigate the function of CLAMP as a regulator of gypsy-dependent insulator activity, we applied genetic and molecular biology approaches. Depletion of CLAMP decreases gypsy-dependent enhancer blocking, indicating a role as a positive regulator of insulator activity. Depletion of CLAMP by RNAi knockdown or null mutation leads to reduced gypsy-dependent barrier activity in all tissues tested. Furthermore, we found that CLAMP associates physically with the core gypsy insulator complex by immunoprecipitation from embryonic nuclear extracts and also partially colocalizes with gypsy components on polytene chromosomes. ChIP-seq of CLAMP demonstrates colocalization with a subset of insulator components throughout the genome. Finally, knockdowns or mutation of clamp disrupt the localization pattern of insulator bodies in nucleus. Altogether, our findings identify a new function for CLAMP in promoting gypsy insulator activity.

832 Determining isoform and cell-type specificity of RNA-binding protein Shep in antagonism of gypsy insulator activity. M. Brovkina, D. Chen, L. Matzat, E. Lei National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Insulators are protein-DNA complexes that organize chromatin structure. They function to block the interactions between promoters and enhancers, as well as to define boundaries between heterochromatin and euchromatin. This physical partitioning of chromatin is required for precise regulation of gene expression during development. We previously identified Shep, a conserved RNA recognition motif (RRM) containing protein, as an interactor of gypsy insulator proteins. Loss of shep leads to synthetic lethality combined with loss of the core gypsy component Mod(mdg4)67.2. Furthermore, mutation of shep improves gypsy insulator activities specifically in the nervous system. Given that loss of shep disrupts neuronal remodeling during metamorphosis, it is of interest to explore the mechanism of this process and how insulator activity may be involved. Here, we investigate regulation of gypsy insulator activities by distinct Shep isoforms. Our results show that restored expression of shep can rescue synthetic lethality with mod(mdg4) and reverse effects on enhancer blocking for the gypsy-dependent allele ct4, both in an isoform-specific manner. Expression of the same isoform of Shep but with point mutations in the RRM domains fails to rescue both phenotypes, suggesting that Shep RNA-binding is essential for insulator antagonism. Further study into its cell type-specificity shows that mutation of shep improves compromised gypsy insulator bodies primarily in two neural cell types: neuroblasts and glia. We are currently exploring Shep inhibition of gypsy insulator activities, regulation of neural development, as well as molecular interactions with RNA and chromatin in a cell-type and isoform-specific manner. Taken together, these data indicate isoform and cell-type specific roles for Shep in insulator antagonism and development of the nervous system.

833 High content genome-wide RNAi screening for factors involved in the formation of gypsy insulator bodies. S Chen, N. Moshkovitch, P Murphy, E Lei Nuclear Organization and Gene Expression Section, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland.

Chromatin insulators regulate gene expression through the formation of higher order chromatin structures, namely chromatin loops. The gypsy insulator body is a highly proteinaceous nuclear structure consisting of core insulator proteins CP190, Su(Hw), and Mod(mdg4)67.2. Although their precise function is still unclear, proper localization of insulator bodies in the nucleus, is highly correlated with gypsy insulator activity. In order to gain insight into these structures, we plan to perform a genome-wide RNAi knockdown screen in a clonal Kc167 cell line that expresses a functional Mod(mdg4)67.2-GFP fusion protein. By examining the formation of insulator bodies using high content imaging, we will identify candidate factors involved in insulator activity and/or nuclear organization.

834 COMPASS-like complex regulation of cell survival in Drosophila eye development. D. Ford1, C. Zraly1, A. Dingwall2,3 1) Molecular Biology/Biochemistry Graduate Program, Loyola Univ Chicago-Stritch School of Medici, Maywood, IL; 2) Oncology Research Institute, Loyola Univ Chicago-Stritch School of Medici, Maywood, IL; 3) Departments of Pathology and Microbiology & Immunology, Loyola Univ Chicago-Chirist School of Medici, Maywood, IL.

COMPASS-like complexes are highly-conserved transcription coregulators responsible for the methylation of histone 3 lysine 4 at transcription enhancer regions. These epigenetic regulators have been identified as interaction partners with various lineage-specific factors and are necessary for transcription activation during cellular reprogramming and differentiation. Unlike in vertebrates, the Drosophila Cmi/Trr COMPASS-like complex contains the histone-binding and enzymatic activity in two distinct, though highly conserved subunits. The genes encoding these subunits split in schizophora dipterans from a single common ancestor gene. Depletion of Cmi and Trr functions in the eye imaginal disc using RNAi produced small and rough eye phenotypes, while overexpression of Cmi led to increased tissue growth. Closer examination of the RNAi phenotypes using TUNEL and immunofluorescent staining, genetic interaction analysis, transgene reporters, and mosaic clones revealed dysregulated developmental signaling pathways and apoptosis in specific regions of the disc. Unexpectedly, we found that altered expression of bantam, a miRNA regulatory target of the complex, likely contributes to these phenotypes.
The recognition of target gene transcriptional state by Polycomb Group Proteins.  Elnaz Ghotbi Ravandi, Piao Ye, Judith Benes, Richard Jones  Dept of Biological Sciences, Southern Methodist University, Dallas, TX.

Polycomb Group proteins are conserved epigenetic transcriptional regulators that maintain the transcriptional repression of silenced genes by altering chromatin structure. Most studies on PcG proteins have been focused on the maintenance phase of PcG silencing, thus the molecular mechanisms by which PcG proteins initially recognize a repressed gene remain unknown. Our lab previously used embryos from bcd osk tsl females, in which giant (gt), a PcG-target gene, is uniformly repressed by PcG proteins. Time course ChIP experiments on bcd osk tsl embryos in different embryonic stages showed the weak presence of PcG proteins at gt in earlier stages prior to their stable binding in later embryonic stages. On the other hand, we generated a genetic system in which gt is ubiquitously expressed by producing embryos that lack the gt repressor Hb and ubiquitously express a maternal gt activator. Preliminary time course ChIP experiments on the resulting embryos demonstrated greatly reduced binding of PcG proteins at the ubiquitously expressed gt gene compared to the PcG-mediated repressed gt gene from bcd osk tsl females. In order to determine whether binding by particular transcription factors or the transcriptional state of a target gene identifies PcG target loci as initially repressed, we will examine whether PcG silencing complexes assemble at a transcriptionally inert gt transgene in a background in which endogenous gt is transcriptionally active. Progress on these studies will be presented.

Early developmental roles of the Cmi/Trr COMPASS-like complex in Drosophila.  T.J. Nickels¹, C.B. Zraly², D. Ford¹, A.K. Dingwall§ 1) Biochemistry and Molecular Biology Graduate Program Loyola University Chicago; 2) Oncology Research Institute Loyola Stritch School of Medicine; 3) Departments of Pathology and Microbiology & Immunology Loyola University Chicago.

The Drosophila Cmi/Trr COMPASS-like complex catalyzes the mono-methylation of histone 4 lysine 4 (H3K4), an epigenetic mark that is associated with transcription enhancers and is critical for proper gene activation during development and cellular differentiation. In D. melanogaster, cara mitad (Cmi/Lpt) and trithorax-related (Trr) are central subunits of a complex orthologous to mammalian mixed lineage leukemia 3 and 4 (MLL3 and MLL4) H3K4 COMPASS-like complexes. Cmi and trr encode essential genes, with mutations causing early lethality and altered regulation of critical signaling pathways. Mutations in the mammalian genes are associated with a large variety of cancers and developmental disorders, but the mechanisms by which these alterations contribute to disease states are unknown. We have carefully characterized the expression pattern of Cmi in oogenesis and embryogenesis using specific antibodies. Additionally, we have used tissue-specific knockdown and null alleles of Cmi to study the resulting phenotypes in ovary and embryo development.

Uncovering a role for the nucleoporin Megator in dosage compensation.  Jennifer Aleman, Maya Capelson  Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

The Nuclear Pore Complex is a large nuclear envelope-spanning protein complex, which consists of ~30 Nucleoporin components or Nups. Nups have recently been shown to play a role in genome organization and transcriptional regulation. These gene regulatory events have been shown to impact Drosophila melanogaster development, yet the mechanisms by which Nups regulate gene expression are not well understood. Our lab has identified a novel nuclear structure formed by specific Nups. We refer to this structure as the nuclear scaffold or nuclear cables since this accurately describes the localization of the Nups – binding in large interspersed domains along chromatin, as observed by immunofluorescence staining of polytene chromosomes of Drosophila larval salivary glands. One of the main components of this structure is a nuclear basket Nup, Megator (Mtor), which has been implicated in both transcriptional regulation and transport functions of the nuclear pore. The goal of my research is to define the biological role of the Mtor-formed nuclear scaffold as well as its effect on gene regulation. We have recently uncovered a role for Mtor in chromatin targeting of a non-coding RNA (ncRNA) roX1, which is required for X chromosome dosage compensation. To determine if Mtor plays a role in determining the distribution of ncRNAs in the nucleus, we have performed single molecule RNA FISH experiments in whole mount larval salivary glands targeting several candidate genes. Results from confocal imaging indicate that upon Mtor knock down, roX1 is not properly targeted to the X chromosome and is instead mis-localized in nuclear space. Preliminary qRT-PCR data indicates that roX1 transcript levels are not significantly changed, supporting our conclusion that Mtor is necessary for targeting of roX1 to the X chromosome. Future directions include investigating whether Mtor binds roX1 ncRNA directly, how improper targeting of roX1 via loss of Mtor is achieved, and what other ncRNA targets depend on Mtor for proper nuclear localization.

Chromatin-bound nuclear pore proteins recruit chromatin remodeling complexes to induce DNA decondensation.  T. Kuhn, S. Little, M. Capelson  University of Pennsylvania, Philadelphia, PA.

Nuclear pore complexes (NPCs) span the eukaryotic nuclear envelope, regulate nucleo-cytoplasmic traffic, and are comprised of about 30 distinct nuclear pore proteins (Nups). In recent years, it has become clear that NPCs and their constituent Nups interact with the genome, and this interaction has a functional impact on gene expression. Genomic targets of nucleoporins tend to be sites of open chromatin associated with active genes, and depletion of Nups can result in loss of open chromatin state, decreased RNA polymerase II (RNAPII) recruitment, and lower gene expression of targets. How Nups
regulate these processes however, and at what stage of gene activation, is not known. Using transgenic Drosophila containing Lacl-Nup fusion proteins and integrated genomic lacO arrays, we have determined that tethering nucleoporins Sec13 and Nup62 to ectopic loci is sufficient to induce visible chromatin decondensation, in a chromatin context-dependent manner. Furthermore, we have determined that tethered Sec13 recruits GAGA Factor, a multi-talented protein associated with formation of open chromatin, as well as Brahma and other components of the PBAP chromatin-remodeling complex. Previously it has been shown that Sec13 is present at two distinct nuclear locations – in an intranuclear pool and at NPCs in the nuclear membrane. Interestingly, our DNA FISH experiments show that Lacl-Sec13-targeted lacO locus is preferentially associated with the nuclear periphery, relative to the control Lacl-GFP. This suggests Sec13 relocates chromatin to NPCs at the nuclear membrane, and demonstrates a correlation between chromatin decondensation and nuclear positioning. As further evidence that this locus is at NPCs, recruitment of additional Nups, including stable, core NPC component Nup93, is also observed upon tethering of nucleoporin Sec13. These data support a long-standing hypothesis within the field that nuclear pore complexes may provide a decondensing environment for genetic elements recruited to them. Furthermore, these experiments provide a likely mechanism for this phenomenon, as they demonstrate a role for NPC components in recruiting specific chromatin remodelers to facilitate chromatin decondensation, regulating a crucial step of an early stage in gene activation.

839 Dissecting the mechanism of X recognition in Drosophila melanogaster using a luciferase reporter. R. Makki, V. Meller Biological Sciences, Wayne State University, Detroit, MI.

Drosophila melanogaster males have one X chromosome while females have two. This creates an imbalance in X to autosome gene dosage between the sexes. To maintain an appropriate ratio of X to autosome gene expression, male fruit flies increase transcription from X-linked genes approximately two-fold. This is accomplished by the Male Specific Lethal (MSL) complex, which is recruited to transcribed X-linked genes and modifies chromatin to increase expression. The MSL complex is thought to assemble at Chromatin Entry Sites (CES), which contain the MSL recognition Element (MRE), and then spread in cis to active genes in the vicinity. Since the MRE is present on autosomes it is unclear how the MSL complex recognizes X-chromatin. We found that repetitive sequences that are near-exclusive to the X chromosome, the 1.688 satellite repeats, promote recruitment of the MSL complex to nearby genes. The 1.688 repeats do not contain MRES. Unlike CES, the 1.688 repeats do not appear to recruit the MSL complex directly. To facilitate dissection of the mechanism of recruitment we are developing a dual-luciferase reporter assay to measure the ability of DNA sequences to recruit compensation. Firefly luciferase is placed on a transgene containing the recruiting element (1.688 repeats or CES) and a normalizing Renilla luciferase is placed on a transgene devoid of recruiting elements. Autosomal inserts of these reporters will be used in an RNAi screen to identify genes necessary for recruitment of compensation by the 1.688 repeats or CES. Measuring recruitment on an autosome avoids the confounding effects of redundant X-linked recruiting elements. We expect to be able to differentiate the recruiting pathways used by different types of DNA sequences. As 1.688 sequences appear to recruit through a different mechanism than the CES, we expect to identify genes not previously known to participate in X recognition.

840 Functional interplay between MSL1 and Cdk7 in transcription regulation. Maria Samata, Sarantis Chlamydas, Herbert Holz, Tomasz Chelmicki, Plamen Georgiev, Friederike Dundar, Gerhard Mittler, Thomas Manke, Asifa Akhtar Chromatin Regulation Department, Max Planck Institut of Immunobiology and Epigenetics, Freiburg, Baden-Wurttemberg, DE.

X chromosome compensation in Drosophila melanogaster serves as a prime example to study the epigenetic control of gene expression. The Male Specific Lethal proteins (MSL) mediate the upregulation of the single male X chromosome by two-fold thus balancing the expression of X-linked genes between male and female flies. Proper gene expression on X chromosome and autosomes requires the coordinated interplay between transcriptional coactivators, transcription factors and the general transcription machinery. We report that MSL1, a central component of the dosage compensation complex displays evolutionarily conserved sex-independent binding to gene promoters. Genetic and biochemical analyses reveal a functional interaction of MSL1 with Cdk7, a subunit of the Cdk-activating kinase (CAK) complex of the general transcription factor TFIH. Notably, MSL1 depletion leads to a sex independent reduction of serine 5 phosphorylation of RNA Polymerase II (Pol II Ser5p) important in early transcriptional events. In addition, we demonstrate that MSL1 is a phosphoprotein and a target of Cdk7 kinase activity in vitro. Inhibition of Cdk7 using small molecule inhibitors leads to reduction of MSL1 localization on chromosomes. Underlining the functional significance of this interaction, transgenic flies expressing MSL1 phosphomutants fail to rescue Pol II Ser5p levels, show mislocalization of MOF and H4K16 acetylation and cause male lethality due to lack of dosage compensation. We propose that by virtue of its interaction with components of the general transcription machinery, MSL1 can exist in different phosphorylation states thereby modulating transcription in flies.

841 Do satellite-binding proteins play a role in D. melanogaster dosage compensation? M. Sneideman, V. Meller Wayne State University, Detroit, MI.

Drosophila melanogaster males carry one X and one Y chromosome, but females have two X chromosomes. This imbalance in X to autosomal gene dosage is potentially lethal. To equalize the expression of X-linked genes between the sexes, males increase transcription from X-linked genes approximately two-fold. This is mediated by the Male-Specific Lethal (MSL) complex, which modifies chromatin to elevate expression. The MSL complex first binds at Chromatin Entry Sites (CES) on the X, and then spreads into nearby active genes. CES contain a short motif, the MSL Recognition Element (MRE) that is bound by the adapter protein CLAMP. CLAMP is necessary to attract the MSL complex to the CES. However, MREs are not limited to the X chromosome. Furthermore, autosomal MREs bind CLAMP but fail to recruit the MSL complex. Another factor must therefore distinguish the X from the autosomes. The X chromosome is strikingly enriched for chromosome-specific repeats. Among these are the 1.688\textsuperscript{b} repeats. Our lab has previously shown that 1.688\textsuperscript{b} repeats play a role in identifying the X. The focus of my project is to test non-histone proteins that bind satellite repeats as a role for a role in X identification. Candidate proteins are selected from known satellite DNA binding factors, and proteins with X-specific or male-specific effects, such as heterochromatin factors. I will determine if these proteins localize to the 1.688\textsuperscript{b} repeats, and knock down candidate genes to determine if a male-specific phenotype, or reduction of dosage compensation, is produced. In preparation for these studies, I am validating knock down and scoring knock down phenotypes for candidate genes. Genetic interaction with loss of function mutations that partially reduce dosage compensation will be used to identify genes that participate in X recognition. Testing satellite-binding proteins for a role in X recognition is an essential step towards understanding how the 1.688\textsuperscript{b} repeats function during dosage compensation.

842 The role of satellite-binding protein, proliferation disrupter, in chromocenter formation. R. Allen. Cummings\textsuperscript{1,2}, M. Jagannathan\textsuperscript{1}, Y. Yamashita\textsuperscript{1,2} 1) Cellular and Developmental Biology, Life Sciences Institute, Ann Arbor, MI; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

Satellite DNAs are ubiquitous tandem repeats that constitute a considerable fraction of eukaryotic genomes. They are located primarily in the pericentromeric heterochromatin of eukaryotic chromosomes and are known to cluster across multiple chromosomes into DAPI-dense nuclear foci known as chromocenters. Although essentially all eukaryotic cells form chromocenters, its function remains obscure. In our recent study, we have found that the Drosophila D1 protein forms chromocenters by binding the AATAT satellite (~8% of the Drosophila genome) on multiple chromosomes and bundling them together. In the absence of D1, cells fail to bundle chromosomes, leading to micronuclei formation. Based on these data, we hypothesize that bundling of heterologous chromosomes by chromocenter formation prevents individual chromosomes from being excluded from the nucleus. D. melanogaster contains a diverse array of distinct satellite DNAs in its genome. Interestingly, the D1-bound AATAT satellite repeat exists primarily on X, Y, and the 4\textsuperscript{th} chromosomes and is minimally present on the major autosomes. This led us to hypothesize that the major autosomes might be bundled into chromocenters by other satellite DNA binding protein(s). Here, we show that the Proliferation disrupter protein (Prod), which is known to bind the major autosomal specific AATAACATAT satellite DNA, localizes to chromocenters in multiple D. melanogaster tissues. We found that RNAi-mediated knockdown of Prod gives similar phenotypes to D1 mutation - chromocenter disruption and nuclear envelope defects. We propose that the chromocenter is a 'modular' system, where satellite binding proteins function co-operatively to bring their individual target sequences together into a network.


Argonaute proteins are an evolutionarily conserved protein family engaged in gene silencing. The key RNA interference (RNAi) pathway protein AGO2 interacts with small RNAs to regulate gene silencing in the cytoplasm. In addition, AGO2 has been shown to regulate gene expression by functioning in the nucleus. In this study, we determined that AGO2 forms a nuclear complex with LaminB, a nuclear scaffolding protein, as well as the transcription machinery. Together, AGO2 and LaminB limit transcription in active or potentially active regions that either do or do not interact directly with the nuclear lamina. We focused on nht, a master control gene of the sperm developmental program, which is up-regulated in the absence of AGO2 or LaminB. The nht gene interacts with the nuclear lamina in somatic cells, and we determined that AGO2 and LaminB control the three-dimensional configuration of the chromatin region in which nht is located. We conclude that AGO2 and LaminB work in concert to regulate how genes are turned on or off by controlling how the genome is folded within the nucleus, and therefore can affect key developmental processes such as the production of sperm.

844 Condensin II drives large-scale chromatin folding and genome compartmentalization in Drosophila. L. Rosin, S. Nguyen, O. Crocker, E. Joyce Genetics, University Of Pennsylvania School of Medicine, Philadelphia, PA.

Proper nuclear organization is essential for development and organism viability. Both chromosome confirmation capture (3C)-based methods and fluorescence in situ hybridization (FISH) have shown that chromosomes occupy distinct nuclear
domains called chromosome territories (CTs). The fact that CTs have been observed in a broad range of species suggests that their formation may play a role in vital cellular processes, such as transcription and DNA repair. In support of this, CT disruption has been seen in several human diseases, including numerous forms of cancer, correlating CT loss with gene misexpression and genome instability. However, the genes involved in the formation and maintenance of CTs remain unknown. Using a combination of forward and reverse genetics, we address these fundamental questions in Drosophila, targeting their relatively small number of chromosomes with whole-genome, multi-color, and chromosome arm-specific Oligopaint FISH probes. Our data reveal that Drosophila cells form largely non-overlapping CTs, indicating that heterologous chromosomes minimize their intermingling during interphase. Additionally, the use of whole-genome Oligopaints on metaphase chromosome spreads illustrate that large chromosomal rearrangements can be correlated with the spatial orientation of DNA during interphase. Finally, we have identified the condensin II complex as an essential factor that inhibits interchromosomal interactions and facilitates chromosome folding during CT formation. Collectively, these analyses show that Drosophila cells and Oligopaint FISH provides a powerful platform for assessing CT function and characterizing the regulatory pathways that control chromosome packaging and positioning in a metazoan.

845 Similarity in replication timing between polytene and diploid cells is associated with the organization of the Drosophila genome. Tatyana Kolesnikova1,2, Fedor Goncharov1, Igor Zhimulev1 1) Institute of Molecular and Cellular Biology, Russian Academy of Sciences, Novosibirsk, Russia; 2) Novosibirsk State University, Novosibirsk, Russia.

Morphologically, polytene chromosomes of Drosophila melanogaster consist of compact “black” bands alternating with less compact “grey” bands and interbands. We developed a comprehensive approach that combines cytology mapping data of the FlyBase-annotated genes and novel tools for predicting cytogenetic features of chromosomes based on their protein composition and obtained the genomic coordinates for all “black” bands of polytene chromosome 2R. By PCNA immunostaining assay we got the replication timetable for all bands mapped. This allowed us to compare replication timing between distinct types of chromosomes and to observe extensive similarity in the global replication patterns on the band resolution level. In both kinds of cells intervals between “black” bands correspond to early replication initiation zones. “Black” bands are depleted for replication initiation events and are characterized by gradient of replication timing so the time of replication completion correlates with the band length. The bands are characterized by low gene density and contain predominantly tissue specific genes and they are represented by silent chromatin types in different tissues. The borders of “black” bands correspond well to the borders of topological domains, and the borders of zones of the enrichment of the SUUR and LAMIN proteins. In conclusion, the characteristic pattern of polytene chromosomes reflects a partition of the Drosophila genome into two global types of domains with contrasting properties. This partition is conservative in different tissues and determines the replication timing in Drosophila.

846 Rif1 inhibits replication fork progression and controls copy number in Drosophila. J.T. Nordman1, A. Munden1, R. Gangula2, S. Mallal2,3 1) Biological Sciences, Vanderbilt University, Nashville, TN; 2) Dept. of Medicine, Vanderbilt University School of Medicine, Nashville, TN; 3) 3Dept. of Pathology, Vanderbilt University School of Medicine, Nashville, TN.

DNA replication must be highly choreographed to ensure the entire genome is accurately replicated in a timely manner. The regulatory mechanisms responsible for ensuring accurate replication and copy number control, however, can be circumvented during development to allow for changes in DNA copy number necessary for cell-type specific processes. During the endo cycle in Drosophila, heterochromatin and defined repressive chromatin regions are inhibited for DNA replication in a developmentally programmed manner, resulting in their underreplication. Underreplication is controlled by the SNF2-domain-containing SUUR protein. We have previously shown that SUUR is recruited to active replication forks, and inhibits their progression, demonstrating that underreplication occurs through direct regulation of the replication fork progression. It is still not known, however, how SUUR is recruited to replication forks and how it inhibits fork progression.

We have now identified a physical interaction between SUUR and Rif1. Rif1 has many roles in DNA metabolism and is a major regulator of the replication timing program. We used CRISPR-mutagenesis generate null alleles of Rif1 and found that repression of DNA replication is dependent on Rif1, suggesting that Rif1 can regulate replication fork progression. Using a model replication system, we showed that loss of Rif1 function results in increased replication fork progression and that Rif1 localizes to active replication forks. Importantly, SUUR associates with replication forks in the absence of Rif1, indicating that Rif1 acts downstream of SUUR to inhibit fork progression. Rif1 has been shown to counteract DDK-dependent helicase activation through recruitment of Protein Phosphatase 1 (PP1) and we have generated a Rif1 mutant predicted to abolish the PP1 interaction. This mutant also fails to promote underreplication, suggesting that Rif1 acts together with PP1 to control replication fork progression. Finally, we have found that the SNF2 domain of SUUR is largely responsible for its recruitment to replication forks, but not heterochromatin where SUUR is constitutively bound. Together, our findings provide new insight into the mechanisms responsible for copy number control during development and demonstrate that Rif1 can modulate replication fork progression.
847 A screen to identify genes that modify cell survival after loss of a single telomere in Drosophila melanogaster.  R.L. Kurzhal1, C. Parks1, H. Lowery1, L.P.D. Pulavarthi1, A.C. Gonzalez-Ebsen2, B. Harvey1, A. Hansen1, D. Beck1, M. Main1 1) Department of Biology, Southeast Missouri State University, Cape Girardeau, MO; 2) Unit for Molecular Medicine, Aarhus University and Aarhus University Hospital, Aarhus, Denmark.

The telomere cap is a complex of proteins and nucleic acids found at chromosome ends which prevents the DNA terminus from being seen as a double strand break in need of repair. HP1, HOAP, and HipHop, among others, are critical components of this capping complex. In most cells, the absence of a single telomere cap is sufficient to trigger apoptosis. Cells that do not die are likely to experience end-to-end fusions of uncapped ends, leading to gross chromosomal rearrangements and genomic instability. The apoptotic response to telomere loss is mediated by the DNA damage response pathway, primarily through Chk2 and p53. Mutation of either of the genes encoding these proteins allows for the survival and proliferation of cells that have lost a telomere. However, even in a wildtype background, a small fraction of such cells manage to evade the apoptotic response. In order to understand how some cells survive telomere loss we used a technique that allows for controlled loss of a single telomere during development and screened for genes that affect cell survival. We identified 16 genes that, when misexpressed, either enhance or suppress the cell death phenotype normally seen after telomere loss, including chk2 indicating the validity of the screen. Many of the identified genes have no characterized role in DNA damage response, cell cycle regulation, or apoptosis. Over-expression of charlatan, jing, or chk2 enhance the cell death phenotype specifically in response to telomere loss in a p53 dependent manner. While chk2 heterozygotes or homozygotes suppress the cell death phenotype, reduction of jing or charlatan do not. Characterization of these genes and the suppressors of the cell death phenotype are ongoing to reveal the underlying mechanism by which these genes modify cell survival after telomere loss.

848 Social isolation mediates epigenetic changes in dopaminergic neurons of Drosophila. Pavan Agrawal1, Clement Kent1,2, Ulrike Heberlein1 1) HHMI, Janelia Research Campus, 19700, Helix Drive, VA; 2) Department of Biology, York University, Toronto Canada.

Social isolation strongly influences animal behavior via transcriptional and epigenetic mechanisms. In Drosophila, dopaminergic neurons play an important role in modulating behaviors influenced by social isolation such as locomotion, sleep and aggression. To look at cell type specific epigenetic changes due to social isolation in adult Drosophila brain, we purified dopaminergic nuclei from socially isolated and socially enriched flies. Because dopaminergic neurons comprise only about 0.1% of the neurons in the fly brain (~120/brain), we adapted the INTACT method to analyze small numbers of defined neurons. Adult flies largely do not utilize DNA methylation mechanisms often used in mammals to mark genes epigenetically. Therefore, we focused on the effects of housing conditions on 6 different histone modifications. We were able to carry out ChIP-seq experiments with as few as 10,000 dopaminergic neurons and compared the transcriptional output using RNA-seq.

Our experiments reveal several clusters of genes with unique epigenetic profiles and with mRNA expression changes due to prior social experiences. Many of these clusters are enriched for chromatin modifying genes, splicing factors, neuropeptides, neural development genes, and genes involved in mating behavior. Chromatin and transcription changes due to long-term social experience were significantly correlated with changes induced by short-term stimulation of DA neurons in an independent study. Our work opens up possibilities for using Drosophila as a model to study epigenetic changes due to stress, social experience and drugs of abuse in small, defined neural population.

849 The histone demethylase KDM5 is essential for larval growth. C. Drelon, H. Belalcazar, J. Secombe  Genetics, Albert Einstein College of Medicine, Bronx, NY.

KDM5 proteins are highly conserved histone demethylases with several conserved domains. In addition of well-described catalytic JmjC domain, KDM5 proteins contain a JmjN domain, an ARID DNA binding domain, a C5HC2 zinc finger of unknown function and two or three histone binding PHD motifs. Whereas mammalian cells encode four KDM5 paralogs KDM5A, KDM5B, KDM5C and KDM5D, Drosophila has a single KDM5 ortholog, making of flies a good model to study the biology of KDM5 family proteins. Dysregulation of KDM5 family proteins is linked to human disease, with loss of function mutations causing intellectual disability and overexpression being linked to several forms of cancers. Therefore, it is important to define the biological functions of KDM5 in order to understand its role in human pathologies. To define the in vivo roles of KDM5, we generated a null allele (kdms140) by imprecise excision of P element. In contrast to previously existing hypomorphic kdms alleles, our new null mutant is 100% lethal (95% for hypomorphic kdms). kdms140 mutants die during the pupal stage, but show a dramatic developmental delay, taking an average of five additional days to pupate. Interestingly, this developmental delay is independent of KDM5's demethylase activity, raising key question regarding which KDM5 functions are essential for development. To characterize this delay phenotype, we analyzed the proliferation and the cell death in kdms140 wing discs. Loss of KDM5 leads to a decreased proliferation and increased cell death. However, those two phenotypes are not cell-autonomous, suggesting that they occur in response to cues from elsewhere in the larva. To complement our phenotypic
analyses of kdm5, we also performed RNA sequencing analysis of 3rd instar larval wing discs. In kdm5 null mutants, 1630 genes are differentially expressed. Of these, 883 genes are downregulated, while 747 genes are upregulated. GO term analysis shown enrichment for cell cycle and proliferation as well as neurogenesis for downregulated genes. For upregulated genes, DNA repair and double-strand break repair via NHEJ came up, suggesting a role of KDM5 in genome stability. Indeed, the loss of kdm5 leads to an increase of irradiation-induced DNA damage and cell death. To conclude, kdm5 is an essential gene involved in development process as well as genome stability.

The microRNA miR-33 is a regulator of pigmentation in D. melanogaster. C. Chan, S. Roman-Holba, J. Kennell Biology, Vassar College, Poughkeepsie, NY.

We have previously identified the microRNA miR-8 as a positive regulator of pigmentation in D. melanogaster. We have now identified the highly conserved microRNA miR-33 as a negative regulator of pigmentation. This microRNA is located within the SREBP locus and is thought to be a highly conserved regulator of lipid homeostasis in animals. In addition to finding impacts of miR-33 on lipids in flies, we observed that overexpression of miR-33 in the developing cuticle decreases abdominal pigmentation in female Drosophila while loss of miR-33 increases pigmentation. Interestingly, we have identified binding sites for miR-33 in the 3'UTR of the AKT mRNA transcript. This suggests miR-33 could indirectly regulate pigmentation through AKT and therefore through the insulin or TOR pathways. In Drosophila, studies have identified AKT as a positive regulator of cuticle pigmentation. Activation of AKT causes increased pigmentation while knockdown of AKT causes loss of pigmentation. AKT may impact pigmentation through its regulation of TORC1; high levels of TORC1 complex activator Rheb results in increased levels of melanin synthesis. On the other hand, FOXO, a transcriptional activator that plays important roles in the insulin signaling pathway, could be another mechanism by which AKT regulates pigmentation. Activation of AKT has been shown to inhibit FOXO and overexpression of FOXO lacking AKT binding sites causes decreased pigmentation. Interestingly, we found that flies with loss of both miR-33 and one copy of Akt show significantly decreased pigmentation compared to miR-33 loss of function alone, suggesting that the increase in pigmentation from loss of miR-33 is due, at least in part, to increased levels of AKT. In our project, we aim to determine whether miR-33 modulates AKT levels directly and further examine how miR-33 affects FOXO target genes 4ebp, ik6 and pudgy as well as genes of the TOR signaling pathway to determine if miR-33 is indeed regulating pigmentation through its impact on AKT.

Fragments of tRNA in Drosophila development. L. Guan, S. Karaiskos, A. Grigoriev Department of Biology Center for Computational and Integrative Biology, Rutgers University, Camden, NJ.

The progress of next-generation sequencing technologies has unveiled various non-coding RNAs that have previously been considered products of random degradation and attracted only minimal interest. Among small RNA families, microRNA have traditionally been considered key post-transcriptional regulators. However, recent studies have reported evidence for widespread presence of fragments of tRNA molecules (tRFs) across a range of organisms. Transfer RNA fragments (tRFs) are a class of small RNA molecules derived from mature or precursor tRNAs. Although tRFs have been discovered very recently, gradually they have been attracting more attention. Similar to microRNAs, there is evidence that tRFs are found across a wide range of organisms in cytoplasmic compartments or loaded to RISC complexes, often in numbers comparable to microRNAs. Despite clear differences between tRFs and microRNAs, there is accumulating evidence that tRFs may play a vital role in post-transcriptional gene regulation and RNA silencing. Aiming to elucidate potential tRF functionality in Drosophila species, we compared non-coding RNA sequencing data derived from multiple developmental stages of D. viridis (including embryo, larvae and adult stages). Using sliding 7-mer windows along a tRF, we searched for putative seed sequences for these fragments with high numbers of conserved complementary sites within 3' UTRs of 12 well annotated fly genomes. Last, we performed Gene Ontology enrichment analysis for predicted tRF targets. We detected tRFs originating from 3'- and 5'-ends of tRNAs across multiple developmental stages at significant levels. Furthermore, we found that individual tRFs display different expression patterns during D. viridis development. Similar to our earlier results in D. melanogaster and compatible with other experimental findings, we found “seed” sequence locations on both ends of different tRFs. Putative targets of these fragments were found to be enriched in a number of biological processes including neuronal and developmental functions.

Functional divergence among adaptively evolving TE regulators in Drosophila. Luyang Wang1, Erin Kelleher1, Daniel Barbash2 1) Department of Biology & Biochemistry, University of Houston, Houston, TX; 2) Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY, USA.

Transposable elements (TEs) are ubiquitous genomic constituents, which can relocate or increase their copy numbers within the host genome through mobilization and replication. Unrestricted TE activity can have serious fitness consequences for the host by creating deleterious mutations and inducing DNA damage. Thus, defending the genome from TEs is essential, particularly in the germline, which transmit genetic information to offspring. In metazoan germelines, genome defense is enacted by piRNAs in complex with PIWI-clade Argonaute proteins, which direct transcriptional and post-transcriptional silencing of TEs. The piRNA pathway also includes other proteins that are involved in piRNA biogenesis or piRNA-mediated TE
The piRNA pathway is proposed to adapt to changes in genomic TEs by changing the composition of the piRNA pool. However, several piRNA pathway proteins exhibit signatures of positive selection along the lineages leading to Drosophila melanogaster and D. simulans. To understand what drives these adaptive changes, we performed interspecific complementation on three adaptively evolving piRNA pathway proteins: Aubergine (Aub), Armitage (Armi) and Spindle-E (SpnE). We compared the ability of D. melanogaster and D. simulans wild-type alleles to complement a D. melanogaster mutant background for female fertility, TE transcript regulation and piRNA biogenesis.

Surprisingly, we observed that although D. simulans alleles of all three proteins did not fully complement D. melanogaster mutants with respect to female fertility, they were robust negative regulators of TE transcript abundance. In contrast, D. simulans alleles for two proteins, Aub and Armi, exhibited defects in piRNA biogenesis. Defects associated with Armi were particularly severe; 81 of 84 examined TE families exhibited decreased abundance of piRNAs in the presence of the D. simulans allele. Our results surprisingly suggest that positive selection has not targeted the ability of piRNA pathway proteins to regulate TE transcripts, but rather has acted on their functions in piRNA biogenesis, implicating interactions between proteins and piRNA precursors as a key interface of genetic conflict.

853 Transposon landscapes in aging Drosophila and hybrid dysgenesis crosses. Nelson Lau1, Nachen Yang2, Satyam Srivastav2, Reazur Rahman2 1) Biochemistry, Boston University School of Medicine, Boston, MA; 2) Biology, Brandeis University, Waltham, MA.

In young animals, genetic mechanisms strongly repress transposable elements (TEs) to ensure genome integrity. However, this silencing capacity declines in aging Drosophila, leading to reactivated TE transcripts. These observations support a hypothesis that TE reactivation during aging can subsequently alter and damage the genome. To test whether aging animal genomes exhibit altered TE Landscape (TLS) reflecting TE transcript reactivation, we deeply sequenced genomes of young and aged Drosophila strains of wild-type and mutant backgrounds. We quantified TLS in aging flies with our TIDAL program, and our approach validated significant increases in new TE insertions in aging Drosophila genomes mutated in the RNAi and Piwi pathways. Interestingly, other pathway mutations that reactivated TE RNA expression exhibit modest genomic TL changes. Our analysis suggests that small RNA surveillance mechanisms still prevent genomic TL expansion despite the increase in transposon transcripts during aging.

We also reexamined the relationship between high transposition activity and transposon genomic load in Drosophila, by focusing on the P-element-mediated gonadal dysgenesis (GD) phenomenon in D. melanogaster. We found that certain Oregon-R substrains have greater P-element genomic loads than the Harwich strain, a prominent strain with strong P-element activity. However, these OreR substrains are less severe as Har males in triggering complete GD in daughters when mated to a P-element-sensitive ‘M’ strain females. To further test this hypothesis, we also generated hybrid strains of Har with iso1 that retain less than 6% of the P-element load as Har, yet retain as strong of a GD effect as Har. We propose that a novel feature of P-elements in Har may distinguish its higher activity compared to other Drosophila strains with high P-element genomic loads.


Dysregulation of the degradative cellular process known as autophagy has been implicating in numerous diseases, including many of the most prevalent neurodegenerative disorders, and has been strongly linked to the aging process. Recent work in disease models is beginning to demonstrate that long non-coding RNAs (lncRNAs) play a role in regulating autophagy pathways, while indicating that novel lncRNAs and their autophagy regulatory mechanisms have yet to be identified. Using the Drosophila Genetic Reference Panel (DGRP), we performed a Genome Wide Association Study and identified numerous autophagy-associated SNPs that target proteins, unannotated IncRNAs, and nearby intergenic regions. RNAi screening verified the key role of SNP-associated protein-coding genes in the regulation of autophagy and lysosomal function. We further confirmed the correlation between the expression of several candidate IncRNAs with lysosomal activity in DGRP fly lines. We are currently generating IncRNA knockout lines using CRISPR/Cas9 technology in order to examine the regulatory role of these IncRNAs in autophagy and lysosomal functions. The goal of this research is to identify novel IncRNAs involved in autophagy regulation, and to determine the mechanisms behind this regulation.


The three genes of the BX-C, Ubx, abd-A and Abd-B, along with 9 segment-specific regulatory domains, specify the identity of the segments that form the posterior thorax and the abdomen of the fly. There is a 4th transcription unit encoded by the BX-C called the iab-8 ncRNA. The iab-8 ncRNA transcription unit is 94kb-long, spanning the entire intergenic region between abd-A and Abd-B. Intriguingly, it is spliced, picking up an exon in each of the iab regulatory domains. In agreement with its promoter located within iab-8, it is expressed in the 8th and 9th abdominal segments (PS13 and PS14). While expression starts in epidermis during early embryogenesis, it is then restricted to the central nervous system. The iab-8 ncRNA is the template
for miRiab-8, a microRNA imbedded within the 5th intron (in iab-3). Deletion of miRiab-8 result in complete sterility in both sexes. This sterility is caused by a behavioral problem. Mutations alleviating the production of the whole iab-8ncRNA leads to de-repression of abd-A in the posterior CNS. This repression is partly mediated by miRiab-8 that target the 3'UTR of abd-A. However, there is a second mechanism to ensure complete abd-A repression. This second repression mechanism was proposed to be transcriptional interference.

To gain further insights on transcriptional interference we made a series of reporter systems reproducing the cis-interaction between the iab-8ncRNA and the abd-A promoter within the BX-C. We show that the iab-8nc RNA partially repress expression of a GFP transgene driven by the endogenous abd-A promoter, even though this transgene does not contain target sites for miRiab8. Furthermore, the iab-8ncRNA can also block interaction between Gal-4 and its target UAS sites, when UAS sites are placed inside the iab-8ncRNA transcriptional unit. Our reporter genes suggest that transcriptional interference is restricted to the CNS and we are presently investigating if it due to the action of the RNA-binding protein elav. Finally, we are interested in the biological significance of the repression of abd-A in the PS13 CNS. It has been previously described that abd-A controls the end of neuroblast proliferation by programmed cell death. The iab-8ncRNA may be important to prevent premature neuroblast apoptosis and misexpression of abd-A in PS13 due to the failure of its repression could be the cause of sterility.

856 Modeling polycystic kidney disease in the fly. C. Gamberti1, David R. Hipfner2, Marie Trudel3, William D. Lubell4 1) Biology, Concordia University, Montreal, PQ, CA; 2) Institut de recherches cliniques de Montréal, Canada; 3) Department of Chemistry, Université de Montréal, Montreal, Canada.

Progressive cystic kidney degeneration underlies diverse renal diseases, including the most common cause of kidney failure, autosomal dominant Polycystic Kidney Disease (PKD). Polycystic kidney disease affects 12.5 million people worldwide and there is no cure. Knowledge of the underlying molecular and cellular mechanisms of PKD is needed; however, progress has been hindered in vertebrate models because of the morphological complexity of the kidney. Drosophila mutants lacking the translational regulator Bicaudal C (BicC, the fly ortholog of vertebrate BICC1 implicated in renal cystogenesis) exhibited progressive cystic degeneration of the Malpighian (renal) tubules and reduced renal function. The BicC protein was found to bind to Drosophila myc mRNA in Malpighian tubules. Elevation of the cognate Myc protein caused tubular degeneration in BicC mutants. Activation of the Target of Rapamycin (TOR) kinase pathway, another common feature of PKD, was found in BicC mutant flies. As observed in PKD patients and rodent models, rapamycin administration substantially reduced the cystic phenotype in flies. We present new mechanistic insight on BicC function and propose that Drosophila may serve as a genetically tractable model for dissecting the evolutionarily-conserved genetic network underlying renal cystogenesis.

857 Muscleblind is a novel modifier of FUS-associated amyotrophic lateral sclerosis (ALS). Ian Casci1, Karthik Krishnamurthy2, Sukhleen Kour1, Nandini Ramesh1, Rogan Grant1, Stacie Oliver1, Lauren Gochenaur1, Krishani Patel1, Piera Pasinelli2, Uhai Pandey1 1) Pediatrics, University of Pittsburgh Medical Center, Pittsburgh, PA; 2) Thomas Jefferson University, Philadelphia, PA.

Pathogenic mutations in FUS lead to amyotrophic lateral sclerosis (ALS) with varying ages of onset, progression and severity, suggesting that additional, unknown factors might contribute to disease pathogenesis. We performed an unbiased genetic screen using a fly model of ALS and discovered muscleblind as an unexpected and novel modifier of FUS toxicity. Muscleblind regulates cytoplasmic mislocalization of mutant FUS, and subsequent accumulation in stress granules, providing insights into pathogenic mechanisms of disease.

858 Drosophila germ granule mRNAs self-organize through a nucleation and cis-regulated recruitment mechanism. M.G. Niepielko, E.R. Gavis Molecular Biology, Princeton University, Princeton, NJ.

The co-packing of different mRNA types into macromolecular structures called ribonucleoprotein particles (RNPs) is a conserved strategy for the regulation of mRNA metabolism. In many animals, the formation of complex RNPs called germ granules is essential for the post-transcriptional regulation of mRNAs that are required for germline maintenance. In Drosophila, the germ granules are assembled at the posterior of the egg and are inherited by the primordial germ cells during embryogenesis. The germ granules contain a common set of core proteins but are heterogeneous with respect to both the identities and amounts of their constituent mRNAs. We and others have shown that within germ granules, mRNAs are organized as spatially distinct clusters, called homotypic clusters, which contain multiple copies of an individual mRNA. It remains unclear, however, how heterogeneity arises and how mRNAs become organized. Here, we have used quantitative image analyses to investigate how two mRNAs with key roles in germline development, nanos (nos) and polar granule component (pgc), are packaged into germ granules. We find that RNPs containing a single nos or pgc transcript can populate the same granule and that these initial RNPs seed homotypic clusters that grow by self-recruitment. Within a granule, homotypic clusters of different mRNAs grow independently of each other and their growth depends on the simultaneous growth of the granule protein core. Investigation of the mechanisms regulating the targeting and recruitment of mRNAs to germ granules revealed a distinct cis-acting element in the nos 3'UTR that is largely responsible for homotypic cluster growth. Together, our results indicate that the delivery of sufficient quantities of mRNAs to the primordial germ cells relies on the growth of homotypic clusters that is regulated, in part, by cis-acting recruitment elements.
Mechanism of mRNA localization to Drosophila germ granules. T. Treck Pulisic¹, T. Douglas¹, M. Grosch¹,², H. Shroff¹, T. Lionnet¹, R. Lehmann¹ ¹) HHMI, Skirball Institute of Biomolecular Medicine, NYU School of Medicine, NY, USA; 2) Ludwig Maximilian University of Munich, Munich, Germany. 3) Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, NIH, MD, USA; 4) Institute for Systems Genetics, NYU School of Medicine, NY, USA.

Germ granules are a hallmark of all germ cells. In Drosophila, an estimated 200 mRNAs are enriched in the germ granules where they regulate germ cell formation, specification, survival and migration. Using single-molecule FISH and super-resolution microscopy, we have shown previously that localized mRNAs are distributed asymmetrically within the granules whereas core germ plasm proteins are distributed evenly throughout granules. Multiple localized mRNAs organize into homotypic clusters that occupy defined positions within the center or periphery of the granule. Moving forward, we aim to understand how these clusters form and what is their biological relevance. Our data demonstrate that mRNAs themselves determine the position of clusters in granules and not germ granule proteins. Our in vitro experiments further show that RNAs homotypically cluster in the absence of proteins and that these trans RNA:RNA interactions are driven by GC-rich palindromic RNA sequences located in the 3'UTR of localized transcripts. Thus, homotypic mRNA clustering is an RNA-driven process. Our data also suggest that mRNAs could also drive their own enrichment to granules. We propose that mRNAs initially seed into germ granule via germ granule proteins and afterwards increase their localized concentration through trans RNA:RNA interactions and mRNA self-entrapment thereby forming homotypic clusters. Our work reveals a new regulatory mechanism for localization and spatial organization of mRNAs to germ granules that may be applicable to other mRNP granules. This work was supported by the Intramural Research Programs of the US National Institute of Biomedical Imaging and Bioengineering awarded to HS. TT is an HHMI Fellow of the Jane Coffin Childs Memorial Fund. RL is an HHMI investigator. This work was also supported by the K99HD099675 Eunice Kennedy Shriver NICHD grant awarded to TT.

Epigenetic control of metazoan transcription and pre-mRNA processing by histone PTMs. Michael Meers¹, Karen Adelman², Robert Duronio¹, Daniel McKay¹, Brian Strahl¹, A. Gregory Matera¹ ¹) Integrative Program in Biological and Genome Sciences, Univ North Carolina, Chapel Hill, NC 27599, USA; 2) Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA.

Histone H3 lysine 36 methylation (H3K36me) is thought to participate in a number of pre-mRNA processing regulatory events. We have developed an innovative genetic platform in Drosophila that allows direct interrogation of the function of specific histone residues. We can now study the biological function of a specific histone PTM, by changing the acceptor residue to an amino acid that cannot be appropriately modified. Our work to date illustrates the strength of this direct approach, revealing that histone point mutants often exhibit only a subset of the phenotypes caused by mutations in their cognate writer enzymes (McKay et al. 2015; Meers et al. 2017a, b; Penke et al. 2016, 2017). In this presentation we focus on the function of H3K36 in Drosophila using a lysine-to-arginine (H3K36R) mutant. We observed global dysregulation of mRNA levels in H3K36R animals that correlates with the incidence of H3K36me3. Similar to previous studies, we found that mutation of H3K36 also resulted in H4 hyperacetylation. However, neither cryptic transcription initiation, nor alternative pre-mRNA splicing, contributed to the observed changes in expression, challenging previously reported roles for H3K36me. Interestingly, knockdown of the RNA surveillance nuclease, Xrn1, and the deadenylase CCR4, restored mRNA levels for a class of downregulated, H3K36me3-rich genes. We propose a post-transcriptional role for modification of replication-dependent H3K36 in the control of metazoan gene expression. References:


The initial studies found that specific mRNA transcripts, encoding postsynaptic domain proteins, were localized to the megaRNP granules in muscle nuclei. Furthermore, it was demonstrated that the formation of these granules is dependent on synaptic activity. The observation that mRNA messages found in the granules were also localized to the postsynaptic domain of the NMJ, led to the proposal that these megaRNP granules would be released from the nucleus and traffic to the synapse for local translation. However, there is currently no direct evidence for this hypothesis.

Interestingly, our recent studies have uncovered that disruptions in splicing lead to increases in the NEB budding process, as assessed at the light microscopy and EM level. Inhibition of core splicing components such as Prp19 and Prp22, via tissue specific RNAi knockdown, led to large increases in the apparent usage of NEB. Moreover, disruption of alternative splicing factors such as Pasilla, Smn and Muscleblind also led to increases in budding. Collectively these results suggest that nuclear envelope budding may represent a method to rid the nucleus of transcripts that are not properly spliced. In particular, we are exploring the possibility that transcripts with retained introns may be targeted to this pathway for ultimate destruction in the cytoplasm. Lastly, using models at the NMJ and in the salivary gland, we are testing the idea that large transcriptional demands on a given gene will lead to increased splicing errors and thus the need for the NEB pathway to clear these transcripts from the nucleus. These studies will ultimately lead to a greater understanding of this enigmatic nucleocytoplasmic transfer mechanism.
involvement of Tet in either myoblast fusion, or muscle pathfinding and attachment, will be presented. Additionally, Tet function is required both for the proper development of sensory organ precursors (SOPs) of the PNS as well as for the function of the mature PNS. Tet mutant larvae exhibit profound defects in mechanical and thermal nociception. These defects arise from the loss of Tet function in larval multi-dendritic sensory neurons.

864  **Convolutional Neural Networks Based Segmentation of in vivo Drosophila Heart Imaging with Optical Coherence Microscopy.** L. Duan, X. Qin, X. Sang, Y. He, J. Pan, T. Xu, J. Men, R. Tanzil, A. Li, Y. Ma, C. Zhou 1-5 1) Department of Electrical and Computer Engineering, Lehigh University, Bethlehem, PA, USA; 2) Department of Electrical Engineering and Computer Science, Hainan University, Haikou, China; 3) School of Precision Instrument and Optoelectronics Engineering, Tianjin University, Tianjin, China; 4) State Key Laboratory of Software Engineering, Wuhan University, Wuhan, China; 5) Department of Bioengineering, Lehigh University, Bethlehem, PA, USA; 6) Genetics and Aging Research Unit, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, MA, USA.

Optical Coherence Microscopy (OCM) is an emerging imaging technology, which enables cross-sectional and 3D structural imaging of biological tissues in vivo. OCM has been used to characterize morphological or functional information about the beating *Drosophila* heart non-invasively. We developed a novel ultrahigh resolution OCM system to acquire time-lapsed OCM images of the Drosophila heart with an axial resolution of ~1.5um and a transverse resolution of ~3.9 um. With the large amount of imaging data collected, there is a great need of an accurate and efficient tool to segment and analyze the OCM images in order to characterize *Drosophila* heart function. Convolutional neural network is a fast developing and powerful machine learning algorithm. Among the different kinds of usage of convolutional neural network, semantic segmentation networks aim to segment the input image and provide pixel based classifications with high accuracy. By using convolutional layers and de-convolutional layers, a well-designed and well trained neural network is capable of giving universal, stable and fast segmentation result of a given input image. In this study, we developed a semantic segmentation neural network to identify and mark the heart regions of *Drosophila* in the cross-sectional OCM images. Five groups of convolutional layers and four groups of de-convolutional layers were used in our model to generate a pixel based prediction mask. Over 20,000 *Drosophila* heart OCM images were labeled with ground truth and used in this model for training. We were able to achieve a high intersection over union (IOU) accuracy rate of ~85%. Based on the automatic segmentation algorithm, accurate heartbeat profiles from different specimens were generated with high efficiency. In summary, we demonstrated a reliable method to automatically segment and quantify *Drosophila* heart regions from OCM images, paving ways for analyzing various morphological and dynamic parameters, such as heart diameters, heart area, and heart rate, of beating *Drosophila* hearts.

865  **Measuring Feeding Behavior of Individual Flies in a 96-well Format.** S.J. Karott, M.D.L.A Jaime, G. Salem, J Krynitsky, M. Garmendia-Cedillos, S. Anderson, S. Harbison, T.J. Pohida, B. Oliver 1) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, MD; 2) Signal Processing and Instrumentation Section, Center for information Technology, NIH, Bethesda, MD; 3) National Heart, Lung and Blood Institute, National Institutes of Health, 10 Center Drive, Bethesda MD 20814 USA.

There is an expanding interest in the scientific community for monitoring devices that can assess Drosophila feeding behavior, but existing systems are limited in terms of fly confinement and assay throughput. To address this, we developed the Monitoring Unit for Fruit Fly Imaging in Ninety-six well-plates (MUFFIN), a tool that is compatible with high-throughput assays due its 96-well plate format. The system consists of an array of 24 cameras, enabling each camera to monitor a 4-well area in the plate. This innovative design allows for 24-7 simultaneous image acquisition and recording of each well. An elaborate 3D CAD design ensured a compact system with a small benchtop footprint that should scale-up to large studies employing multiple MUFFINS. Algorithms are used to detect fly silhouettes in the image. The silhouette is used to determine proximity to food and eating bouts. Furthermore, the centroid of the silhouette is used to compute an activity measure based on image distance traveled. In our experiments, we used the Whole Animal Feeding Flat (WAFFL), to house and feed the flies. The WAFFL consists of a 96-well plate with enough space for flies to move and have access to a food plate that is present beneath it through the pores present in each well. We quantified fly survival as well as differences in behavior between starved and fed flies in terms of proximity to food. We observed that flies exhibited higher activity when introduced to 100 kcal media after being starved for 6 hours with PBS in comparison to flies that were instead fed during the time preceding introduction to food. We also determined the frequency at which the flies were feeding and the duration of each feeding period based on whether the flies were starved or not. The versatility of MUFFIN will help expand the scope of many existing high-throughput assays using Drosophila, as well as allow for the collection of new data and the design of behavioral and pharmacology studies previously not practical to implement.


Sequencing of pooled individuals (Pool-Seq) is a powerful method to address important questions in evolutionary biology and population genetics. By aggregating the sequences of tens to thousands of individuals, Pool-Seq allows detection of
patterns in allele frequencies across samples. However, one complication of this method is that the individuals in the pool may contribute different amounts of DNA, leading to misrepresentation of certain individuals in the resulting sequences. Such differences in DNA may arise from variation in size, stage of development, or tissue sampling. One possible solution to this problem is through arresting the embryos at a specific stage of development, where each individual is expected to have the same number of cells, and thus DNA, contributing to the pool.

Here, we test a maternally-transmitted shRNA targeting the *dorsal* gene for inducing embryo arrest in multiple genetic backgrounds of *Drosophila melanogaster*. The shRNA is produced maternally and transferred to the oocyte during oogenesis, leading to RNA interference of the *dorsal* gene during early embryogenesis. Both components of the gene-silencing mechanism, which includes a UAS-drive shRNA and mata4-Gal4 protein, are located on the X chromosome. Thus, in crosses of males carrying the transgenes to females without it, we expected F1 daughters to express the shRNA and their progeny to arrest as embryos. We tested the efficiency of this strategy by crossing males carrying the silencing construct to females from each of five divergent strains from the DGRP and GDL. We then crossed F1 females to their brothers and measured the efficacy of arrest by quantifying the ratio of unhatched to hatched eggs (i.e. arrested embryos). Preliminary results in a single strain suggest a highly efficient rate of arrest (>99%).

If this gene-silencer proves to be successful across strains, it could be utilized for reducing the error in the pool-sequencing method. Thus, embryo arrest could be used in conjunction with this technique to make it an even more reliable tool for population genetics.

867  **Cell-based screen technologies at the Drosophila RNAi Screening Center.**  S. Mohr1,2, Y. Hu1,2, R. Viswanatha1, B. Housden3, K. Sierzputowska4, G. Amador1,2, A. Comjean1,2, V. Chung3, J. Zirin1,2, N. Perrimon1,2,4 1) Dept Gen, Harvard Med Sch, Boston, MA; 2) DRSC, Harvard Med Sch, Boston, MA; 3) Living Systems Institute, University of Exeter, UK; 4) HHMI, Boston, MA.

Since 2003, the Drosophila RNAi Screening Center (https://fgr.hms.harvard.edu/) has been serving as a high-throughput cell-based screening facility and technology transfer center. We recently identified two new technologies as a focus for development: CRISPR pooled screening with sgRNA libraries and variable dose analysis screening with shRNA libraries. Both technologies can be used to identify essential genes under baseline or perturbed conditions, and each has experimental and practical advantages. We will present these methods and their requirements, as well as example applications, to help facilitate experimental design and use of these approaches for genome-scale cell-based screens by the community. RNAi screening in arrayed formats continues to be supported at the DRSC through custom, focused, and genome-wide libraries useful for off-site or on-site screens using luminescence, fluorescence, confocal imaging, and other readouts. Together with our bioinformatics and other resources, as well as through collaborations with other centers and laboratories, we provide access to the full suite of resources needed for a functional genomics workflow, from gene and reagent identification to low-throughput screening and data analysis and integration. We will present standard workflows for a variety of project types in order to get researchers thinking about how to use our resources for efficient and effective experimental studies exploring diverse topics.

868  **Optimized tissue-specific knockout via CRISPR/Cas9 reveals gene perdurance and redundancy in neuronal morphogenesis in Drosophila.**  Amy Poe, Bei Wang, Chun Han  Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY.

Loss-of-function (LOF) analysis in a tissue-specific manner is instrumental for studying developmental roles of essential genes, determining cell autonomy, and dissecting cell-cell interactions. The CRISPR/Cas9 system has the potential to surpass current methods of tissue-specific LOF due to its simplicity and efficiency in creating gene knockout. In this study, we aim to optimize CRISPR/Cas9-based tools to achieve highly efficient knockout of one or more genes in a tissue-specific manner in *Drosophila*. For this purpose, we developed convenient tools for generating and evaluating enhancer-driven Cas9 lines, and identified a multi-gRNA design that is more effective in generating gene knockout in somatic cells than previously described designs. By detecting large DNA deletions induced by two gRNAs in individual neurons, we found that the efficiency of large DNA deletion is affected by the target locus and by the distance between the gRNAs. Furthermore, we compared enhancer-driven Cas9, Gal4/UAS-driven Cas9, and RNAi in tissue-specific LOF and found that enhancer-driven Cas9 resulted in the least perdurance and cytotoxicity in post-mitotic neurons. Lastly, by using our optimized tissue-specific CRISPR/Cas9 tools, we found that the receptor protein tyrosine phosphatase Ptp69D has very little perdurance in dendrite morphogenesis of larval peripheral sensory neurons whereas genes of the SNARE complex exhibit high perdurance and perdurance.

869  **A new resource from the TRiP: sgRNA stocks for gene overexpression and knockout by CRISPR-Cas9.**  J. Zirin1, B. Ewen-Campen1, Y. Hu1, L. Liu1, D. Yang-Zhou1, R. Colbeth1, E. Vogt1, C. Villalta1, G. Amador1, A. Comjean1, V. Chung1, S. Kondo2, J. Ni1, S. Mohr1, N. Perrimon1,2,4 1) Dept of Genetics, Harvard Medical School, Boston, MA; 2) Invertebrate Genetics Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan; 3) Tsinghua Fly Center, Tsinghua University, Beijing, China; 4) Howard
Hurdle to make data integration a common laboratory practice. There is a treasure trove of underused data sitting in public repositories and this work addresses the first hurdle to make data integration a common laboratory practice. Genomics groups will be able to use QC metrics and technical metadata to identify high quality and useful samples for use in downstream analysis. This resource will classify samples by library strategy (i.e., RNA-seq, DNA-seq, ChIP-seq) and provide both coverage counts and browser tracks for all samples mapped using an identical workflow to FlyBase release 6.11. We use a data driven approach to describe both technical and biological metadata for each sample, and determine optimal mapping parameters. Our pre-alignment workflow provides a set of more than 10 quality metrics, including library layout (i.e., single or pair-ended) and library strandedness (i.e., first, second, or unstranded). Next our alignment workflow re-aligns all samples using HISAT2, aggregates technical replicates (SRRs) to the library level (SRX), and generates both coverage counts and normalized genome browser tracks. We then use a machine learning approach to classify samples by library strategy (i.e., RNA-seq, DNA-seq, ChIP-seq). Finally we use another classifier to separate samples into tissue or cell types. This resource will allow any researcher to visualize browser tracks for any publicly available dataset. Genomics groups will be able to use QC metrics and technical metadata to identify high quality and useful samples for use in their own research. There is a treasure trove of underused data sitting in public repositories and this work addresses the first hurdle to make data integration a common laboratory practice.

870 Functional characterization of ultra-conserved small open reading frame (smORF) genes. J. A. Bosch1, F. Escobedo1, B. Brown2, S. Celinker1, N. Perrimon1, 2) Lawrence Berkeley National Laboratory, Berkeley, CA.; 3) Howard Hughes Medical Institute, Boston, MA.

Naturally produced small peptides (Drosophila). This finding is extraordinary and suggests that there are many more genes in the genome than previously thought. However, characterizing these genes remains a daunting challenge, as each smORF must be tested for translation and function in vivo. In order to enrich for smORFs with a high likelihood of translation and functional importance, we have identified a set of 171 smORF genes with protein sequence conservation between human and Drosophila, most of which are conserved among other bilaterian species. Our goal is to functionally characterize this select list of ultra-conserved smORF genes in Drosophila. Toward this end, we have been generating transgenic smORF lines to perform loss-of-function (CRISPR knockout) and gain-of-function (CRISPR activation) genomics for each smORF gene in vivo. From our ongoing phenotypic analysis, we have so far identified two genes that are lethal during larval development when knocked out. We found that one gene is expressed in the brain and encodes a protein that is secreted from cultured cells, suggesting it may act as an extracellular ligand or neurotransmitter. The second gene encodes a membrane-spanning protein and its human homologue is frequently lost in cancer lines. Importantly, these two genes are uncharacterized in any organism. These initial results validate our approach to discover functionally important novel genes. Taken together, our gene dataset of 171 ultra-conserved smORF genes, construction of genetic tools, and phenotypic analysis represents a genome-scale effort to characterize smORFs in a model organism. We expect this on-going study will provide a wealth of information on the biological functions of this poorly characterized class of genes.

871 482 Billion Reads and Counting: Re-mapping the Entire Drosophila Sequence Read Archive. Justin Fear, Zhen-Xia Chen, Brian Oliver National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

To maximize the durability reuse of omics data to generate new knowledge, data needs to be updated to new genome annotations and treatment in a common analysis pipeline. The sequence read archive (SRA) is a public repository of high throughput sequencing data, which contains over 52 terabases or 482 billion reads of Drosophila melanogaster data in >35k samples (as of Nov 2017). This is more than 15 times the size of the modENCODE datasets and is massively underused by the community. We have reprocessed this data to make them easier to find, access, and use. Specifically, we present sample characteristics of all 35,263 D. melanogaster SRA runs (SRRs) to allow users to find high quality datasets that meet specific needs and provide both coverage counts and browser tracks for all samples mapped using an identical workflow to FlyBase release 6.11. We use a data driven approach to describe both technical and biological metadata for each sample, and determine optimal mapping parameters. Our pre-alignment workflow provides a set of more than 10 quality metrics, including library layout (i.e., single or pair-ended) and library strandedness (i.e., first, second, or unstranded). Next our alignment workflow re-aligns all samples using HISAT2, aggregates technical replicates (SRRs) to the library level (SRX), and generates both coverage counts and normalized genome browser tracks. We then use a machine learning approach to classify samples by library strategy (i.e., RNA-seq, DNA-seq, ChIP-seq). Finally we use another classifier to separate samples into tissue or cell types. This resource will allow any researcher to visualize browser tracks for any publicly available dataset. Genomics groups will be able to use QC metrics and technical metadata to identify high quality and useful samples for use in their own research. There is a treasure trove of underused data sitting in public repositories and this work addresses the first hurdle to make data integration a common laboratory practice.
Segregation distortion and meiotic drive, deviations from expected Mendelian transmission ratios, can cause rapid allele frequency shifts in populations. While strong distorters can be identified relatively easily, subtle distortion can be difficult to detect because of the number of individuals required for screening. Subtle distorters may be more common than strong ones in nature and their genetic basis and evolutionary dynamics remain understudied.

Sequencing the combined DNA from pools of individuals (Pool-seq) is a cost-effective way to estimate allele frequencies across a large number of samples. This technique can be used to estimate allele frequencies in any sample of individuals, including detecting segregation distortion in pools of offspring. However, to detect subtle deviations, many (1000s of) individuals must be pooled and sequenced to high depth, thus increasing sequencing costs and decreasing throughput. Here, we show that sequencing a reproducible subset of the genome across pooled samples using double-digest restriction-site association DNA sequencing (Pool-ddRADseq) enables detection of subtle segregation distortion in Drosophila melanogaster. By sequencing only a subset of the genome, we significantly lower costs and increase the number of samples that can be assessed in a single lane of sequencing. First, we develop a pipeline that statistically tests for distortion due to the effect of a single locus in sequencing data. We then investigate the effects of recombination, sequencing depth, the number of loci sequenced, and the number of individuals in the pool on our power to detect distortion using simulated Pool-ddRADseq data. Finally, we apply the pipeline to Pool-ddRADseq data from a controlled cross that emulates 5% distortion. Preliminary results suggest that this can be applied to conduct high-throughput screens for segregation distortion. Thus Pool-ddRADseq can greatly accelerate the discovery of subtle distorters in manipulative experiments or surveys of wild-caught strains.

Deconvolution of bulk sequencing variation profiles into constituent clonal genotypes in tumors remains a formidable challenge. We present a novel phylogenetic method (CloneScape) to deduce clone genotypes at a single base resolution, along with clonal and subclonal frequencies in each sample from a single individual. CloneScape accurately reconstructs the most recent common ancestor of all clone genotypes, which is a direct descendant of the earliest stem clones, as well as intermediate and sample-specific subclonal genotypes. CloneScape performs better than current approaches and will enable reliable inference of clonal phylogenies, origins, and mutations involved in cancer progression.

The power of sequencing experiments such as RNA-seq, ChIP-seq, methyl-seq, and other seqs, comes from their ability to survey the entire genome, transcriptome, methylome (and other -omes) at once. Furthermore, these experiments almost always produce more data than is used for a particular study. These data can therefore be potentially used to answer other, drastically different questions without the need for additional sequencing. The biggest problem with re-using existing data is identifying datasets that are relevant. The current size of the Short Read Archive (SRA) at NCBI—a central repository for sequencing data—is ~5.3 × 10^15 nucleotides and such amount of data cannot be searched with current methods. The Sequence Bloom Tree (SBT), introduced last year is a data structure to efficiently index and search a set of sequences. SBT index is compact and can be searched orders of magnitude faster compared to previous approaches. Here we present a Galaxy-based web portal that can be used to search through SRA-sized datasets in minutes to identify relevant datasets. We illustrate the utility of this system for Drosophila research community by applying it to curated sets of RNA-seq datasets for a number of fruit fly species.

FlyAtlas.org is an Affymetrix microarray-based tissue expression atlas, covering 26 adult and larval tissues of Drosophila melanogaster (1,2). Its release revealed a substantial appetite for such data, with over 1700 citations (Google Scholar) since 2007. Generated in the same lab, with the same fly line raised on the same diet, as FlyAtlas, the FlyAtlas2.org resource employs a completely new set of expression data based on RNASeq and miRNASeq, rather than microarray analysis, and so provides information on the expression of different transcripts of a gene (3). Furthermore, the data for somatic tissues are now available for both male and female adult flies, allowing studies of sexual dimorphism. Gene coverage has been extended by the inclusion of microRNAs. The web interface has been modified to accommodate the extra data, but at the same time
has been adapted for viewing on small mobile devices (4). Users can inspect the RNAseq reads alongside the annotated Drosophila genome in the (external) UCSC browser, and are able to link out to the previous FlyAtlas resource to compare the data obtained by RNAseq with that obtained using microarrays. In this poster, we present tutorials on how to access the richness of the data, together with comparisons of FlyAtlas v FlyAtlas2 datasets. We also show substantial and enlightening systematic differences between male and female somatic transcriptomes across multiple adult tissues; and similar specificity in miRNA somatic adult expression.

In keeping with the open access ethos of the FlyAtlas project, these data have been deposited in the European Nucleotide Archive (5).

Acknowledgments: This work was supported by the Biotechnology and Biological Sciences Research Council [BB/K019953/1]. RNA sequencing was carried out by Edinburgh Genomics, The University of Edinburgh. Edinburgh Genomics is partly supported through core grants from Natural Environment Research Council (R8/H10/56), Medical Research Council (MR/K001744/1) and Biotechnological and Biological Research Council (BB/J004243/1).

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4)  http://flyatlas2.org
5)  https://www.ebi.ac.uk/enas/data/view/PRJEB22205

876  DRSC Informatics Tools for Functional Genomics Studies – 2018 update.  C. Y. Hu1, A. Comjean1, V. Chung1, A. Vinayagam1, A. Nand1, N. Nipun1, T. Hao2, FlyBase Consortium2, N. Perrimon1, S. E. Mohr1 1) Genetics, Harvard Medical School, Boston, MA; 2) Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Boston MA; 3) Howard Hughes Medical Institute, 77 Avenue Louis Pasteur, Boston, MA; 4) Harvard University, Cambridge, MA.

A set of online informatics tools has been developed at Drosophila RNAi Screening Center (DRSC) to help scientists identify genes, select RNAi reagents, analyze high-throughput datasets and validate results. Here, we present recent updates to existing tools and new tools. DIOPT (flyrnai.org/diopt) was developed for query of predicted orthologs among 9 common model systems by integrating 14 ortholog prediction approaches. We extending DIOPT by building a resource called gene2function (gene2function.org). DIOPT can help researchers to quickly identify orthologous genes while gene2function brings information of all orthologs together in a table format so that research can quickly survey what is known about each ortholog. The information includes disease association, drug target, GO, publication, interaction data, RNAi/CRISPR data, phenotype and expression info, ORF clones as well as researchers. At Gene2Function, a graphical overview comparing evidence-based GO annotation across all orthologs is also provided.

DRSC officially launched MIST (Molecular Interaction Search Tool) in Aug 2017. MIST integrates protein-protein interactions and genetic interactions of many public resources eg BioGrid, intAct, FlyBase, DroID as well as mapping interaction data among major model organisms as interologs. The user-interface allows users to search single gene, a list of genes or a list of gene pairs, then identify all the interactions and build network. The user-interface was built with different filters so that user can build different types of networks or network at different confident level.

iProteinDB (flyrnai.org/tools/iproteindb/web/) is a new integrated resource of post-translational modification (PTM) data. For example, users will be able to query phosphorylation sites identified in large-scale phosphoproteomics studies as well as annotation from literature curation.

877  FlyExpress 7: A novel discovery platform that integrates genome sequence data with spatiotemporal patterns of expression from in situ hybridizations in Drosophila.  Rob Kulathinal1,2, Stuart Newfeld3, Maxwell Sanderford1, Sudhir Kumar1,2 1) Institute for Genomic and Evolutionary Medicine, Temple University, Philadelphia PA; 2) Department of Biology, Temple University, Philadelphia PA; 3) School of Life Sciences, Arizona State University, Tempe AZ.

Examining developmental gene expression patterns provide key clues about a gene’s potential function and its regulatory interactions. Drosophila melanogaster is ideal for such investigations as multiple individual and high-throughput efforts have captured the spatiotemporal patterns of thousands of embryonic expressed genes in the form of in situ images. FlyExpress (www.flyexpress.net), a knowledgebase based on a massive and unique digital library of standardized images and a simple search engine to find co-expressed genes, was created to facilitate the analytical and visual mining of these patterns. Here, we demonstrate the next generation of FlyExpress resources to facilitate the integrative analysis of sequence data and spatiotemporal patterns of expression from images. FlyExpress 7 now includes over 100,000 standardized in situ images and implements a more efficient, user-defined search algorithm to identify co-expressed genes via Genomewide Expression Maps (GEMs). Shared motifs found in the upstream 5' regions of any pair of co-expressed genes can be visualized in an
interactive dotplot. Additional webtools and linkouts to assist in the downstream validation of candidate motifs, in addition to beta-tested prototypes of cis-regulatory motif discovery tools, are also demonstrated. Together, FlyExpress 7 represents our largest effort yet to accelerate discovery via the development and dispersal of new webtools that allow researchers to perform data-driven analyses of co-expression (image) and genomic (sequence) data.

878 New supervised and unsupervised machine-learning methods on p38 MAPK longevity reveals regulation of age-dependent disease proteins. B. Becerra1, S. Ryan2,3, J. Hill1, M. McCall1, K. Kojima4, J. Mobley5, N. Mortimer1, A. Vraillas-Mortimer1,2 1) Biological Sciences, Illinois State University, Normal, IL; 2) Department of Biological Sciences, University of Denver, Denver CO; 3) Chemical and Biological Engineering Department, Colorado School of Mines, Golden, CO; 4) Cancer Center Mass Spectrometry/Proteomics Shared Facility, University of Alabama Birmingham, Birmingham, AL.

Maintaining protein homeostasis is an important aspect of cellular health, however, this process becomes impaired with aging leading to cellular dysfunction. We have previously found that the stress response protein p38 MAPK (p38kb) regulates lifespan and age-dependent locomotor behaviors in Drosophila, via a novel role in mediating protein homeostasis. We have developed new machine-learning approaches to perform proteomic analysis of muscle and brain tissues across the entire lifespan of the long-lived p38K over-expression animals, short-lived p38K mutants, and genetic background controls to determine the changes in the proteome that may be influencing longevity versus accelerated aging. Using unsupervised cluster analysis of differentially expressed proteins, we have discovered sets of proteins enriched with functional annotations. We also used cluster analysis to infer regulatory interactions between differentially expressed proteins. Using supervised machine learning to identify predictors of aging in control flies, we find that the brain and muscle age differently with unique suites of proteins driving the aging process. We also used our machine learning model to predict the age of p38K over-expression and mutant animal samples. We find that the long-lived p38K over-expression animals exhibit a younger profile. Conversely, loss of p38K leads to an accelerated shift with animals prematurely entering older profiles. Interestingly, we find that p38K regulates the levels of a subset of these muscle and brain aging predictor proteins, suggesting that p38K may coordinate a node that drives the normal rate of aging.

879 Combining the auxin-inducible degradation system with CRISPR/Cas9-based genome editing: a novel tool for the conditional depletion of endogenous proteins in Drosophila melanogaster. M. Bence, F. Jankovics, M. Erdélyi Institute of Genetics, Biological Research Centre, HAS, Szeged, HU.

Inducible protein degradation techniques have considerable advantages over classical genetic approaches, which generate loss-of-function phenotypes at the gene or mRNA level. The plant-derived auxin-inducible degradation (AID) system is a promising technique to induce conditional protein depletion. This method is based on the ability of the plant TIR1 protein to incorporate into the highly conserved SKP1/CUL1/F-box (SCF) ubiquitin ligase complex in non-plant cells. In the presence of auxin, the heterologously expressed TIR1 protein is able to induce the proteasomal degradation of target proteins tagged with the auxin-inducible degron (AID) motif. Here, we present a detailed characterization of this method employed during the adult oogenesis of Drosophila.

To examine the effectiveness of the AID system in Drosophila germ line, we inserted the AID motif into the vasa locus by CRISPR/Cas9-based homologous recombination. The AID-tagged Vasa protein showed proper expression and subcellular localization. Furthermore, the protein was fully functional since in homozygous condition it resulted in wild type viable and fertile phenotype. We demonstrated that neither TIR1 overexpression nor auxin treatment cause harmful effects on the adult flies. Our results revealed that the AID system is able to induce efficient and reversible degradation of Vasa protein in the ovary and in the early embryo. The pattern of Vasa depletion correlated well with TIR1 expression, demonstrating that the AID system is able to achieve a fine spatial control of protein destruction. The efficiency of Vasa degradation and the severity of resulted loss-of-function phenotypes were dependent on the applied auxin concentration. Therefore, the auxin not only provides temporal control, but by altering its concentration we can achieve distinct phenotypes and various degrees of penetrance.

In summary, we demonstrated that the AID system is able to induce efficient and conditional degradation of endogenously expressed proteins in Drosophila. This method provides fine spatiotemporal control of protein depletion and allows for the generation of different levels of protein knockdown in a well-regulated manner. These features of the AID system allowed us to gain deeper insight into functions of the highly complex and pleiotropic vasa gene. Furthermore, as the application of AID system can be extended to any protein, it can be a valuable tool in all fields of Drosophila genetics.

This study is supported by grant from the National Research, Development and Innovation Office (K-117010; PD 124446; GINOP-2.3.2-15-2016-00001; GINOP-2.3.2-15-2016-00032). M.B. is supported by the János Bolyai Research Fellowship of the Hungarian Academy of Sciences.

880 Toward an automated systematic approach to multicellular motif analysis in tissue organization. T. Stern1,4, M. Krajnc2, S. Y. Shvartsman1,2,3, E. F. Wieschaus1,3,4 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 4) Howard Hughes Medical Institute.
Over the last three decades, an increasing repertoire of cellular motifs, i.e. conserved behaviors that drive morphological movements during development, has been identified. Thus far, identification and analysis of these behaviors has required meticulous visual inspection of long sequences of microscopic images of thousands of moving and reshaping cells, which is both time-consuming and limited by the observer's perception and discretion. Here, we present the first automated approach for the search and analysis of multicellular motifs of arbitrary complexity and duration based on time-lapse images of cell membranes.

The core of our approach is an algorithm for alignment of two groups of neighboring cells over time, based on dynamic time warping. By comparing the geometries, spatial organization and adjacency relations of constituent cells at each time point, the algorithm recovers an optimal match between the sequences, such that corresponding stages within the motif can be related to one another, while providing a score for overall similarity. Then, similarly to a BLAST search, a “Motif Search” algorithm allows automatic detection of all appearances of a previously documented or newly defined motif in a time-lapse of a tissue, with accuracy levels comparable to a human operator. Search results can be used to generate an accurate dynamic heat-map of the spatiotemporal distribution of the motif within the tissue. Furthermore, hierarchical clustering and variable selection analyses enable uncovering quantitative phenotypic differences in motif behaviors in tissues subject to different genetic perturbations, which may reveal the regulatory roles of specific genes.

Using our approach, we performed a comprehensive mapping of two central motifs, T1 transitions and rosettes, during germ-band extension, showing significant differences in their spatiotemporal distributions. Then, we analyzed altered motif distribution in the maternal triple mutant bicoid nanos torso-like and embryos homozygous for the even-skipped mutation. Lastly, we present our advances toward a “Motif Discovery” algorithm, which is designed to automatically recover a complete dictionary of all elementary multicellular behaviors, including previously unseen ones, within a given tissue.

Our methods can be applied on time-lapses of any imaging modality, using standard computers, and require no previous computational background. The proposed approach will promote a new quantitative, objective and holistic view of tissue organization through cellular behaviors and advance the exploration of underlying regulatory mechanisms.

881 Transgenesis 2.0: Selection-based genome manipulation in Drosophila melanogaster. N. Matinyan1,2, A. Sarrigon-Perdigones1, Y. Gonzalez1, O. Pena Ramos1, K. J.T. Venken1,2,3,4 1) Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; 2) Integrative Molecular Biomedical Sciences Graduate Program, Baylor College of Medicine, Houston, TX; 3) Department of Pharmacology, Baylor College of Medicine, Houston, TX; 4) Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX.

We have developed a drug-based selection and counterselection system for expedited transgenesis and in vivo genome engineering in Drosophila melanogaster. Our system has adapted commonly used drug resistance/sensitivity markers, identical to those used in bacteria and cell culture, for Drosophila. Unlike traditional physical markers which couple phenotypic differences to genetic changes and require extensive screening or counterscreening, drug-based selection markers do not require any screening. Instead in the presence of their corresponding drug, these markers can select for or counterselect against particular genotypes. Furthermore, selection markers are dominant, do not require any special genetic background and are largely independent of each other allowing simultaneous use of multiple markers for complex, multiplex genome manipulations.

We tested several selection and counterselection markers derived from bacteria, viruses, and yeast in vivo in Drosophila melanogaster. Using the ΦC31 transgenesis platform we generated transgenic fly lines bearing either, a drug resistance selection marker, a drug sensitivity counterselection marker, a chimeric fusion of a selection and a counterselection marker, or a fluorescent marker as control. We observed effective selection of transgenic, drug resistant flies expressing selection markers conferring resistance to Geneticin, puromycin, blasticidin, or phleomycin. Furthermore, we were able to selectively kill transgenic Drosophila expressing sensitivity markers for ganciclovir, an antiviral, or the antifungal drug 5-fluorocytosine. In addition, using transgenic flies expressing chimeric fusion markers, we were able to select or counterselect the same strain depending on the drug or drug combination used. Moreover, since each marker only confers robust resistance or sensitivity to its corresponding drug, we crossed several drug resistant transgenic strains together and were able to specifically and robustly select for particular genotypes using combinations of drugs. Finally, we are probing combinations of selection markers to carry out multiplex genome engineering using both ΦC31 and CRISPR/Cas9 platforms. We anticipate selection-based genetics will greatly enhance the speed, complexity, and efficiency of transgenesis and genome engineering overall not only in Drosophila melanogaster, but most likely in many other insect model systems.

882 Drosophila larval fat body preparations to reveal regionalized gene expression. D. Khalili1, R. Krautz2, I Söll1, G Hauptmann1, U Theopold1 1) Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, SE; 2) Gurdon Institute and Department of Physiology, Development and Neuroscience, University if Cambridge, UK.

The insect fat body functions as the central organ to coordinate metabolic activities, detoxification, systemic energy...
homeostasis and innate immune responses. Despite indications of regionalized differences in morphology, biochemical properties and gene expression, the fat body is generally treated as a uniform tissue. Typically, grind-and-bind methods like qPCR, RNA-seq and microarrays have been used to obtain high-throughput gene expression data of wild-type and aberrant fat body samples, which were however devoid of cellular and regional information. We therefore devised a versatile dissection procedure to obtain isolated whole larval fat bodies suitable for detection of differential gene expression at cellular and regional resolution. The fat body preparations were subjected to an optimized and adapted in situ hybridization procedure, which took into account the high lipid content and the delicate structure of the tissue samples. This allowed us to visualize and document regionalized mRNA transcript distribution patterns within whole larval fat bodies.

**883  You're a fly scientist? How do I get the flies out of my kitchen?**  
T.J. S. Merritt  Chemistry & Biochemistry, Laurentian University, Sudbury, Ontario, CA.

Through a completely unscientific survey, I have determined that every scientist that has ever admitted to working on fruit flies has been asked how to get flies out of a kitchen.

Millions of dollars in research support notwithstanding, what people really want to know is how to get flies out of their fruit bowl. Recently, I wrote an on-line piece about trapping fly – and 265,770 people clicked on the link (roughly and I'm not making this up: https://theconversation.com/how-to-kill-fruit-flies-according-to-a-scientist-81740). Capitalizing on this interest, I have created an educational teaching lab based on the version of the fly trap that we use in my lab (and my kitchen). In this lab exercise, students create different types of traps and bait them with a variety of substances. The materials are simple and flexible and the design encourages students to try their own ideas. The exercise allows expansion, to fit the level of the classroom, into the science of chemoreception and various aspects of life history ecology and behaviour. Students learn the fundamentals of experimental design and data analysis, and possibly reduce the number of flies flying in their kitchens at home.

**884  Why the Fly? A K-12 outreach program that promotes the use of Drosophila as a model organism.**  
A. Nagengast¹, H. Mistry¹, J. DiAngelo¹  ¹Biochemistry & Chemistry, Widener University, Chester, PA; 2) Biology & Biochemistry, Widener University, Chester, PA; 3) Division of Science, Penn State Berks, Reading, PA.

Outreach to the public about the use of *Drosophila* as a model organism is critical for continued support of biomedical research. Early exposure at the elementary to high school level increases the public's awareness of model organisms in general. To address this need, we have developed a collection of publically available fly mutants to build excitement and enthusiasm about *Drosophila* at different grade levels. Elementary school students view a subset of the mutants with simple magnifying glasses while high school students can use dissecting microscopes. Students identify the difference between male and female wild type flies and observe flies with genetic mutations that alter wing shape, eye color, body color, body shape and muscle function. Additionally, transgenic flies that express Green Fluorescent Protein (GFP) in different body structures are displayed. Specific fly lines and grade-level appropriate talking points will be addressed.

**885  Sequencing of a novel mutation in Stat92E by a student-led, open-ended research course.**  
J.A. Armstrong, H.L. Pinson, students of BIOL173L Spring 2016  Keck Science Dept. Claremont McKenna, Pitzer and Scripps Colleges, Claremont, CA.

Our half credit course BIOL173L *Molecular Biology Seminar with Laboratory* introduces undergraduates to basic techniques in molecular biology and to independent research. It is designed for students in the Molecular Biology major, typically in their sophomore year, but is open to all interested students. Faculty rotate through the course and often incorporate their own research. In the spring of 2016 I taught the class in a student-led, open-ended format. I presented the students with flies carrying a novel allele in *Stat92E*, which encodes the single STAT transcription factor of the JAK/STAT pathway. I asked them to begin with the flies and end the semester with the determination of the DNA sequence of the Stat92E mutant. My goal was for the 13 students in the course to take full ownership of the project. The students, most of whom had no research experience, determined much of the experimental approach, created protocols, performed the necessary fly crosses, selected and ordered appropriate reagents, designed PCR primers, performed all lab prep work, conducted the work, and interpreted the resulting sequence data. The class designed the syllabus as the semester progressed and their timeline evolved. Flexibility and a willingness to work with change was a necessity. The students successfully sequenced the mutant allele, discovering a point mutation that resulted in an amino acid substitution within the Stat92E DNA binding domain.

**886  Identification of genes required for viability through undergraduate research experiences.**  
C. Vanderfeltz, J. Dyer  Department of Biology, Rockhurst University, Kansas City, MO.

In order to expose undergraduate students to authentic research experiences, we incorporated a lethal mutant mapping project in our Genetics Laboratory courses and as independent study projects at Rockhurst University. The overall scientific goal for this project is to identify the genetic mutations associated with more than 1,500 unknown lethal mutant strains
currently present at the Bloomington *Drosophila* Stock Center at Indiana University. We began by examining lethal mutant strains on chromosome 2 using deficiency mapping to narrow down the genetic locations of these mutations. After mapping to relatively small regions of chromosome 2, complementation testing was performed in order to identify any specific genes that have been mutated in the lethal mutant strains. After identification of potential genes that might contain mutations through these genetic crossing methods, DNA sequencing was performed to identify any mutations present in coding regions of these genes in the lethal mutant strains. Rescue experiments will be used to verify that the lethality phenotypes present in the lethal mutant strains are due to the identified mutations. Through this ongoing project, undergraduate students were able to gain experience in genetic crossing of model organisms in attempts to identify novel information regarding genes required for viability. Our goal is to use the collective information from our crosses to contribute to the scientific community by producing valuable genetic information related to lethality.

887  First Year Research Experience [FYRE, course based research]: Introducing undergraduates to the research enterprise using *Drosophila* adult myogenesis as a model. J.J. Fernandes, Divyalakshmi Sunderajan, Sedlack Kole, Crookes Justin, Brad Brorosky, Sam Lewis, Jeffery Rhoades Biology Dept, Miami Univ, Oxford, OH.

A 2 semester course sequence was developed to engage up to 20 students per academic year in the process of research, raise awareness about research programs in the department and resources at the institution. During the first semester, students were exposed to examples of ongoing research in the neuroscience program, made aware of library resources, microscopy and imaging facilities, developing short presentations based on departmental research topics, and a grounding in basic concepts surrounding the research project to be conducted in the Spring. Students also examined a research poster on Drosophila myogenesis, and developed a basic understanding of ongoing work in the lab. By the end of the first semester, students were able to identify research questions based on cell-cell interactions that are involved in regulating development of the Dorsal Longitudinal muscle fibers in Drosophila. The research project examined a role for the FGF signaling pathway during DLM development using the Gal4-Gal80 system to manipulate heartless using htl-RNAi and DN-Htl. During the second semester, aided by undergraduates working in the research lab, students in the course monitor the project- transfer crosses, collect animals for temperature shifts, perform sectioning and staining of thoracic specimens, collect data and perform analysis. The results are presented at the institution’s annual undergraduate research forum, and the posters are also used to conduct an outreach session at the local high school. Students from the course will present the 2 semester research experience and will also present qualitative outcomes of their course experience. Student presenters: Allaire, Danielle M., Ashner, Emily R., Carrier, Christian H., Carson, William P., Chander, Muskan, Clayton, Callihan A., Neupane, Raghavee, Packett, Jessica S., Parras, Peter R., Reitz, Sarah M., Stoeva, Luiza E., Young, Olivia T. Additional researchers: Bennett, John P., Carew, Jermaine N., Cavalco, Natalie G., Curren, Emily G., Jackson, Megan L., Jacques, Isaiah M., Kaplevatsky, Ryan L., Kuhn, Leah R., Lewis, Samuel D., Megura, Matthew C., Moon, Justin T., Peters, Jennifer L., Rhoades, Jeffrey A., Simbartl, Nicholas A., Singstock, Mitchell D., Brooke Buckingham.

888  Exploring PCR and RT-PCR as wet-bench alternatives to augment student experiences in a genomics based CURE. P.M. Visconde. D.W. Paetkau Saint Mary’s College, Notre Dame, IN.

The Genomics Education Partnership (GEP) provides a classroom undergraduate research experience (CURE) in genomics. Students traditionally engaged in finishing (sequence improvement) and gene annotation using bacterial artificial chromosome (BAC) libraries. These libraries provided student finishers with gel electrophoresis evidence (wet bench results) to verify in silico gene models. Next-generation sequencing projects do not require BAC libraries, resulting in a more mechanical and less evidence-based finishing project. In order to add wet bench evidence to this CURE, gDNA PCR and Sanger sequencing was attempted. Twelve students attempted to fix 14 gaps found in ~1 million bases of *Drosophila ficusphila* DNA. One gap was fixed without PCR. Of the remaining 13 gaps, 8 PCR bands were successfully produced, 6 of these produced usable sequence, but only one successfully closed the gap region. Students found the low success rate (14 %) to be frustrating. Reverse-transcriptase PCR (RT-PCR) verification of in silico gene models was explored as an alternative method to alleviate student frustration.