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Full text of Abstracts

1 Xenbase: genomic, transcriptomic, phenotype and disease integration. *Peter Vize*[1], Kevin Burns[2], Praneet Chaturvedi[2], Stanley Chu[1], Malcolm Fisher[2], Joshua Fortreide[2], Vaneet Lotay[1], Mardi Nenni[2], Troy Pells[1], V.G. Ponferrada[2], Dong (Joe) Wang[1], Ying Wang[1], Christina James-Zorn[2], Kamran Karimi[1], Zorn Aaron[2] 1) Department of Computer Science, University of Calgary, Calgary, Alberta, Canada; 2) Division of Developmental Biology, Cincinnati, OH, USA.

The Xenopus model organism database, Xenbase (www.xenbase.org), supports biomedical research using frogs of this genus, and makes data generated using Xenopus visible to researchers exploring other model organisms or human biology. Content is gathered by a variety of automatic data pipelines, semi-automated curation systems, and manual annotation by a team of dedicated domain experts. The various types of content range from genomes, proteomes, expression patterns and interaction networks to models of human disease. New features leverage the flood of sequence data to visualize changes in gene expression in response to experimental manipulations, changes over developmental time, co-regulation and more. New genome annotations of both X. laevis and X.tropicalis will be presented, as will new custom tracks on the Xenbase browser and a UCSC track hub. Tree views, similar to those in the recent single-cell papers (doi 10.1126/science.aar5780), are now available to browse and explore the Xenopus Anatomical Ontology. Features due for release soon include Expression as a Phenotype and Anatomical Phenotypes.

Xenbase staff are available at the conference to provide one on one training and to demonstrate a wide range of features ranging from text-mining, custom date visualizations in genome browsers and RNA-seq data viewers, small molecule libraries and more.

If you use Xenbase resources in your research please consider citing us, for example
Nucleic Acids Res. 2018 46(D1):D861-D868.

2 National *Xenopus* Resource – serving the *Xenopus* research community. Marcin Wlizla[1], Sean McNamara[1], Nikko-Ideen Shaidani[1], Robert Grainger[2], *Marko Horb*[1] 1) National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA; 2) Department of Biology, University of Virginia, Charlottesville, VA.

Since its establishment in 2010, the National *Xenopus* Resource (NXR) has focused on development of three facets in particular: resources, services, and research, all of which promote advancement of *Xenopus* research. Our resources include wild type and inbred strains of both *Xenopus laevis* and *Xenopus tropicalis* as well as more than 150 distinct mutant and transgenic lines. The NXR services include generation of custom mutant and transgenic lines for *Xenopus* researchers who may not have the means or the expertise to do so on their own, laboratory space available for visiting scientists, and promoting dissemination of cutting edge techniques in *Xenopus* research by hosting the biennial '*Xenopus* Resources and Emerging Technologies' meeting as well as through on-site held workshops giving hands-on instruction in bioinformatics, genome editing, and imaging. The research that the NXR does in-house focuses on developing techniques that will be of considerable use to the *Xenopus* community. As we grow, we continue to develop these facets further with the goals of serving the *Xenopus* community and promoting the use of *Xenopus* in the study of developmental processes and disease. Here, I describe the recent progress we have made in expanding and optimizing our operations as well as provide information on how investigators can best take advantage of the resources and services we provide.

3 Distributing male *Xenopus* as frozen sperm and the European *Xenopus* Resource Centre's service offering. *Matt Guille*[1], Ania Noble[1], Maya Piccinni[1], Annie Godwin[1], Gretel Nicholson[1], Alan Jafkins[1], Colin Sharpe[1], Viki Allan[2] 1) EXRC, University of Portsmouth, UK; 2) University of Manchester, UK.

The EXRC makes, collects, quality assures, stores and distributes both molecular and animal resources to support researchers using the *Xenopus* model for biochemistry, developmental and cell biology, supplying thousands of resources annually. In a joint project with NXR we optimized a robust method for *X. laevis* sperm freezing and have more recently been trialing the distribution of both wild-type and genetically altered male gametes using frozen sperm, rather than live animals. Since more fertilisations can be performed per male with frozen rather than fresh sperm, distributing male gametes as frozen sperm has positive impacts for the community and animal welfare. First the cost of research is reduced: fewer males are used, there is no need to house males and the cost of transporting sperm is much less than that of frogs; second, male frogs are not exposed to the stress of travel, and finally fewer animals are used overall. We will present the results of this trial and data concerning the long-term effects on a *Xenopus* colony of using animals derived from frozen sperm.
This year we have applied for a further five year's core funding for the EXRC and we will know whether this has been successful before the meeting. If it has, then we will explain what we intend to do over this period and seek feedback from the community regarding the exact resources we should focus on.

4 The launching of Amphibian Research Center (ARC) at Hiroshima University as the core facility of *Xenopus* resource in Japan. *H. Ogino*, A. Suzuki Amphibian Research Center, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan.

Recent progress in the genome projects and genome editing technologies has made a striking breakthrough in *Xenopus* research. To support this movement, Hiroshima University has launched Amphibian Research Center (ARC) in 2016. The steering committee includes not only the faculties of Hiroshima University but also outside established researchers in Japan. The objectives of the mission are to perform leading-edge studies on development, regeneration, evolution and disease, and to develop living and non-living amphibian resources with the international collaborative network. The resource targets include *X. tropicalis*, *X. laevis* and other amphibians useful for molecular biology studies. The grant proposal of *X. tropicalis* resource project was especially adopted by Japan Agency for Medical Research and Development (AMED), as part of the fourth National BioResource Project (NBRP) that will continue by 2021.

As the *Xenopus* resource project, we will collect, maintain and distribute the following resources. We will also perform dissemination activities, such as technical seminars, for further development of the *Xenopus* research community in Japan.

(A) Living resources

・Inbred strains of wild type *X. tropicalis*

(Nigerian A, Nigerian H, Ivory Coast, Golden)

・Inbred strains of wild type *X. laevis* (J-strain) and *X. borealis*

・Genetically modified lines

(transgenics for live-imaging analysis, mutants generated by genome editing, etc.)

(B) Non-living resources

・A comparative genome browser for *X. tropicalis* and *X. laevis*.

・A *Xenopus* - human orthology database

・Plasmids, genomic DNA, experimental protocols, etc.

Moreover, our ARC/NBRP has been working together with EXRC, NXR, Xenbase and the international *X. laevis* genome project consortium to develop the system to share reagents and information essential for basic research, such as the distribution of *X. laevis* inbred strain (J-strain) and BAC clones worldwide and the improvement of gene annotation. We have completed the analysis of the genetic backgrounds of *X. tropicalis* strains commonly used in the US, Europe and Japan through a collaborative efforts between three resource facilities, ARC/NBRP, EXRC and NXR. We summarize and update the advancement of these efforts for the international *Xenopus* community.

5 Production and characterization of monoclonal antibody to Xenopus proteins. *Dominique Alfandari*, Alfandari Vet.&Animal Sciences, University of Massachusetts, Amherst, MA.

Loss of function experiments are key to understand the role of genes and proteins during development and disease. To demonstrate the specificity of knock down or Knock out strategies, antibody that recognize the endogenous proteins are essential. Most antibodies available commercially do not cross react with Xenopus proteins. More recently, the ubiquitin ligase trim21 together with specific antibody was shown as an effective way to deplete proteins in embryos, making the generation of new antibody that recognize the native protein even more important. Our goal is to produce monoclonal antibodies to at least 100 Xenopus proteins. To achieve this we are developing three approaches. First, Xenopus embryo extracts are used to immunize mice. The fusions are done at the Fred Hutchinson Research Cancer Center and IgG positive hybridoma picked grown and frozen. Screening involves ELISA on Xenopus extract, whole mount immunostaining and western blot. Interesting clones are characterized by LC/MS/MS to identify the protein target. The second approach is to immunize with purified protein for target that are requested by the Xenopus community. The fusions and screening are performed in the Alfandari Lab. The third approach uses phage display library of Rabbit immunoglobulin variable chains. This approach will be tested first on purified proteins but also on Xenopus explants to obtain tissue specific cell surface markers. The progress made during the first 9 month of this grant will be presented together with the initial list of targets.

6 An update of the *Xenopus* ORFeome project. D. Hill[1], M. Gilchrist[2], A. Zorn[3], T. Stukenberg[4], *W. Pearson*[4] 1) Center for Cancer Systems Biology, Dana Farber Cancer Institute, Boston, MA; 2) The Francis Crick Institute, London, UK; 3) Developmental Biology, Cincinnati Children's Hospital, Cincinnati, OH; 4) Biochemistry and Mol. Genetics, U. of Virginia, Charlottesville, VA.

The *Xenopus* ORFeome project completed the first version of the ORFeome two years ago, moving close to 90% of the available cDNA clones from the publicly available full-length *X. laevis* collection (XGC) to Gateway donor vectors. We have also moved publicly available full-length *X. tropicalis* clones (XGC clone set) into Gateway clones. The *X. laevis* and *X. tropicalis* ORFeomes are available to the community through both commercial distributers and the three Xenopus stock centers. Each ORFeome clone is fully integrated into the *Xenopus* community database, Xenbase.

Supported by the NIH, the *Xenopus* ORFeome project will clone an additional 11-12,000 ORFs de novo from RT-mRNA. This second version will generate the first cDNA clones for the half of the *Xenopus* gene repertoire, which is currently unavailable to the community in any format. The lack of cDNA clones for around 1/2 the *Xenopus* genes represents a major roadblock for almost all *Xenopus* projects. While a simple strategy for finding *X. tropicalis* ORFs would simply extract the ORF mRNA sequence from the *X. tropicalis* genome, previous analysis has shown that the genome assembly has errors, and many apparent ORFs are incomplete. We are reducing the truncated ORF problem by comparing a comprehensive *X. tropicalis* RNAseq assembly with not only *X. tropicalis* proteins, but also with human and mouse homologs. *X. tropicalis* and humans diverged less than 400 Mya, and more than half of human/*X. tropicalis* protein alignments are 50% identical, a distance where protein lengths are usually similar, and alignments have few gaps. Integrating mammalian and X. tropicalis RNA-seq alignments allows high-quality genome assemblies and protein predictions to improve the *X. tropicalis* ORFeome.

The extensive sequencing required to build the ORFeome will improve the annotation of both the *X. laevis* and *X. tropicalis* genomes. Part of

the sequencing of *X. tropicalis* RT-mRNAs employs PacBio to obtain full transcript sequences as well as to obtain full insert sequences of clones derived from PCR amplification and Gateway cloning. By using RT-mRNA as the source material, there is also potential to identify and clone isoforms as well. The completion of the *Xenopus* ORFeome should decrease the time to characterize most proteins in the myriad of functional assays carried out by the entire *Xenopus* community.

7 Strategies for generating and manipulating immortal *Xenopus* cell lines with normal ploidy derived from embryos. *Gary J Gorbsky*[1], John R Daum[1], Marko E Horb[2] 1) Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA.

Many cell biological and molecular pathways are highly amenable to study through the use of permanent cell lines. For cell biology studies involving live cell microscopy, *Xenopus* cell lines have specific advantages including large cell size, proliferation at room temperature, and growth in media that do not require a high CO_2 atmosphere. Derivatized lines expressing fluorescent proteins or lines manipulated through CRISPR-based gene editing can be permanently stored in liquid nitrogen. Gene editing of euploid cell lines followed by nuclear transfer into enucleated host eggs holds the potential for rapid generation of non-mosaic F0 mutant frogs. Previously, cell lines derived from tissues or embryos of *Xenopus laevis* and *Xenopus tropicalis* have been reported. However, with one exception, all of those that have been analyzed were found to be aneuploid. Moreover, the currently available *Xenopus* cell lines were derived from outbred stocks of *X. laevis* and *X. tropicalis*. Cell lines from the inbred strains used for genome sequencing would simplify their use in gene editing. Therefore, we have endeavored to produce novel *Xenopus* cell lines from inbred strains. Using neurula stage embryos, we optimized methods for dissociating cells while minimizing damage. Other technical challenges included avoiding microbial contamination and preventing primary cells in culture from undergoing terminal differentiation. We generated nine novel immortal cell lines from *X. tropicalis* embryos of the Nigerian strain. In preliminary analyses, chromosome spreads prepared from these lines showed that the majority of cells in all but one line have normal, diploid ploidy. We chose four lines with disparate morphology for analysis by RNA seq. All four lines express telomerase, a necessary requirement for immortalization. We have optimized conditions for electroporation to obtain efficiencies over 85%. We have generated sublines expressing fluorescent α-tubulin and carried out gene editing with CRISPR methods. We are extending our approaches to develop novel cell lines from *X. laevis* embryos of the inbred J strain.

8 Profiling of alternative polyadenylation: the newest genomic resource for functional annotation of the *Xenopus tropicalis* genome. *Zhihua Jiang*[1], Xiang Zhou[1], Yangzi Zhang[1], Jennifer Michal[1], Lujiang Qu[1], Shuwen Zhang[1], Mark Wildung[2], Weiwei Du[2], Derek Pouchnik[2], Hui Zhang[3], Yin Xia[3], Honghua Shi[4], Guoli Ji[5], Jon Davis[6], Richard Harland[7] 1) Department of Animal Sciences, Washington State Univ, Pullman, WA; 2) Laboratory for Biotechnology and Bioanalysis, Center for Reproductive Biology, Washington State University, Pullman, WA; 3) School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China; 4) State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai, China; 5) Department of Automation, Xiamen University, Xiamen, China; 6) Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA; 7) Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA.

Although usage of alternative polyadenylation sites (APSs) in *Xenopus* genes was observed more than three decades ago, genome-wide research has yet to be conducted. In fact, alternative polyadenylation of RNA is an evolutionarily conserved mechanism that regulates genes for multi-functions, adding sophisticated complexity to the information transfer from genome to phenome. In the present study, we report the first *X. tropicalis* alternative polyadenylation site (APS) resource with a total of 127,914 sites derived from embryos and adults. This study is the first attempt to classify APSs into categories relevant to the maternal-to-zygotic transition (MZT) events and revealed pathways in embryos enriched in reprogramming before MZT. In comparison, development and response to stimuli pathways were enriched for zygotic genome activation (ZGA). In addition, we found dramatic gender differences in APS usage. Males were transcriptionally less active, but more diverse than females, particularly when they are young. However, the transcriptional activities of males and females were comparable in old age. In addition, APS profiles in embryos before MZT were distinct from both embryos at ZGA and adults. In particular, young females and embryos before MZT were transcriptionally similar. Use of genes, intronic APSs and APS lengthening increases with age. Validation revealed that 97,411 APSs had evidence from other sources, such as 19,116, 80,759, 28,144 and 82,699 APSs supported by EST-seq (expressed sequence tag sequencing), HATT-seq (head and tail tag sequencing), Iso-seq (isoform sequencing) and RNA-seq (RNA sequencing) reads, respectively. Overall, our results suggest that the minimal functional unit in genomes be the alternative transcripts rather than the genes.

11 Xenopus as a model for investigating transcription factor regulation in development and cancer. *A. Philpott* Oncology, University of Cambridge, Cambridge, GB.

Xenopus is an excellent model system to investigate control of transcription factors in response to cell cycle and developmentally-dependent post-translational modification. We have used a variety of embryo and extract approaches to demonstrate that multi-site CDK-dependent phosphorylation of basic helix-loop-helix transcription factors controls their ability to drive differentiation in developing tissues including central nervous system, peripheral nervous system and muscle. Moreover, we see that regulation by multi-site phosphorylation is shared by both tissue specific transcriptional activators and more widely inhibitory bHLH factors. We are now using Xenopus as a powerful model to understand how perturbation of transcription factor post-translational control contributes to stalling of the normal developmental programme in the pediatric cancer, neuroblastoma.

12 Transcriptional regulatory dynamics in neural progenitors of the regenerating spinal cord. A.D. Kakebeen, A. Chitsazan, *A. E. Wills* Biochemistry, University of Washington, Seattle, WA.

Regeneration represents a unique challenge with respect to gene regulation. In some respects, regeneration parallels embryogenesis, with re-activation of many of the same pathways, but in the divergent context of already-differentiated tissues. In *Xenopus*, amputation of the

tadpole tail leads to rapid regeneration of multiple complex tissues, including the spinal cord. Neural progenitors in the amputated spinal cord have to balance the competing requirements of differentiation and self renewal to replace tissue and restore function. To understand the gene regulatory programs driving these distinct cellular priorities, we isolated neural progenitors over a regeneration timecourse. We applied an Assay for Transposase Accessible Chromatin (ATAC-Seq) to profile transcriptional regulatory dynamics in these cells as regeneration progresses. We find that ATAC-Seq is an effective assay for capturing epigenetic dynamics in this rare cell type. We also show that neural progenitors prioritize distinct phases of gene activation as regeneration progresses, beginning with reformation of the epithelial tube, followed by neuronal differentiation and then self-renewal. Closer inspection of the genes and transcription factors associated with each transition reveals many regulators that are shared with early embryogenesis such as Wnt, BMP and Shh signaling, but also fundamental differences in the timing and manner with which these programs are initiated. Overall we present a tractable method for querying epigenetic dynamics in progenitor cells that highlights the unique regulatory environment of the regenerating spinal cord, and the ways in which it parallels and diverges from embryonic development.

13 Graded mechanical strain directs cilia differentiation in the *Xenopus* left-right organizer. *Christopher Kintner*[1], Shyam Srinivasan[1], Raymond Keller[2], Yuan-Hung Chien[1] 1) Salk Institute, La Jolla, CA; 2) Department of Biology University of Virginia Charlottesville, VA 22904 USA.

The Xenopus left-right organizer (LRO) requires different ciliated cell types to produce and sense flow during left-right patterning. Flow is produced in the LRO by cells located medially that extend long motile cilium that are further positioned along the anterior-posterior (A-P) planar axis in a manner required for leftward flow. Cells located within the lateral LRO sense this flow by extending short, immobile cilia that remain in a central planar position. Cilia planar positioning in the LRO requires the core planar cell polarity (PCP) pathway, but how this pathway is aligned to the A-P body axis is still unclear. We previously showed that mechanical strain produced by gastrulation acts on the ventral side of the embryo to promote and align the formation of PCP axis in the developing skin. Here, we report that the presumptive LRO is also subjugated to oriented mechanical strain during gastrulation and that this strain is both necessary and sufficient to promote an A-P planar axis requiring for cilia positioning. We also find that strain is graded in magnitude along the LRO medial-lateral axis with the highest levels occurring at the midline, tapering to lower levels laterally. In both loss and gain-of-function analyses, we show that this graded strain instructs cilia length, motility and planar positioning. We further show that strain directs cilia differentiation based on a prepattern in the presumptive LRO involving Foxj1, the master regulator of motile cilia differentiation. We propose the model where differential strain is a graded, developmental cue, linking the establishment of an A-P planar axis to cilia length, motility, and planar location, during formation of the *Xenopus* LRO.

14 Mitotic chromosome congression requires the microtubule-associated protein RECQL4, mutated in the Rothmund-Thomson syndrome. *H. Yokoyama*[1], D. Moreno-Andres[2], W. Antonin[2] 1) ID Pharma, Tsukuba, Japan; 2) RWTH Aachen University, Germany.

RecQ like-helicase 4 (RECQL4) is mutated in patients suffering from Rothmund-Thomson syndrome, a genetic disease characterized by premature aging and high cancer susceptibility. Patient cells show chromosome instability, suggesting abnormal chromosome segregation. Here we show that RECQL4 is a microtubule-associated protein localizing to the mitotic spindle. RECQL4 depletion in frog egg extracts does not affect spindle assembly per se, but prevents chromosome alignment at the metaphase plate. Low doses of nocodazole depolymerize RECQL4-depleted spindles more easily, suggesting abnormal microtubule-kinetochore interaction. Indeed, inter-kinetochore distance of sister chromatids is larger in depleted extracts and patient fibroblasts. Consistently, RECQL4 downregulation in HeLa cells causes chromosome misalignment and delays mitotic progression. Importantly, these chromosome alignment defects are independent from RECQL4's reported roles in DNA replication and damage repair. Our data both elucidate a novel function of RECQL4 in chromosome alignment and provide an intriguing molecular explanation for the disease-associated phenotypes of Rothmund-Thomson syndrome.

15 The left-right asymmetry of liver lobation is generated by Pitx2c-mediated asymmetries in the hepatic diverticulum. *Nanette Nascone-Yoder* Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Internal organs exhibit left-right asymmetric sizes, shapes and anatomical positions, but how these different lateralities develop is poorly understood. Here we use the experimentally tractable *Xenopus* model to uncover the morphogenetic events that drive the left-right asymmetrical lobation of the liver. On the right side of the early hepatic diverticulum, endoderm cells become columnar and apically constricted, forming an expanded epithelial surface and, ultimately, an enlarged right liver lobe. In contrast, the cells on the left side become rounder, and rearrange into a compact, stratified architecture that produces a smaller left lobe. Side-specific gain- and loss-of-function studies reveal that asymmetric expression of the left-right determinant Pitx2c elicits distinct epithelial morphogenesis events in the left side of the diverticulum. Surprisingly, the cellular events induced by Pitx2c during liver development are opposite those induced in other digestive organs, suggesting divergent cellular mechanisms underlie the formation of different lateralities.

16 *Xenopus* as a system to elucidate evolutionally changing functions of Meis TALE-Homeobox family proteins during metazoan nervous system development. Pavel Federenchik, *Dale Frank* Biochemistry, Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, IL.

Highly conserved Hox and TALE homeobox proteins are required for AP axis formation in bilaterians. However, Hox and TALE homeobox proteins are also expressed in radial Cnidarians, like sea anemones, corals and jelly fish, as well as single celled organisms like amoeba and fungi. It is unclear when the interactions between TALE and Hox proteins were first utilized to generate the body plan during metazoan evolution. We previously showed that the *Xenopus* TALE-protein, Meis3 induces posterior neural tissue such as hindbrain and spinal cord in *Xenopus* embryonic cells and whole embryos. The Homothorax protein (*Drosophila* Meis) also induces hindbrain and spinal cord cell fates in *Xenopus*, despite being from an invertebrate. In this study we have asked: At what point in metazoan evolution did Meis proteins acquire the ability to induce the vertebrate-specific hindbrain tissue? In our assay, ectopic Meis protein induces expression of hindbrain and spinal cord

markers in animal cap explants and embryos. We initially discovered that the sea anemone (*Nematostella*) Meis protein did not induce hindbrain, but only spinal cord in *Xenopus*, suggesting that Cnidarian Meis proteins may be less advanced than in bilaterians. This proved to be incorrect. By dissecting Meis protein into various domains, we found that the highly conserved Meis specific and Meis-Homeobox DNA-binding domains are sufficient to induce spinal cord, but not hindbrain. The *Nematostella* Meis protein lacks a carboxyl terminus that is conserved in vertebrates. This region has a transcription-activation domain that induces hindbrain. In chimeric *Xenopus-Nematostella* proteins, *Nematostella* DNA-binding domains fused to the *Xenopus* carboxyl terminus robustly induce hindbrain. Moreover, we found that coral and jelly fish Meis proteins contain the "vertebrate" conserved carboxyl terminus, and they act like bilaterian Meis proteins to induce hindbrain in *Xenopus*. Surprisingly, Meis proteins from amoeba (*Acanthamoeba*) and placozoa (*Trichoplax*) also induce hindbrain. Early in metazoan evolution, the ability to induce a "vertebrate" structure was built in or pre-evolved, suggesting that the formation of the basic building-blocks for regulating AP axis formation pre-date the structures themselves. The earliest metazoans started off with highly powerful protein machinery (TALE, Hox) that were later utilized along the evolutionary path to create some of our most advanced and specific vertebrate structures, like the hindbrain.

17 Neil2 stimulates Tdg-mediated BER in active DNA demethylation in *Xenopus* neural crest formation. *D. Han*[1], M. Musheev[1], C. Niehrs[1,2] 1) Institute of Molecular Biology gGmbH (IMB), Mainz, Germany; 2) Division of Molecular Embryology, DKFZ-ZMBH Alliance, Heidelberg, Germany.

DNA demethylation plays an important role in development and animal physiology. In active DNA demethylation, 5-methylcytosine (5mC) is iteratively oxidized into 5-hydroxylmethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-caboxylcytosine (5caC) by Ten-eleven-translocation (TET) enzymes. For demethylation, 5fC and 5caC are removed by Thymine DNA glycosylase and base excision repair (BER). Our recent *in vitro* data indicate that Nei-like (NEIL) glycosylases play a crucial role in this context by increasing the enzymatic turnover of TDG in BER. Here we analyzed the role of TET, TDG, and especially NEILs in *Xenopus laevis* embryos. Expression analysis shows that *tet3, tdg,* and *neils* are expressed in the brain. Antisense Morpholino knock down of *tet3, tdg, neil2,* and *neil3* induces microcephaly and neural crest defects. Analysis of 5mC and its oxidative derivatives by mass spectrometry supports the cooperation of *tet3, tdg*, and *neil2* in active DNA demethylation *in vivo*.

18 The reprogramming and stability of cell differentiation as revealed by nuclear transfer. *John Gurdon* Zoology [Gurdon Institute], University of Cambridge, Cambridge, GB.

After brief comments on my background working with Xenopus, I will review current experiments on eggs and oocytes that relate to mechanisms of reprogramming and stabilization of cell differentiation.

19 Dynamic cell contacts as regulators of neural crest migration. Marie-Claire Kratzer[1,2], Anita Grund[1], Hanna Berger[1], Anne Merks[2], Sarah Becker[2], Jubin Kashef[2,3], *Annette Borchers*[1] 1) Philipps-University Marburg, Department of Biology, Molecular Embryology, Marburg, Germany; 2) Karlsruhe Institute of Technology (KIT), Zoological Institute, Cell- and Developmental Biology, Karlsruhe, Germany ; 3) Karlsruhe Institute of Technology (KIT), Institute for Photon Science and Synchrotron Radiation, Eggenstein-Leopoldshafen, Germany.

Neural crest cells are pluripotent, highly migratory cells contributing to a broad range of vertebrate tissues and failure in their migration can result in severe birth defects and malformation syndromes. Neural crest migration is controlled by various means including chemotaxis, guidance cues and dynamic cell-cell contacts. Non-canonical planar cell polarity (PCP) Wnt signaling has been shown to play a role in contact inhibition of locomotion, a phenomenon whereby neural crest cells change their directionality upon neural crest cell-cell contact. Although dynamic cell contacts have been acknowledged as regulators of neural crest migration, their molecular dynamics as well as the means by which cell contact information is translated into cell polarity and directional migration remains a topic of current research. Here we present our recent data on the role of the Rho-GEF Trio as a mediator of transmembrane factors dynamically localized at neural crest cell-cell contact sites.

20 *c-Answer*, the gene lost in evolution in warm-blooded animals, controls regeneration and brain development via Fgf and purinergic signaling in *Xenopus laevis*. *D. Korotkova*[1,2], A. Ivanova[1], L. Rubanov[3], A. Seliverstov[3], O. Zverkov[3], N. Martynova[1], M. Tereshina[1], A. Nesterenko[1,4], V. Lubetsky[3], A. Zaraisky[1] 1) Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Moscow, RU; 2) Lomonosov Moscow State University, Moscow, Moscow, RU; 3) The Institute for Information Transmission Problems, Russian Academy of Sciences, Moscow, Moscow, RU ; 4) Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Moscow, RU.

Major genetic network restructuring within virtually the same set of genes is considered to underlie the evolutionary transformations in vertebrates, such as the progressive forebrain development and the loss of the appendage regeneration capacity. We suggest that the mechanism that influence greatly this genetic network restructuring is the elimination of some important genes (e.g. regulators of brain development or regeneration) in vertebrate evolution. Accordingly, we developed the bioinformatics approach that allowed us to detect genes eliminated in evolution of vertebrates.

We report here that one of the genes, eliminated in warm-blooded animals, encodes a transmembrane protein that binds to FGFR1-4 and P2Y1 receptors. Due to its expression in the presumptive forebrain in the *Xenopus laevis* embryos and in the wound epithelium and blastema in the regenerating tails and limb buds in tadpoles, we named this protein c-Answer (**c**old**-**blooded **An**imals **S**pecific **W**ound **E**pithelium **R**eceptor-binding protein).

In order to investigate physiological functions of c-Answer we carried out experiments on the overexpression of various c-Answer deletion mutants, *c-Answer* KD with the help of antisense morpholino oligonucleotides and *c-Answer* KO via CRISPR/Cas9 system. The obtained results proved that c-Answer provides a stimulating effect on appendage regeneration and brain development.

Having analyzed these data, we suggested that c-Answer could be involved in Fgf and purinergic signaling pathways. CoIP followed by Western blotting showed that c-Answer interacts with FGFR1-4 and P2Y1 receptors. Furthermore, we have demonstrated that c-Answer stimulates MAPK/ERK and purinergic signaling.

Thus, the obtained data suggest that elimination of *c-Answer* in the ancestors of warm-blooded animals conditioned the decreased activity of at least two signaling pathways activated by FGFR and P2Y1 receptors, which in turn could contribute to changes in the mechanisms that regulate the forebrain development and regeneration.

21 An unexpected requirement of a ciliary dynein for early mesoderm development and gastrulation in *Xenopus laevis*. *P. Vick*, S. Mantino, L. Gerstner, J. Kreis Institute of Zoology, University of Hohenheim, Stuttgart, DE.

Motile cilia are required for a multitude of physiological functions during embryonic development and adult life. Thus, naturally occurring, or experimentally induced disruption of ciliary movement causes severe health-related phenotypes. A human syndrome, primary ciliary dyskinesia, for example, results in chronic infections of the respiratory tract, sterility, and *situs inversus*, i.e. defects in left-right (LR) asymmetry. Accordingly, when cilia motility is manipulated during early development, *Xenopus* embryos develop LR axis defects, lack of epidermal mucus-based clearance, neural tube defects like hydrocephalus, and massive edema due to kidney failure. One major component required for cilia movement, are axonemal dynein motor protein complexes, which are attached to one microtubule doublet while 'walking' on another to cause bending of the cilium.

Previously, we could demonstrate that the dynein axonemal heavy chain gene *dnah9*, a major component of the this motor complex, is expressed in all tissues developing motile cilia, and knockdown caused the above-mentioned defects related to immotile cilia. Surprisingly, we also found it to be expressed maternally before gastrulation, although first motile cilia – and motile cilia-related phenotypes – have only be described from neurulation onwards.

Here, we show an early requirement of *dnah9* for proper gastrulation movements and axis development. While at first, bottle cell formation was initiated properly, knockdown of *dnah9* then caused arrest of gastrulation movements due to lack of involution and axial elongation. Interestingly, this correlated with a lack of expression of mesodermal marker genes during gastrulation, implicating a role for mesoderm induction or patterning. Accordingly, knockdown blocked Activin-induced elongation, and FGF-induced expression of brachyury in animal caps. Further, lack of mesodermal marker genes could be rescued by introduction of constitutive-active intracellular components of the FGF-pathway, implicating a role of Dnah9 for FGF-mediated mesoderm development. We suggest, in this context, the ciliary motor protein Dnah9 participates in a cilia-independent ancient role, potentially as a cytoplasmic component for FGF-mediated gastrulation movements in *Xenopus*.

22 Cell-cell and cell-matrix interactions during neural crest EMT and migration. *S. Nie*, T. Garmon, M. Wittling, P. Ho School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA.

Neural crest represents a great model to study the mechanism of cell migration in vivo. During neural crest migration, they constantly modify their interactions with neighboring cells and the extracellular matrix. Here, we examined the activity of matrix metalloproteinase 14 (MMP14 or MT1-MMP) in neural crest cell migration. MMP14 is highly expressed in neural crest cells just before they commence migration. When MMP14 was knocked down, the migration of neural crest cells was impaired. To determine whether MMP14 is required to process extracellular matrix for neural crest cells to migrate forward, we examined the migration of isolated cranial neural crest tissue in vitro. Neural crest explant can still spread in culture at the loss of MMP14, but cells cannot break apart from each other and migrate individually. When the expression of cadherins was examined by immunofluorescence, we found that the expression of E-cadherin is reduced with excessive MMP14, while the expression of N-cadherin is increased at the loss of MMP14. In addition to be regulated by MMP14, we further showed that E-cadherin is also post-translationally modified on their extracellular domain during neural crest cell migration. We detected the enrichment of two N-glycans on E-cadherin during neural crest cell migration. Comparing wild type and mutant E-cadherins lacking these glycosylation sites, we hope to determine how these modifications affect neural crest EMT and migration.

23 Identifying phenotypic convergence among autism risk genes. *H.R. Willsey*[1], C.R.T. Exner[1], Y. Xu[1,2], A. Kim[2], J. Mandell[3], Q. Qi[3], A.J. Willsey[1,3], R.M. Harland[2], M.W. State[1] 1) Department of Psychiatry, Weill Institute for Neurosciences, University of California, San Francisco; 2) Department of Molecular and Cell Biology, University of California, Berkeley; 3) Institute for Neurodegenerative Diseases, Weill Institute for Neurosciences, University of California, San Francisco.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder of unknown etiology and without pharmacological therapeutics. Recent advances in gene discovery have identified 65 large effect size ASD risk genes. These genes are functionally heterogeneous and likely pleiotropic, so it is critical to identify phenotypes in common to multiple genes, as those are most likely to be relevant to ASD pathology. To identify such 'convergent phenotypes,' we leveraged CRISPR/Cas9 genome editing in *Xenopus tropicalis*to study many loss of function variants in ASD risk genes in parallel during brain development. Strikingly, among the 10 most strongly associated genes, we observe that loss of function commonly disrupts brain size and neural progenitor cell (NPC) number. Furthermore, gene expression profiling of dissected brain reveals common developmental pathways and processes disrupted after ASD gene loss. These convergent phenotypes set the stage for small molecule screening approaches. Hence, this study provides a path forward for understanding the molecular underpinnings of ASD and other psychiatric disorders, and for identifying possible therapeutic targets.

24 Collective cell movements driven by actomyosin contractility. *A. Shindo*[1], Y. Inoue[2], A. Anastasia[1], J. Wallingford[3], M. Kinoshita[1] 1) Nagoya University, Nagoya, JP; 2) Kyoto University, Kyoto, JP; 3) University of Texas at Austin, TX.

Actomyosin-mediated contractility drives collective cell movement during tissue formation and repair. We have focused on two models of collective cell movement, the convergent extension and wound closure, to understand how the contractile force is spatio-temporally regulated in each cell.

We found that each collective cell movement establishes a distinct actomyosin behavior to generate the appropriate force to shape the tissue. During the process of convergent extension, actomyosin oscillates asymmetrically beneath the specific cell–cell junctions and contracts the cell junction. We found that the planar cell polarity pathway locally controls the asymmetric actomyosin pulses to drive the cell movement efficiently. In contrast, we observed the non-oscillating contraction of actomyosin during wound closure. Interestingly, the pace of contraction

of the cell junction by actomyosin appears faster during wound closure than that during convergent extension.
In this talk, I will highlight the local behaviors of actomyosin as a cellular machinery to alter tissue shape and discuss how the simple contractile force of actomyosin is utilized for controlling the shapes of various tissues *in vivo*.

25 Xenopus is electrifying: congenital heart disease, K channels, cell fate, and gastrulation. Emily Sempou, Jie Zhu, David Zenisek, *Mustafa Khokha* Yale University, New Haven, CT.
Congenital heart disease (CHD) is the most common birth defect, yet its primarily genetic causes continue to be obscure. Six out of eight members of the KCNH family of voltage-gated potassium (K^+) channels have recently emerged in a large patient exome sequencing study as candidate disease genes for CHD and specifically heterotaxy. Heterotaxy is a disorder in which organs, including the heart, are mispatterned relatively to the left-right (LR) axis. It frequently results in very severe heart defects because the heart has a distinct and intricate LR architecture. Even though voltage-gated K^+ channels are well known for their connection to cardiac arrhythmias, a role in *structural* heart disease is undefined. We depleted candidate heterotaxy gene kcnh6 in *Xenopus* embryos using CRISPR and morpholino and recapitulated the patient phenotype. Knockdown embryos were impaired in their LR development due to changes in plasma membrane potential at stages as early as gastrulation. Specifically, distinct meso- and ectodermal cell populations in the gastrula failed to acquire their fate, while the endoderm differentiated correctly. This conflicted with our initial hypothesis that electrical charge of cells is more likely to affect their morphogenetic behavior rather than cell fate. We also determined that the basis of these defects was aberrant activation of voltage-gated calcium channels and dramatically increased Ca^{2+} signaling, triggered by changes in cell membrane potential. Finally, we recapitulated differentiation defects caused by Kcnh6 depletion and cell membrane depolarization outside the context of embryonic structure using embryonic stem cells that we differentiated *in vitro* into endo-, meso- and ectoderm. Altogether, our findings identify potassium channels and membrane potential as novel instruments in the embryonic patterning "toolbox" as well as a potential causes of left-right patterning defects in human patients.

26 Cytoplasmic volume and limiting nucleoplasmin scale nuclear size during *Xenopus laevis* development. P. Chen[1], K. Nelson[2], M. Tomschik[1], J. Gatlin[1], J. Oakey[2], *D. Levy*[1] 1) Molecular Biology, University of Wyoming, Laramie, WY; 2) Chemical Engineering, University of Wyoming, Laramie, WY.
Early *Xenopus laevis* embryogenesis is characterized by dramatic reductions in both cell and nuclear sizes. Between fertilization and the midblastula transition (MBT, stage 8), there is an ~4-fold reduction in the volume of individual nuclei, and an additional ~4-fold reduction from the MBT to gastrulation (stage 10.5-12). While cytoplasmic activities, including nuclear import and protein kinase C, have been implicated in regulating nuclear size, we were interested in testing the contribution of cytoplasmic volume to nuclear scaling during embryogenesis. To investigate this question, we coupled *X. laevis* embryo extracts with microfluidic devices that allow us to generate cytoplasmic droplets of defined size and shape. We isolated embryonic cytoplasm containing endogenous embryonic nuclei from gastrula stage embryos, and then encapsulated nuclei and extract in droplets of differing volumes. Nuclei in droplets of embryonic cytoplasm expand to a new steady-state size after ~3 hours. In droplets ranging in volume from 0.02 - 0.5 nl, nuclear volume increased by 1.4- to 3-fold, with larger increases occurring in larger droplets. These data indicate that the volume of embryonic cytoplasm is limiting for nuclear growth. In droplets greater than ~0.5 nl (average blastomere volume at stage 8 is 0.6 nl), the increase in nuclear volume reaches a threshold of ~3-fold. Nuclei exhibit similar growth trends in spherical and flattened droplets of comparable volume, indicating droplet shape does not influence nuclear growth and supporting the idea that nuclear size is sensitive to volume and not boundary sensing. To identify cytoplasmic components limiting for nuclear growth, we biochemically fractionated extract and identified fractions capable of enhancing nuclear growth in gastrula stage extracts. Histone chaperone nucleoplasmin (Npm2) was identified as a promising candidate, as the nuclear scaling curve shifted upward when extract droplets were supplemented with Npm2 protein. Consistent with Npm2 being limiting for nuclear size in vivo, embryos microinjected with Npm2 exhibited larger nuclei. Having ruled out transcription-dependent effects of Npm2 on nuclear size, our data suggest that the amount of Npm2 influences nuclear size by controlling nuclear histone levels and chromatin structure. Indeed, the amount of Npm2 per cell decreases over development, correlating with reduced nuclear histone levels that we propose alter the mechanical properties of chromatin in a way that minimizes nuclear growth. Thus, reductions in cell volume with concomitant decreases in the amounts of Npm2 and nuclear histones represent a novel mechanism of developmental nuclear size scaling.

27 Role of p53 in Kidney Development: Modeling Renal Anomalies of Li-Fraumeni Patients. Alexandria Blackburn[1,2], Nicholas Cho[1,3], Mark Corkins[1], Amisheila Kinua[1,4], Zubaida Saifudeen[5], *Rachel Miller*[1,2,6,7] 1) Pediatric Research Center, Department of Pediatrics, UTHealth McGovern Medical School, Houston, TX; 2) Program in Genetics and Epigenetics, University of Texas MD Anderson UTHealth Graduate School of Biomedical Sciences, Houston, TX; 3) Program in Bioengineering, Rice University, Houston, TX; 4) Program in Biochemistry and Cell Biology, Rice University, Houston, TX; 5) Department of Pediatrics- Nephrology, Tulane School of Medicince, New Orleans, LA; 6) Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX; 7) Program in Biochemistry and Cell Biology, University of Texas MD Anderson UTHealth Graduate School of Biomedical Sciences, Houston, TX.
Patients with Li-Fraumeni syndrome are predisposed to cancer resulting from germline mutations in the tumor suppressor, *p53*. Inheritance of mutations in *p53* occurs in an autosomal dominant pattern and results in an increased lifetime risk for developing multiple malignancies with a significant likelihood of early onset. Although prior studies using mouse knockouts have demonstrated that p53 plays a role in kidney formation, developmental kidney anomalies have not been associated with *p53* mutations seen in Li-Fraumeni patients. Preliminary MRI data indicate that these patients have a higher incidence of urogenital anomalies than the general population. Thus, the goal of this study is to utilize *Xenopus* embryos to assess p53's fundamental role in kidney development and to generate animal models to determine whether p53 mutations seen in Li-Fraumeni patients result in renal anomalies. Previous studies showed that *p53*-null mouse embryos have developmental kidney defects, including renal hypoplasia. Our data indicate that similar nephric developmental defects are also present when p53 levels are reduced in the kidneys of *Xenopus* embryos as assessed by targeting morpholino knockdown and CRISPR knockout of *p53* to the *Xenopus* kidney. In the future, we will determine whether *p53* mutations seen in Li-Fraumeni patients result in renal anomalies, thereby generating

animal models of Li-Fraumeni kidney anomalies for future research. Overall, these experiments will facilitate our understanding of how p53 affects nephron formation generally and how Li-Fraumeni patient mutations in *p53* may disrupt this process.

28 Tril activates a non-canonical Toll-like receptor signaling cascade to coordinately dampen Nodal and enhance Bmp activity. Yangsook Green, Hyungseok Kim, Isabellle Cooperstein, Autumn McKnite, *Jan Christian* Neurobiology and Anatomy, University of Utah, Salt Lake City, UT.

Embryonic development and adult homeostasis are critically dependent on a precise balance of Bone morphogenetic protein (Bmp) and Tgfß/nodal signaling. During gastrulation, nodal ligands induce mesoderm and specify anterior fates, while Bmps specify ventral fates such as blood. Smad7 is a target gene induced downstream of activated nodal and Bmp receptors, and is a central hub for negative regulation of both pathways. We identified Tril (Toll-like receptor 4 interactor with leucine-rich repeats) as a novel transmembrane protein that triggers degradation of Smad7 during gastrulation, thereby relieving repression of endogenous Bmp signaling. Paradoxically, although Smad7 levels are elevated when expression of Tril is knocked down, nodal signaling is enhanced rather than inhibited, and this leads to defects in gastrulation and head development. Thus, Tril coordinately promotes Bmp signaling while dampening nodal signaling. We show that Tril uncouples the effects of Smad7 on the Bmp and nodal pathways by stabilizing and activating the ubiquitin ligase, Nedd4l. Nedd4l dampens the ability of nodal to activate downstream signaling by targeting nodal receptorss for degradation in a Smad7 dependent manner, and terminates signaling by targeting phosphorylated Smad2 for degradation. Nedd4l accumulates at high levels in multivesicular bodies (MVBs) anchored on the basolateral surface of *Xenopus* cells, and is rapidly degraded when formation of MVBs is blocked in vivo. Finally, we used a yeast two hybrid screen to identify Tril binding partners. We show that Tril activates an NF-kB independent Toll-like receptor (Tlr) signaling cascade to trigger stabilization/activation of Nedd4l and degradation of Smad7. Tlrs are known to play non-immune roles in developmental patterning and morphogenesis in insects, and our studies provide evidence that Tlr-dependent signaling is also required for embryonic patterning in vertebrates.

29 Identification of cis-regulatory elements in Xenopus neural crest formation. Marta Marin-Barba[1], Leighton Folkes[1], Vicky Hatch[1], Simon Moxon[1], Gert Veenstra[2], Ruth Williams[3], Tatjana Sauka-Spengler[3], *Grant Wheeler*[1] 1) School of Biological Sciences, University of East Anglia, Norwich, UK; 2) Radboud University, Department of Molecular Developmental Biology, Nijmegen, NL; 3) Weatherall Institute of Molecular Medicine, University of Oxford, UK.

The Neural Crest (NC) are a transient multipotent and migratory cell population unique to vertebrates that give rise to several derivatives such as melanocytes, neurons and craniofacial structures. NC are being widely studied in the Xenopus animal model focusing on the gene regulatory networks involved in NC specification and differentiation. We have shown that regulation at the level of transcriptional elongation is important for NC development and have carried out PolII CHIP-seq which highlighted polymerase pausing of the c-Myc gene as being crucial for expression of NC genes. To further this analysis we have looked to identify CIS-regulatory elements involved in NC transcriptional regulation.

We took advantage of ATAC sequencing technology. ATAC sequencing (Assay for Transposase-Accessible Chromatin) is a high throughput technique which reveals the open chromatin regions on the genome, known to be enhancers and promoters. We have used ATAC sequencing in neural crest induced animal caps grown to two different stages of development, St 13 (NC specification) and St18 (NC differentiation) to identify active enhancers, and transcription factors bound to them. Sce1 mediated transgenesis has been used to validate these novel NC specific enhancers. We are using this data to currently identify relevant potential enhancers involved in specification or differentiation.

30 Genome-wide analysis of polyadenylation and translation during *Xenopus* oogenesis. *M.D. Blower*[1,2], Fei Yang[1,2], Murat Cetinbas[1,2], Wei Wang[1,2] 1) Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Genetics, Harvard Medical School, Boston MA.

Organisms regulate protein expression using both transcriptional and posttranscriptional mechanisms. The most prominent example of posttranscriptional mRNA regulation involves control of mRNA poly-A tail length by cytoplasmic polyadenylation and deadenyaltion. During *Xenopus* oogenesis and early embryogenesis the vast majority of protein expression changes are orchestrated by controlled lengthening and shortening of mRNA poly-A tails. Decades of work has shown that cytoplasmic polyadenylation plays a key role in oogenesis and early embryogenesis. However, we do not have a genome-wide view of all mRNAs that are controlled by poly-A tail regulation and how changes in mRNA poly-A tail length are translated into changes in protein expression. To address these questions we have developed novel experimental and computational methods for poly-A tail length measurement to study the transcriptome during *Xenopus* oogenesis. We have used Illumina sequencing to directly measure the poly-A tail length of the *Xenopus* transcriptome during oocyte maturation. We found that the transcriptome undergoes dramatic changes during oocyte maturation with hundreds of mRNAs showing increased poly-A tail length. To examine mRNA translation during oogenesis we have measured mRNA association with poly–ribosomes. Consistent with our poly-A sequencing data we find that oocytes undergo large changes in protein translation during oogenesis and that increased poly-A tail length correlates with increased mRNA engagement by poly-ribosomes. Using this large dataset we have identified conserved and novel groups of proteins that show regulated protein expression during oocyte maturation. Additionally, our dataset has allowed us to identify and annotate alternative poly-A site usage. Taken together we have developed a novel experimental and computational framework to analyze control of protein expression by cytoplasmic polyadenylation that has yielded novel insights into the proteins expressed during oocyte maturation.

31 Spindle to Cortex Communication in Frog Eggs. *C.M Field*[1,2], J.F. Pelletier[1,2], T.J. Mitchison[1,2] 1) Systems Biology, Harvard Medical School, Boston, MA; 2) Marine Biological Laboratory Woods Hole, MA.

A central question in cytokinesis is how spatial information is communicated from the metaphase spindle to the cortex to position the cleavage furrow. In frog eggs, this occurs over hundreds of microns. Using confocal microscopy of fixed Xenopus zygotes after normal and polyspermic fertilization we discovered a central role for the planar boundary between sister microtubule asters that grow from the poles of the spindle at anaphase. Proximity to anaphase chromatin triggers assembly of CPC and Centralspindlin onto stable microtubule bundles at

the aster boundary. The resulting CPC-positive state of the aster boundary propagates outwards as the asters grow towards the cortex. We are investigating initiation and propagation of CPC-positive aster boundaries using an egg extract system, and also the influence of these boundaries on other components of cytoplasm. Actin and keratin networks disassemble in the vicinity of CPC-positive microtubule bundles, dependent on AurkB kinase activity, which we interpret as a mechanism for softening the cytoplasm in preparation for furrow ingression. Using active CPC immobilized on beads, and actin disassembly as a readout, we estimate that kinase activity extends ~20 microns as a reaction-diffusion gradient. We hypothesize this kinase activity gradient plays a key role in propagating CPC recruitment outwards from chromatin, towards the cortex, via CPC auto-activation on microtubule bundles.

32 TBC1d24-ephrinB2 interaction regulates contact inhibition of locomotion in neural crest cell migration. *Jaeho Yoon*, Yoo-seok Hwang, Moonsup Lee, Jian Sun, Ira Daar Cancer & Developmental Biology Laboratory, NCI-frederick, FREDERICK, MD.

Although Eph-ephrin signalling has been implicated in the migration of cranial neural crest (CNC) cells, it is still unclear how ephrinB transduces signals regulating this event. We provide evidence that TBC1d24, a putative Rab35-GTPase activating protein (Rab35 GAP), complexes with ephrinB2 via the scaffold Dishevelled (Dsh), and mediates a signal affecting contact inhibition of locomotion (CIL) in CNC cells. Moreover, we found that in migrating CNC, the interaction between ephrinB2 and TBC1d24 negatively regulates E-cadherin recycling in these cells via Rab35. Upon engagement of the cognate Eph receptor, ephrinB2 is tyrosine phosphorylated, which disrupts the ephrinB2/Dsh/TBC1d24 complex. The dissolution of this complex leads to increasing E-cadherin levels at the plasma membrane, resulting in loss of CIL, and disrupted CNC migration. Our results indicate that TBC1d24 is a critical player in ephrinB2 control of CNC cell migration via CIL.

33 The Response of Wild-Type Epithelial Cells to Oncogene-Expressing Clusters. *M.E. Moruzzi*[1], A. Nestor-Bergmann[2], O. Jensen[1], K. Brennan[1], S. Woolner[1] 1) University of Manchester, Manchester, GB; 2) Department of Physiology, Neuroscience and Development, Cambridge, GB.

In early stage cancer, oncogenic lesions exist within a largely intact epithelial tissue. At later disease stages, tumours are known to alter their proteomic and mechanical microenvironment; this changes the behaviour of wild-type fibroblasts and immune cells, aiding disease progression. It remains largely unknown whether early oncogenic lesions alter their local environment or the behaviour of surrounding cells. To investigate the response of wild-type epithelial cells to neighbouring oncogenic lesions, we established an early carcinoma model using *Xenopus laevis* embryos, creating clusters of oncogene-expressing cells within otherwise normal *in vivo* tissue. Surrounding wild-type epithelial cells respond to either *k*Ras^{V12} or *c*MYC clusters with a significant increase in cell division rate. Furthermore, wild-type cell divisions close to *k*Ras^{V12} clusters acquire directionality, orienting towards the cluster. Cell shape analysis indicates *k*Ras^{V12} clusters induce directional tensile stress in surrounding tissue. Both the changes in mechanical stress and cell division depend on non-muscle myosin II expression in the *k*Ras^{V12} cluster, suggesting the response of wild-type cells is downstream of cytoskeletal contractility in *k*Ras^{V12} cells. *c*MYC clusters do not alter tissue mechanics and the increased division rate in surrounding cells is not dependent on myosin II, implying the wild-type cell response to *c*MYC clusters is likely due to a change in the chemical environment. Our results indicate deregulated cell division may occur in surrounding wild-type, as well as oncogene-expressing, epithelial cells from the very earliest stages of carcinoma.

34 Modeling tracheoesophageal birth defects: the role of Hedgehog/Gli signaling in foregut morphogenesis. *T. Nasr*[1,2,3,5], P. Mancini[1,2,3], J. Vardanyan[1,2,3], J. Kinney[1,2,3], K. Daniels[1,2,3], P. Chaturvedi[1,2,3], S. Cha[1,2,3], S. Rankin[1,2,3], J. Shannon[1,2,3,4], A. Zorn[1,2,3,5] 1) Center for Stem Cell & Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 4) Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 5) Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, OH.

The trachea and esophagus (TE) arise from a single foregut tube in early fetal development. Defects in TE morphogenesis result in a spectrum of life-threatening congenital tracheoesophageal birth defects (TEDs) that prevent proper breathing or feeding in newborns. The genetic basis of TEDs, as well as the molecular and cellular pathways controlling normal TE morphogenesis, are poorly understood. We have used a combination of immunostaining, small molecule treatments, and genetic manipulation of Xenopus and mouse embryos to define the conserved cell biological events and biomechanical processes that control separation of the trachea and esophagus from the common foregut tube. We have defined a novel step-wise process of TE morphogenesis, including dorso-ventral patterning of the foregut tube; mesenchymal-driven medial constriction; and epithelial remodeling concurrent with foregut elongation and differentiation. We have examined how this cell biology is regulated by the Hedgehog (Hh) pathway; one of the few pathways that, when mutated, is known to cause TEDs in human patients and mouse models. Hh acts through the transcription factors Gli2 and Gli3. In the absence of signaling, Gli3 acts as a transcriptional repressor; with Hh signaling Gli2 and Gli3 become transcriptional activators. Xenopus and mouse models of Pallister-Hall Syndrome (OMIM 146510) caused by a constitutive *GLI3*-repressor mutation exhibit delayed medial constriction and defects in epithelial remodeling resulting in a tracheoesophageal cleft similar to some patients. This work defines for the first time the cell biological basis of TEDs. Ongoing genomic studies focus on identifying the Gli-regulated genes that control TE morphogenesis, which may be novel candidate genes for TED-causing mutations in human patients.

35 The Ran-GTP Gradient Acts as a Rheostat to Promote XCTK2 MT Cross-Linking and Sliding. Stephanie Ems-McClung[1], Lesley Weaver[2], Stephanie Zhang[2], *Claire Walczak*[1] 1) Medical Sciences, Indiana University, Bloomington, IN; 2) Department of Biology, Indiana University, Bloomington, IN.

The mitotic spindle is critical for the accurate segregation of chromosomes into daughter cells. A major regulator of spindle assembly is the

small GTPase Ran, which forms a gradient of RanGTP around the chromatin that extends to the spindle poles and activates a number of spindle assembly factors. While the existence of the RanGTP gradient has been clearly established, it is not known whether there are effector gradients of spindle assembly factors that respond differentially to RanGTP. Our previous work demonstrated that XCTK2, a Kinesin-14 that cross-links and slides microtubules (MTs), is a key Ran effector, but how its activity is controlled by Ran has not been established. Here, we report that enhancing the RanGTP gradient in spindles assembled in *Xenopus* egg extracts promotes the localization of XCTK2 to the spindle. This localization is mediated by the tail of XCTK2, whose MT binding is tunable by the Ran-regulated import receptors, importin alpha/beta. *In vitro*, importin alpha/beta inhibit XCTK2 anti-parallel MT cross-linking and sliding activity, suggesting that importin alpha/beta could promote a bias toward parallel MT sliding in the spindle. To elucidate where the importins interact with XCTK2 in the spindle, we generated a series of FRET biosensors of XCTK2 and importin alpha. Through its tail domain, YPet-XCTK2 interacts directly with importin alpha-CyPet and importin beta, resulting in a high FRET ratio that can be reduced by the addition of the RanGTP analog RanQ69L. Using fluorescence lifetime imaging microscopy, our preliminary results suggest that importin alpha interacts with XCTK2 in a gradient in the spindle with the highest interaction occurring near the spindle poles. Together, our results suggest that the RanGTP gradient is used to promote XCTK2 binding to the spindle and acts as a rheostat to modulate MT cross-linking and sliding along the length of the spindle. We propose that this activity is most critical to bias XCTK2 activity toward parallel MT cross-linking where it facilitates spindle pole focusing.

36 Dynamic signals of p300 binding in early development. Rosa Gomes Faria, *Mike Gilchrist* The Francis Crick Institute, London, GB.

Xenopus is an ideal system for studying the regulation of transcription in early development. Here we follow up our earlier work on dynamic signals of gene activation with a similarly time-resolved, global study of p300 binding in the immediate post-MBT embryo. The p300 protein is implicated in promoter-enhancer DNA looping, and although its precise role is not well understood, it is routinely used as a mark of active enhancers. We generated p300 ChIP data from *Xenopus tropicalis* embryos at 30 minute intervals between 5.0 and 17.5 hours post fertilisation (Stages 9 to 19). Once we had developed an appropriate normalisation method, we were able to see dynamic signals of p300 binding at candidate enhancers, which we could then compare with p300 binding and transcription rates at nearby genes. This allowed us to make predictions for likely enhancer-gene pairs, and showed, somewhat counter-intuitively, that p300 binding correlates better with transcription levels than with transcription rates. Furthermore, we were able to use transcription factor motif analysis of the DNA sequence underlying our dynamic enhancer marks to make candidate target gene predictions. We propose that this approach will yield insights into the general mechanisms of transcriptional control, as well as specific details of gene-enhancer interactions.

37 Dual roles for ATP in modulatiing nucleolar protein aggregates in *X. laevis* oocytes. M. H. Hayes[2], *D. L. Weeks*[1,2] 1) Biochemistry, University of Iowa, Iowa City, IA; 2) Molecular Medicine, University of Iowa, Iowa City, IA.

For many proteins, aggregation is one part of a structural equilibrium that can occur. Balancing productive aggregation versus the pathogenic aggregation that leads to toxicity is critical and known to involve ATP dependent action of chaperones and disaggregases. However, recently, adenosine triphosphate (ATP) was identified as a hydrotrope which, independent of hydrolysis, was sufficient to shift the equilibrium between soluble and aggregated protein *in vitro*. This novel function of ATP was postulated to help regulate proteostasis *in vivo*. We tested this hypothesis on amyloid and liquid droplet aggregates found in *Xenopus* oocyte nucleoli. We found evidence that ATP is important for a destabilizing event in two ways, one that includes an energy dependent process reliant upon one or more soluble nuclear factors after which, ATP continues to change aggregate structure independent of hydrolysis.

38 Sensory activity mediated by GluN2B-containing NMDARs facilitates structural and functional recovery of the tectal circuit following injury in Xenopus tadpoles. *H.T. Cline*, A.C. Gambrill, R.L. Faulkner, C.R. McKeown Department of Neuroscience, The Scripps Research Institute, La Jolla, CA.

Traumatic brain injuries introduce functional and structural circuit deficits that must be repaired in order for an organism to regain function. In many systems, repair is dependent on neurogenesis and the integration of new neurons into the existing circuit. However, the mechanisms governing this integration are poorly understood. We have developed an injury model in which Xenopus laevis tadpoles are given a penetrating stab wound which damages the optic tectal circuit and results in a loss of behavior. The developing Xenopus tectal circuit is highly plastic and its maturation is driven by sensory activity. We tested whether providing additional visual input could affect circuit recovery. Visual stimulation introduced 24 hours following injury drove increases in spontaneous activity, circuit integration, and sped behavioral recovery, but failed to affect structural plasticity. In order to investigate the mechanism of activity-mediated post-injury plasticity, we knocked down GluN2B-containing NMDA receptors. Loss of GluN2B-NMDARs resulted in smaller dendritic arbors and deficits in functional integration in neurons born post-injury. GluN2B knockdown also prevented behavioral recovery. Visual activity-provoked changes in functional plasticity and behavioral recovery were occluded by loss of GluN2B function. We conclude sensory activity mediated by GluN2B-containing NMDARs mediates structural and functional recovery of the tectal circuit following injury in Xenopus tadpoles.

39 Regulation of integrin signaling in chondrogenesis and osteoarthritis development. Eun Kyung Song[1], Jimin Jeon[2], Sofia Medina-Ruiz[3], Taejoon WKwon[1], Siyoung Yang[1], *Tae Joo Park*[1] 1) School of Life Sciences, Ulsan Natl. Inst. of Sci. &Tech., Ulsan, KR; 2) Department of Pharmacology, Ajou University School of Medicine, Suwon 16499, Korea; 3) Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, United States.

Basic research and clinical trials have investigated the function of integrin signaling in chondrocyte differentiation and cartilage disorders, although the exact functions of integrin signaling during chondrogenesis are not well understood. Integrin signaling is necessary for cartilage development, as the loss of known mediators of integrin signaling causes abnormal cartilage and endochondral bone formation. In contrast, integrin-extracellular matrix (ECM) contacts promote the dedifferentiation of cultured primary chondrocytes, and several studies suggest that integrin signaling serves different roles depending on the chondrogenic stage. Furthermore, integrin signaling is a key source of the inflammatory reactions responsible for joint destruction. Given the ECM-rich environment and the expression of multiple integrin subunits, it is

challenging for chondrocytes to minimize integrin-ECM interactions to allow chondrogenic differentiation to proceed and also maintain chondrogenic properties and protect from dedifferentiation or destructive signals. Here, we found a secreted integrin modulator expressed in prechondrocytes and promoting chondrogenesis in vertebrate. Integrin signaling is not only involved in cartilage disorders, but also contributes to various other human disorders such as inflammatory bowel disease, cardiovascular disorders, and cancers. Our discovery on a unique secreted integrin modulator should attract attention from researchers in many fields of biomedical science and will lead to new approaches for treating integrin-related human diseases, including destructive cartilage disorders.

40 How do Wnt proteins regulate planar cell polarity? *Yusuke Mii*[1], Ritsuko Takada[1], Makoto Matsuyama[2], Shinji Takada[1] 1) National Institute for Basic Biology, Okazaki, Aichi, JP; 2) Shigei Medical Research Institute, Okayama, Okayama, JP.

Wnt is a family of secreted signaling proteins involved in various processes of animal development. Among these processes, embryonic patterning is mediated by Wnt/beta-catenin signaling and planar cell polarity (PCP) is mediated by so-called noncanonical Wnt or Wnt/PCP signaling. However, it is still controversial how Wnt proteins regulate these processes, largely due to the lack of visualization of Wnt proteins. Recently we reported visualization of endogenous Wnt8 protein and essential roles of "heparan sulfate clusters" in distribution and signaling of Wnt8 protein in *Xenopus* embryos (Mii et al., *Nat. Commun.* 2017). Here we examined spatial distribution of Wnt11, which mainly activates noncanonical Wnt signaling. Interestingly, most of endogenous Wnt11 protein was arranged in "parallel pattern" along the medio-lateral axis in the dorsal marginal zone. This pattern is similar to polarized localization of GFP-Pk3 co-expressed with Vangl2, which is a recently reported method to visualize PCP (Chu and Sokol, *eLife* 2016). When Wnt11 was overexpressed in the animal cap region, where cell polarity is not evident in normal embryos, Wnt11 was unevenly accumulated showing a polarized pattern around a single cell. This polarized accumulation was dependent on Wnt signaling. At the edge of Wnt11-expressing cells, endogenous Frizzled7, a core PCP component as well as a Wnt receptor, was also accumulated together with Wnt11. On the other hand, another core PCP component Pk3, which shows opposite localization to Frizzled, was reduced at the edge of Wnt11-expressing cells. These observations suggest a local regulation of PCP by Wnt11. Indeed, a membrane-tethered form of Wnt11 can direct PCP within a short range (a few cell diameters). However, secreted wild-type Wnt11 can direct PCP over a long range (15-20 cell diameters), suggesting diffusion of the Wnt protein is essential for the long range, global coordination of PCP. Importantly we found that core PCP components can affect localization of Wnt11. Thus we propose that local and mutual regulation of Wnt11 and core PCP components could coordinate cell polarity in a long range, through a domino-like polarization of cells and relocation of Wnt11 protein.

41 Spatiotemporal patterning of zygotic genome activation in vertebrate embryogenesis. *H. Chen*[1], L Einstein[1], M Good[1,2] 1) Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA; 2) Bioengineering, University of Pennsylvania, Philadelphia, PA.

Thousands of zygotic genes are activated during maternal-to-zygotic transition (MZT); however, how massive gene expression is spatially regulated within an embryo is still unknown. Using *Xenopus*as a model, we detected nascent transcripts by labeling RNA with 5-ethynyl uridine (5-EU). In combination with click chemistry and confocal imaging, we were able to track zygotic genome activation (ZGA) at the single-cell level in an embryo during the mid-blastula transition (MBT). As expected, we found that the EU-RNA was not detected in embryos prior to cleavage 10 (C10), consistent with published gene profiling data derived using RNA extracted from whole embryos. Interestingly, ZGA is not uniform in space or time – high levels of zygotic transcription were evident in only a few cells in the animal pole at C12, increasingly more cells at C13-C14 and nearly entire embryo at C15. This progressive zygotic gene transcription argues against a timer model, in which ZGA onset corresponds to a specific time post-fertilization. To validate our labeling approach, we co-microinjected 5-EU and α-amanitin. We found that α-amanitin treatment eliminated nearly all nascent transcription, suggesting that the EU-labeled RNAs we image are predominantly RNA polymerase II dependent. The spatial patterns of ZGA prompted us to characterize whether ZGA is cell size dependent. By quantifying the EU-RNA level in single cells at different stages, we found that the EU-RNA level was inversely correlated with cell size, supporting a cell-size sensing model for ZGA. To differentiate between the effects of cell size and number of cell divisions, we generated mini-embryos that are one fourth to one eighth volume of the normal embryos. Large-scale transcription initiated as early as cleavage 10 in mini-embryos but it was still off in wild-type embryos. These data suggest that by reducing cell size below a size threshold, genome activation is triggered. Finally, a higher percentage of cells in the presumptive ectoderm compared to the presumptive endoderm were transcriptionally active at the MBT, suggesting a potential link between spatial onset of ZGA and germ layer specification. Taken together, our results provide new insights into ZGA regulation and a spatial map of ZGA onset during early embryogenesis in vertebrates.

42 A novel function of the ubiquitin-independent proteasome system in controlling *Xenopus* germline development. Hyojeong Hwang, Zhigang Jin, *Jing Yang* Comparative Biosciences, University of Illinois, Urbana, IL.

Germline development is essential for the continuation of every species. In *Xenopus,* which specifies the germline through inheritance of germ plasm, genetic information responsible for early primordial germ cell (PGC) development is carried by a subset of RNAs and proteins synthesized during oogenesis. These maternal factors are stored in the oocyte in a quiescent state. After fertilization, they become activated and act in a precisely regulated fashion to coordinate early PGC development. Currently it is unclear how these maternal factors are regulated during the oocyte-to-embryo transition.

Dead-end1 (Dnd1), is a vertebrate specific RNA-binding protein essential for germline development. In *Xenopus*, Dnd1 is expressed at a very low level in the oocyte. After fertilization, the expression of Dnd1 protein increases dramatically to facilitate translation of *nanos*, which is essential for preventing somatic differentiation of PGCs. The expression Dnd1 protein must be tightly regulated. Overexpression of Dnd1 in the oocyte induces premature *nanos* translation and impaired embryonic development. Here we report that Dnd1 protein is intrinsically unstable in the oocyte. Degradation of Dnd1 in the oocyte is mediated by an evolutionarily conserved degron through a ubiquitin-independent and proteasome-dependent mechanism. Inside the cell, un-ubiquitinated proteins are recruited to the proteasome for degradation by three proteasome activators. These include PA28a/b hetero-heptamer, PA28g homo-heptamer, and PA200, which acts a monomer to open the gate

of the proteasome. We found that Dnd1 was stabilized in the oocyte when dominant negative (dn) PA28a, dnPA28b, dnPA28g, and dnPA200 were co-overexpressed. Strikingly, we found that the localization, but not the total level of PA28a, PA28b, PA28g, and PA200 mRNAs was dynamically regulated during the oocyte-to-embryo transition. In fully-grown oocytes, these proteasome activators mRNAs are distributed in the entire oocytes. By 2-cell stage, however, they become restricted to the animal hemisphere, completely separated from vegetally localized *dnd1*. Importantly, we found that ectopic expression of PA28a/b, PA28g, and PA200 in the vegetal pole severely interfered with PGC development. Based on these results, we conclude that the vegetal-to-animal translocation of proteasome activator mRNAs during the oocyte-to-embryo transition, which separates proteasome activators from vegetally localized germ plasm, is a prerequisite for germline development after fertilization.

43 Genetic and Epigenetic Control of Centromere Function in the Early Xenopus Embryo. Owen Smith[1], David Jukam[1], Magdalena Strzelecka[2], Andrew Grenfell[2], Rebecca Heald[2], *Aaron Straight*[1] 1) Department of Biochemistry, Stanford University, Stanford, CA; 2) Department of Molecular and Cellular Biology, University of California at Berkeley, Berkeley, CA.

In eukaryotes, accurate chromosome segregation during cell division requires the attachment of each replicated chromosome pair to the microtubules of the mitotic spindle. The interaction site between the chromosome and the microtubules is the chromosomal centromere and kinetochore. Disruption of centromere and kinetochore function result in chromosome missegregation, aneuploidy and cell death. A defining feature of the centromere is the replacement of histone H3 in nucleosomes with the centromere specific histone variant CEntromere Protein A (CENP-A). CENP-A is essential for viability and is required for the formation of centromeres and kinetochores. Centromere formation is thought to be strongly epigenetically determined by the presence of CENP-A and once a centromere is formed its chromosomal location is maintained, independent of the sequence of the underlying DNA. In vertebrates, CENP-A is retained on chromatin through meiosis so that the location of centromeres is preestablished in sperm and egg nuclei. This has made it difficult to independently evaluate the importance of DNA sequence and CENP-A dependent epigenetic control in the process of centromere formation. To study the de novo formation of centromeres we have developed a system using Xenopus laevis egg and embryonic extracts that can assemble new CENP-A nucleosomes in vitro. We have recently isolated centromeric sequences in Xenopus that selectively associate with CENP-A nucleosomes. Using these sequences, we have been able to reconstitute arrays of Xenopus centromeric repeats in the absence of CENP-A nucleosomes and independently test the importance of DNA sequence and CENP-A chromatin in centromere formation. These artificial neocentromeres provide a unique approach to study the formation of centromeres and the role of genetic and epigenetic forces in controlling chromosome segregation.

44 Exploiting CRISPR/Cas9 mediated genome editing to model human hereditary retinal disease and cancer in *Xenopus tropicalis*. Thomas Naert[1,4], Marjolein Carron[1,2], Dionysia Dimitrakopoulou[1], Dieter Tulkens[1], Giulia Ascari[2], Frauke Coppieters[2], Chris Guerin[1,3], Pieter Van Vlierberghe[2], Elfride De Baere[2], *Kris Vleminckx*[1,2,4] 1) Department for Biomedical Molecular Biology, Ghent University, Ghent, BE; 2) Center for Medical Genetics, Ghent University, Ghent, BE; 3) VIB-UGent Center for Inflammation Research, Ghent, BE; 4) Cancer Research Institute Ghent, Ghent, BE.

The last couple of years have seen unbiased and systematic analysis of gene mutations, genomic rearrangements and RNA expression in patients afflicted by Mendelian disorders or cancer. While whole exome and whole genome sequencing (WES and WGS) allow detailed and fast identification of mutations and variants in the afflicted patients, it mostly remains a major challenge to identify the affected gene that is linked to the disease. Due to its diploid genome that is highly syntenic to the human, *Xenopus tropicalis* is ideally positioned for modeling human disease and interrogate gene function. We have recently generated a model for human syndromic retinal dystrophy by inactivation of the *rcbtb1* gene, encoding a putative substrate adaptor for cullin 3 (CUL3), the major component of the CULLIN3-RING ubiquitin ligase complex. Immunohistological analysis and 3D electron microscopy confirmed the occurrence of retinal dystrophy in the knockout animals. Functional studies, including electroretinography, are established to follow disease progression in living animals and molecular analysis is performed to document the physiological retinal processes affected by rcbtb1 dysfunction to find anchor points for therapeutic intervention. In a parallel on-going effort, we continue to build models for human cancers, primarily by using multiplexed CRISPR/Cas9 mediated targeting of combinations of tumor suppressor genes. As such we already modeled several human solid tumor types including retinoblastoma, desmoid tumors, choroid plexus cancer, pancreatic neuroendocrine carcinoma, bladder cancer and small cell lung cancer. Furthermore, by combining the introduction of activating mutations in *notch1* and inactivating mutations in the tumor suppressor gene *pten*, we obtained a model for human T-cell acute lymphoblastic leukemia (T-ALL). We will present our experimental pipeline for identification of additional driver mutations in T-ALL as well as for screening potential dependency factors that may be amendable for therapeutic intervention.

45 Coordinated regulation of the dorsal-ventral and anterior-posterior patterning of *Xenopus* embryos by the BTB/POZ zinc finger protein Zbtb14. Kimiko Takebayashi-Suzuki, Misa Uchida, *Atsushi Suzuki* Amphibian Research Center, Hiroshima University, Higashi-Hiroshima, JP.

During early vertebrate embryogenesis, bone morphogenetic proteins (BMPs) belonging to the TGF-β family of growth factors play a central role in dorsal–ventral (DV) patterning of embryos, while other growth factors such as Wnt and FGF family members regulate formation of the anterior–posterior (AP) axis. Although the establishment of body plan is thought to require coordinated formation of the DV and AP axes, the mechanistic details underlying this coordination are not well understood. Here we show that a *Xenopus* homologue of *zbtb14* plays an essential role in the regulation of both DV and AP patterning during early *Xenopus* development. We show that overexpression of Zbtb14 promotes neural induction and inhibits epidermal differentiation, thereby regulating DV patterning. In addition, Zbtb14 promotes the formation of posterior neural tissue and suppresses anterior neural development. Consistent with this, knock-down experiments show that Zbtb14 is required for neural development, especially for the formation of posterior neural tissues. Mechanistically, Zbtb14 reduces the levels of phosphorylated Smad1/5/8 to suppress BMP signaling and induces an accumulation of β-Catenin to promote Wnt signaling. Collectively, these results suggest that Zbtb14 plays a crucial role in the formation of DV and AP axes by regulating both the BMP and Wnt signaling pathways during early *Xenopus* embryogenesis.

46 The transcription factor Hypermethylated in Cancer 1 (Hic1) interacts with Wnt signaling pathways during neural crest migration. *H.J. Ray*, C. Chang Cell, Development and Integrative Biology Department, University of Alabama Birmingham, Birmingham, AL.

Many members of the POZ-ZF transcription factor (TF) family are known regulators of both developmental processes and cancer. The POZ-ZF TF Hypermethylated in Cancer 1 (HIC1) is an epithelial tumor suppressor that is recognized as a valuable prognostic marker, although little is known about HIC1 function in normal epithelial cells. Additionally, Hic1 loss-of-function mouse alleles show embryonic lethality accompanied with developmental defects, including craniofacial defects, that are reminiscent of human Miller-Dieker syndrome, a complex developmental disorder resulting from a large genomic deletion that includes the *HIC1* gene. While this data gives clues that HIC1 may play an important role in early developmental processes, the mechanisms of HIC1 function in embryogenesis are currently unknown. A previous RNA-sequencing study in our lab found that *hic1* expression is enriched within the presumptive neuroectoderm domain during gastrulation when compared to both presumptive ectoderm and earlier ectodermal precursor populations, prompting us to use the power of the *Xenopus laevis* model system to investigate Hic1 functions in development of neuroectodermal derivatives. We find that *hic1* is expressed throughout early development and in developing tissues, including the cement gland and branchial arches. Targeted micro-injection of either *hic1* mRNA (overexpression) or a translation-blocking morpholino (*hic1* MO, knockdown) to the neural-neural crest domain results in craniofacial defects including malformation of the craniofacial cartilages. In situ hybridization (ISH) reveals that early patterning of neural and cranial neural crest (CNC) domains is mostly unaffected, but migration of the CNC population is altered. Transplant and explant studies using CNC cells from *hic1* morphants further demonstrate that overall CNC migration is decreased and morphant cells exhibit defects in lamellipodial formation and persistence. Mechanistic studies using ISH, RT-PCR and secondary axis formation assays reveal that *hic1* interacts with Wnt signaling pathways. Our data help to shed light on the mechanisms of *hic1* function during development. Ongoing studies are aimed at elucidating the molecular nature of *hic1-wnt* interaction during CNC migration, with implications for our understanding of HIC1 function in both developmental processes and cancer.

47 Role of the genetic pathway Lin28/*let-7* in the thyroid hormone homeostasis during *Xenopus* metamorphosis. Daniel Guzmán-Gundermann[1], Jimena Martínez[2], Cristal Muñoz[2], Juan Larrain[1], *Fernando Faunes*[2] 1) Center for Aging and Regeneration, Millennium Nucleus in Regenerative Biology, Faculty of Biological Sciences, P. Universidad Católica de Chile, Chile; 2) Faculty of Life Sciences, Universidad Andrés Bello, Chile.

Amphibian metamorphosis is triggered by the hypothalamus-pituitary-thyroid gland (HPT) axis, which results in the synthesis of thyroid hormones (TH). However, the genetic mechanisms involved in the activation of the HPT axis and how these axes are connected to environmental conditions have not been described. We previously showed that the heterochronic gene Lin28 is downregulated during metamorphosis and the ubiquitous overexpression of Lin28 delays Xenopus metamorphosis. Lin28 regulates the translation of multiple genes and inhibits the biogenesis of the microRNA *let-7*. Our aim is to determine the molecular targets downstream of Lin28 during metamorphosis. Proteome analysis showed that the TH-transporter Albumin is downregulated after Lin28 overexpression, indicating defective TH-transport in Lin28-overexpressing animals. Transcriptome analysis showed that several pituitary hormones are downregulated in Lin28-overexpressing animals compared to controls.

Importantly, a truncated form of Lin28A (Lin28A-DeltaC) that is not able to regulate translation also delayed metamorphosis. These results suggest that Lin28 control the timing of metamorphosis through *let-7*.

To study if food availability regulates Lin28 expression, we performed starvation experiments during pre-metamorphosis. The presence of food for two days downregulates Lin28 expression compared to starved controls. We propose that food availability, the genetic pathway Lin28/*let-7* and the HPT axis are integrated to allow the proper timing of metamorphosis.

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48 A new model for the molecular mechanism of regulating Nodal signaling range to generate left-right asymmetry in vertebrates. *T. Ikeda*[1], Takayoshi Yamamoto[2], Hiroyuki Takeda[1], Masanori Taira[3] 1) Laboratory of Embryology, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo; 2) Graduate School of Arts and Sciences, The University of Tokyo, Tokyo; 3) Faculty of Science and Engineering, Chuo University, Tokyo.

In vertebrates, left-side specific *nodal* expression in the lateral plate mesoderm (LPM) is essential for the formation of left-right asymmetry. Previous studies have suggested that the Nodal protein forms a heterodimer with Gdf1 and is secreted from the cells beside the left-right organizer (LRO), then induces its own expression in the left LPM. However, the molecular mechanism of this long-range Nodal signaling from the LRO to the left LPM is unknown. Here we found that *Xenopus* Nodal1, a functional homolog of mouse Nodal, shows dot-like distribution in the intercellular space upon heterodimerization with Derrière, one of the *Xenopus* Gdf1 ortholog, and that the Nodal1-Derrière heterodimer interacts with heparan sulfate (HS). In addition, Nodal1 pro-domain enhanced the interaction with Nodal1 and heparin *in vitro*. We also found that Pcsk6, which is formerly known as a Nodal1 pro-domain convertase, accumulates Nodal1-Derrière on its expressing cells. Pcsk6 were found to interacts with both HS and the full-length Nodal1, but with the mature Nodal1 to a lesser extent. Considering these results and a previous study in which Pcsk6 was reported to cleave Nodal1 pro-domain, but not Derriere, it was suggested that Nodal1-Derrière on the Pcsk6 expressing cells largely retains its pro-domain. Based on these findings, we concluded that Pcsk6 may have a novel function as an adaptor between Nodal1-Derrière and HS rather than convertase. Together, our results suggest that the long-range activity of the Nodal1-Derrière heterodimer is accomplished through the interaction with HS, and this interaction may be enhanced by the pro-domain of Nodal1 and Pcsk6. We are currently examining which amino acid residues within Nodal1 or Derrière are responsible for their interaction with HS and Pcsk6, by examining the distribution pattern of various point mutants. Furthermore, we also investigated how Nodal signaling is inhibited on the right side. We found that a secreted Nodal inhibitor Coco/Dand5, which is preferentially expressed on the right side of the LRO, also interacts with HS. This result suggests that Coco may accumulate on the right side to inhibit Nodal signal unilaterally. Taken together, our study has not only revealed that HS is involved in both Nodal signaling range expansion on the left side and Nodal signal inhibition on the right side, but also unified three proposed regulatory mechanisms of Nodal signaling range, namely, the heterodimerization, the pro-domain

cleavage, and the interaction with extracellular matrix, thus proposing a new model for the molecular mechanism of regulating Nodal signaling range to generate left-right asymmetry. We are now verifying the generality of this model within vertebrates, focusing on the distribution pattern of the orthologs of Nodal1 and Derrière in zebrafish and mice.

49 Genome-wide analysis of topoisomerase II cleavage sites on condensed mitotic chromosomes. J. Paiano[2], J. Haase[1], A. Canela[2], W. Wu[2], M.K. Bonner[1], A. Nussenzweig[2], *A.E. Kelly*[1] 1) Laboratory of Biochemistry and Molecular Biology, NIH/NCI, Bethesda, MD; 2) Laboratory of Genome Integrity, NIH/NCI, Bethesda, MD.

Mitotic chromosome condensation requires the condensin I and II complexes as well as topoisomerase II (topo II). Current evidence suggests that condensins organize chromosomes by extruding DNA into large loops, which results in helical arrays arranged along a central scaffold. However, much less is known about what roles topo II plays in the condensation process, and how it collaborates with condensins. To study the role of topo II in condensation, we mapped etoposide-dependent double-strand breaks across unreplicated mouse chromosomes in *Xenopus* egg extracts using END-seq. As a first step, we analyzed chromosomes that had reached steady-state condensation, wherein topo II and condensin II are localized to the central axis. Across multiple replicates, we found 7,482 highly reproducible topo II cleavage sites spread evenly over all chromosomes. This results in one cleavage every ~370 kb, which is highly similar to the reported ~400 kb loops formed by condensin II. Indeed, we found that a significant subset of these topo II cleavage sites (~900) overlap with condensin II locations found in mouse embryonic stem cells. Altogether, our results demonstrate a non-random distribution of topo II-dependent cleavage sites within condensed chromosomes, and suggest that a subset of these sites may be located at the base of condensin II-formed loops. We are investigating the possibility that specific topo II and condensin II "founder sites" may be important regulators of chromosome condensation.

50 The Extreme Anterior Domain regulates head size: a target for microcephaly. *Hazel Sive*[1,2], Justin Chen[1,2] 1) Whitehead Institute, Cambridge, MA; 2) Massachusetts Institute of Technology, Cambridge, MA.

As the complex architecture of the head develops, many tissues grow and collectively contribute. This process is interrupted in microcephaly, a significant anomaly associated with attenuated brain size. Interestingly, in both syndromic and induced microcephaly, the facial skeleton is often also anomalous, suggesting coordination between development of the brain and facial skeleton. We show that the *Xenopus* Extreme Anterior Domain (EAD), a conserved embryonic region, is an organizer of overall head size and morphology. Extirpation of the EAD reduces head size, including a smaller forebrain and midbrain as well as truncated facial cartilages derived from the first, second and third neural crest streams. These data suggest that the EAD plays a global signaling role and extends a broad 'sphere of influence' during head development. Part of this organizer function depends on the beta-catenin Wnt inhibitors Frzb and Crescent that are produced in the EAD, since EAD-specific loss of function (LOF) embryos have small brains and truncated facial cartilages. Transgenic reporter analysis indicates that the EAD has a signaling range of at least 600um, with activity initiating at tailbud stages. Interruption of EAD signaling alters pharyngeal arch regulatory gene expression in the neural crest (*sox9*), mesoderm (*tbx1*) and endoderm (*fgf8)*. A key target of EAD signaling is cell proliferation, as pH3 immunostaining was markedly reduced in both the brain and neural crest after EAD-specific LOF in *frzb1* and *crescent*. Our findings identify a mechanism for coordinating the size and differentiation of multiple tissues that contribute to the head. Although microcephaly has been viewed as due to cell autonomous effects, this study identifies a global mechanism that may be impacted by agents associated with microcephaly, with accompanying implication for therapeutic intervention.

51 Probing the local mechanical architecture of the metaphase meiotic spindle. *Y. Shimamoto* Center for Frontier Research, National Institute of Genetics, Mishima, Shizuoka, JP.

Metaphase spindles, required for proper segregation of chromosomes during cell division, are arrays of microtubules whose movement and overlap length are controlled by diverse force-generating activities such as those of motor proteins, while the overall size and bipolarity of the structure are robustly maintained. Through decades of studies, we now have advanced knowledge of filament architecture, dynamics, and associated proteins. However, it is still unclear how the steady bipolar structure is maintained, because of poor understanding of the responses of spindle microtubules to force. Previously, we had developed a microneedle-based quantitative micromanipulation assay in *Xenopus* cell-free egg extract to study the viscoelastic nature of the metaphase meiotic spindle. Here, we integrated this assay with fluorescence-based filament motion tracking to analyze the movement of spindle microtubules in response to force. Our analysis revealed submicron-scale microtubule motion dynamics arising associated with applied forces of hundreds of piconewtons, and their local heterogeneity in terms of spindle location, such as the pole and the equator. We further found that the regional variation in the microtubule's motion responses depends on activities of dynein and kinesin-5, two key motor proteins required for proper spindle assembly. Based on these results, we discuss the local mechanical architecture of the metaphase spindle that would maintain its structural and functional stability for error-free cell division.

52 Development of New Quantitative Proteomics Technology and its Application to Xenopus Oocytes and Embryos. M. Sonnett, E. Yeung, *M. Wühr* Princeton University, Princeton, NJ.

Quantitative analysis of proteomes is essential for understanding both normal biology and disease states, but systematic measurements of proteins lag far behind other omics approaches, such as transcriptional profiling. This lag is due to multiple technical limitations, including difficulties in reliably quantifying rare but important proteins. A major advancement for proteomics was the introduction of multiplexing, which allows the simultaneous quantification of thousands of proteins among multiple conditions. However, accurate multiplexed proteomics currently requires highly specialized and expensive instrumentation. Many important but low abundant proteins like transcription factors remain prone to measurement artifacts or can't be detected.

I will talk about a new quantitative proteomics technology (TMTc+), which will increase the sensitivity, accuracy, and precision of quantitative multiplexed proteomics while making it less expensive and more accessible. We evaluated our new technique by quantifying a developmental time-series of Xenopus development and comparing it to our previous publication acquired with the current state-of-the-art (TMT-MS3). We significantly increase the number of low abundant proteins (e.g. the number of quantified transcription factors increases from 95 to 305).

Lastly, I will talk about our attempt to combine the newly developed methods with targeted proteomics. With this method we aim to further increase proteomics sensitivity down to the level of the lowest abundant transcription factors.

53 A pathway linking the microbiome, host immune system and regeneration. *C. Beck*, T. Bishop, T. Devine, X. Morgan University of Otago, Dunedin, NZ.

Amphibians are the masters of regeneration, effortlessly regrowing severed tails, limbs and other organs for over 300 million years. Although tadpoles of the frog *Xenopus laevis* are an established model of spinal cord (tail) regeneration, there is a short life stage, just before tadpoles begin feeding, when regeneration often fails. We call this the refractory period, and it has been used by ourselves and others to study the differences between successful and unsuccessful regeneration.

Our recent work suggests that the regenerative outcome in response to partial removal of the tail might depend on the composition of the tadpole microbiome. We have found that tadpoles treated with the antibiotic gentamycin are less likely to regenerate, but if heat killed *E.coli* or lipopolysaccharide (LPS) is added to the tadpole media, more tadpoles regenerate. These results may indicate a role for Gram negative bacteria in regeneration competence, likely through interaction with Toll like receptors such as TLR4, key regulators of the immune response.

The idea that the immune system determines regenerative outcome is well established in amphibians and recent evidence suggests this is also the case in mammals. Tadpole tail regeneration is accompanied by rapid and sustained production of reactive oxygen species (ROS). Inhibition of NADPH oxidase (Nox) prevents both sustained ROS production and tail regeneration. Furthermore, in axolotls, another species of amphibian that is capable of regeneration even as an adult, macrophages have been shown to be required for regeneration. We have discovered that the transcription factor NF-kB is rapidly activated following tail amputation. This suggests a pathway where partial amputation of the tail of the tadpole leads to unusual exposure of microbiota to macrophage TLR4, resulting in activation of NF-kB. NF-kB then orchestrates the pro- and anti-inflammatory mechanisms that determine the wound environment, sustain production of ROS, and determine regenerative outcome.

Currently, we are working to define (using 16S rRNA sequencing) and manipulate (by altering media and food source) the microbiome of *Xenopus* tadpoles, to determine the relationships between regeneration success and *Xenopus* skin, gut or environmental microbiota.

54 Proteomic and transcriptomic analysis of neural cell fate in developing *Xenopus laevis* embryos and explants. K. Dubiak, E. Peuchen, M. Champion, N. Dovichi, *P. Huber* Dept of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN.

Descendants of the D1 blastomere in the 8-cell *Xenopus* embryo make major contributions to the brain, retina, and spinal cord of the animal. We have tracked the emergence of the nervous system at the protein level using isobaric labeling combined with mass spectrometry and at the transcript level using RNA-seq technology. D1 progeny were taken from 16- and 32-cell embryos and at four subsequent developmental stages: midblastula (stage 8), gastrula (stage 11), early neurula (stage 14), and mid-neurula (stage 18). In order to understand how extrinsic factors released from surrounding cells influence cell fate, D1 explants were allowed to develop in parallel with sibling embryos to the same developmental stages. A total of 5,967 proteins and 41,368 transcripts (18,803 at TPM >1) were identified and quantitated. Whereas, the protein profiles of progeny or explants do not change substantially over this period, the pattern of RNA expression exhibits marked changes beginning at gastrula stage for both. Gene ontology (GO) analysis of genes differentially expressed between progeny and explants shows high enrichment in progeny cells for biological processes involved in nervous system development, neurogenesis, and morphogenesis; quite distinctly, the explants show enrichment for cilium organization/assembly and microtubule-based processes. In the majority of cases, the temporal expression of a protein follows that of the cognate mRNA. However, we have identified some examples of translational regulation where the transcript is inactive. An interesting example in this regard is Churchill (Churc), which regulates early neural gene expression. The mRNA encoding Churc is present in both progeny and explant cells, but the protein is present only in progeny. Bioinformatics analysis of the data is continuing with a focus on translational regulation and transcription factor network building.

55 CRISPR/Cas9-based *in vivo* identification of therapeutic targets in *Xenopus tropicalis* cancer models reveals EZH2 as novel drug target in desmoid tumor-type fibromatosis. *Thomas Naert*[1,2], Dionysia Dimitrakopoulou[1,2], Suzan Demuynck[1], Sven De Grande[1], Liza Eeckhout[1], Marnik Vuylsteke[1,3,4], David Creytens[5], Kris Vleminckx[1,2,6] 1) Department of Biomedical Molecular Biology, Ghent University, Ghent, BE; 2) Cancer Research Institute Ghent, Belgium; 3) Gnomixx, Melle, 9090, Belgium; 4) Inflammation Research Center (IRC), VIB, Ghent, 9052, Belgium; 5) Department of Pathology, Ghent University and Ghent University Hospital, Ghent, Belgium; 6) Center for Medical Genetics, Ghent University, Belgium.

Desmoid tumors (DT) are a highly invasive, but non-metastasizing tumor type, caused by mutational activation of the Wnt/β-catenin pathway. There are at this time no clinically actionable, rationally driven, targeted therapies available for DT patients, and as such standard of care remains insufficient.

In our group, we deployed CRISPR/Cas9 technology in *Xenopus tropicalis* to establish fast (< 3 months) and efficient genetic cancer models. We have recently established models for desmoid tumors, retinoblastoma, choroid plexus cancer and pancreatic neuroendocrine cancer, amongst others.

Evidently, the translational value of these novel preclinical cancer models will rely on how they can be used to advance treatment options for patients. As such, we generated a methodology allowing semi-high throughput screening of new candidate dependency factors (therapeutic targets) via multiplexed CRISPR/Cas9-mediated genome editing. We have already successfully applied this approach, CRISPR/Cas9-mediated Negative-Selection Identification of Drug targets (CRISPR-NSID), to identify EZH2 as a new therapeutic target in DT. Next, we performed experiments to address the clinical relevance of our findings. We found EZH2 protein is highly expressed in clinically resected human DT samples and we performed drug treatments with inhibitors of EZH2 in primary human desmoid tumor cultures and in our *Xenopus* DT model.

Our results show that desmoid tumors are dependent on EZH2 expression, revealing pharmacological inhibitors as novel candidates for a drug

trial in DT. We believe that the CRISPR-NSID technology in *Xenopus tropicalis* will find broad applicability for *in vivo* identification of novel dependency factors in different types of cancer, thereby exposing new therapeutic strategies for cancer patients. This should further establish *Xenopus tropicalis* as a valuable pre-clinical cancer model.

56 Regulation of cohesin function during cell cycle progression. Eulália Lima da Silva[1,2], Dawn E. Bender[1,2], Annelise Poss[1], Daria Klusa[1], Jingrong Chen[1], *Susannah Rankin*[1,2] 1) Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Cell Biology Department, OU Health Sciences Center, Oklahoma City, OK.

During cell cycle progression the cohesin complex contributes to proper chromosome dynamics in multiple ways. In its namesake role, it tethers together sister chromatids as they emerge from the replication fork. This sister chromatid cohesion ensures ensures accurate chromosome alignment and segregation at anaphase. In higher eukaryotes, cohesin also promotes the formation of chromosome loops that ensure orderly compaction of the genome in the interphase nucleus. In this topological role cohesin is critical for proper regulation of gene expression. We have been analyzing how these two distinct roles of cohesin are independently regulated.

The stability of cohesin's association with chromatin is in part controlled by acetylation of its Smc3 subunit by members of the Eco1 family of acetyltransferases. Vertebrates express two Eco1 isoforms, called Esco1 and Esco2. Using a combination of biochemistry and functional analysis in cultured cells and *Xenopus* embryos and extracts we have shown that Esco2 associates with the DNA replication machinery, and is dedicated to tethering sister chromatids together as they emerge from the replication fork. In contrast, Esco1 is active throughout interphase and causes local chromatin rearrangements in a phosphorylation-dependent manner. These results help to provide an explanation for how the distinct roles of cohesin in vertebrate systems can be differentially controlled during cell cycle progression to ensure proper chromosome structure and segregation.

57 Physical constraint induces cell division during neurulation. *Lance Davidson*, Deepthi Vijayraghavan University of Pittsburgh, Pittsburgh, PA.

Tissue cohesion is critical during morphogenesis where cell sheets move en mass. Cohesion is maintained through cell-matrix or cell-cell affinities while cell cycle checkpoints prevent tissue dissolution when shear, tension, or compressive stresses are highest. Checkpoints such as the one maintained by Wee2 in the mesoderm inhibit cell division during gastrulation (Leise and Mueller, 2002). We have uncovered a potential G2/M checkpoint that operates during neurulation. Here we show most cells in the *Xenopus laevis* neural plate complete S-Phase after gastrulation. Consistent with a generic block of cell division during morphogenesis, only a few cells in the neural pate complete M-phase during neural convergence and extension. Since cell proliferation can be sensitive to mechanical cues, e.g. in MDCK and zebrafish tail epidermis (Gudipaty et al., 2017), we tested whether neural plate cells could be driven through the G2/M checkpoint by mechanical perturbation. We placed isolated dorsal tissues, containing the neural plate, into confining stiff agarose cells. In response, neural plate cells under confinement increased their rate of cell-division from less than 2% per hour to more than 12% per hour. Dorsal isolates in confinement exhibit 3-fold greater stress than those in low confinement (Zhou et al., 2015) suggesting increased stress in the dorsal isolate release neural cells from a G2/M block. We are using the gel confinement assay to identify pathways that integrate environmental mechanical cues with cell cycle control systems to increase the numbers, and potential diversity of neural progenitor cells in the neural tube.

58 Expanding the Ciliary Complex Map: Integration of 9k Mass Spectrometry Experiments Improves Coverage of Ciliopathy Disease Genes. *Kevin Drew*[1], Chanjae Lee [1,2], Ryan L. Huizar [1,2], Fan Tu [1,2], Blake Borgeson [1,2], Claire D. McWhite [1,2], Yun Ma [3], John B. Wallingford[1,2], Edward M. Marcotte [1,2] 1) Center for Systems and Synthetic Biology, University of Texas at Austin, Austin, TX; 2) Department of Molecular Biosciences, University of Texas at Austin, Austin, TX; 3) The Otolaryngology Hospital, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

Understanding the molecular network of developmental processes is crucial to our ability to develop therapeutics for developmental diseases. Unfortunately, we currently lack knowledge of the composition, formation and function of the human molecular network. Recent high throughput protein interaction studies identify many new protein complexes but coverage of protein interactions important in development such as ciliary complexes is limited. Here we take an integrated approach by reanalyzing and combining > 9k mass spectrometry experiments from published datasets and construct the most comprehensive human protein complex map to date covering over a third of the proteome and producing over 4k complexes. We expand coverage of the ciliary proteome, identifying > 230 complexes and sub-complexes involving > 150 ciliary proteins. Additionally, our integrated complex map, Hu.MAP (http://proteincomplexes.org) outperforms all other maps with a precision of >80% at a recall of 50% for predicted test interactions. We find many new complexes including ones with enrichment for cilia related developmental disorders (e.g. Joubert Syndrome, Bardet-Biedl Syndrome, Meckel-Gruber Syndrome) that have novel members suggesting candidate disease genes. We experimentally validate several disease candidates in a vertebrate model system (*X. laevis*) and show they localize to cilia as predicted by our map. Specifically, we identify ANKRD55 as a novel member of intraflagellar transport machinery and discover CCDC138, WDR90 and KIAA1328 as members of a large centriolar complex that localizes to the basal body. The expansiveness and accuracy of Hu.MAP yields greater understanding of cellular function and provides avenues for better disease characterization.
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59 From Tetra-amelia to Polymelia: RSPO2 antagonizes RNF43/ZNRF3 without the help of LGR4/5/6. Emmanuelle Szenker-Ravi[1], Hülya Kayserili[3], Celia Bosso-Lefèvre[1], Thomas Naert[2], Umut Altunoglu[4], Marc Leushacke[1], Kris Vleminckx[2], *Bruno Reversade*[1] 1) Institute of Medical Biology, A*STAR, Singapore; 2) Department of Biomedical Molecular Biology, Ghent University, Belgium; 3) Medical Genetics Department, Koç University School of Medicine (KUSOM), Istanbul, Turkey; 4) Medical Genetics Department, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey.

The four secreted ligands RSPO1–RSPO4 act via their cognate LGR4, LGR5 and LGR6 receptors to amplify WNT signalling. Here I will present an allelic series of recessive RSPO2 mutations in humans that cause Tetra-Amelia syndrome, which is characterized by lung aplasia and a total absence of the four limbs.

Functional studies revealed impaired binding to the LGR4/5/6 receptors and the RNF43 and ZNRF3 transmembrane ligases, and reduced WNT potentiation, which correlated with allele severity. Unexpectedly, however, the triple and ubiquitous knockout of Lgr4, Lgr5 and Lgr6 in mice did not recapitulate the known Rspo2 or Rspo3 loss-of-function phenotypes.

Instead, we found that the concurrent deletion of rnf43 and znrf3 in *Xenopus* embryos was sufficient to trigger the outgrowth of supernumerary limbs. Our results establish that RSPO2, without the LGR4/5/6 receptors, serves as a direct antagonistic ligand to RNF43 and ZNRF3, which together constitute a master switch that governs limb specification.

60 Control of ciliated cell distribution in the embryonic epidermis. Alexandre Chuyen, Raphael Clément, Virginie Thomé, Andrea Pasini, *Laurent Kodjabachian* IBDM, CNRS, Aix-Marseille University, Marseille, FRANCE.

While the contribution of gene regulatory networks to pattern formation during development has been intensively studied, less is known about the contribution of individual and collective cell behaviors to the emergence of organized patterns. The *Xenopus* embryonic ciliated epidermis represents an attractive model to study this problem. The mature epidermis consists of an epithelium that contains regularly spaced multiciliated cells (MCCs), which produce a polarized and powerful flow of water at the surface of the tadpole. MCCs are born in a chaotic pattern in the deep layer of the epidermis and intercalate into the surface layer during neurulation. We have used a combination of quantitative analyses from still pictures, time-lapse fluorescent imaging and in silico simulation to understand the transition of MCC distribution from chaotic to ordered. Our data suggest that this transition first involves contact-mediated repulsion between highly motile immature MCCs in the deep layer, followed by stable association and intercalation into vertices of surface layer cells. Perturbation of actin-based lamellipodia formation reduces repulsion between MCCs, leading to the formation of stable aggregates and loss of the regular pattern of MCC distribution. We further show that the SCF/KIT signaling pathway underlies the two behaviors that we have uncovered, planar repulsion and vertical chemotaxis. The SCF ligand is expressed by surface layer cells, while the KIT tyrosine kinase receptor is specifically expressed in immature MCCs, soon after their birth. KIT receptor inhibition by multiple means invariably leads to loss of MCC repulsion but does not prevent intercalation. Consequently properly ciliated MCCs eventually form an irregular pattern in mature epidermis. In contrast, SCF knockdown appears to reduce vertical chemotaxis, such that MCCs eventually form clumps in regions devoid of morpholino. Based on current knowledge, we propose that SCF/KIT signaling is required for actin-based protrusions and MCC directional motility. This work illustrates how individual cell behaviors can be coordinated to allow the emergence of a global pattern.

61 Neural crest early development and multipotency. *A.H. Monsoro Burq*[1,2,3] 1) Univ. Paris Sud, Université Paris Saclay, F-91405, Orsay, France; 2) Institut Curie, CNRS UMR 3347, INSERM U1021, F-91405, Orsay, France; 3) Institut Universitaire de France, 75005 Paris.

Neural crest cells are induced at the border between the neural and the non-neural dorsal ectoderm of vertebrate embryos. These migratory and multipotent cells generate a vast assay of different derivatives, including pigment cells, neurons and glia of the peripheral nervous system, craniofacial mesenchyme and skeleton, and endocrine cells, among others. In the last decade, the complex gene network governing neural crest development has been explored, and the frog embryo has been instrumental to decipher the regulatory logic of neural crest early induction within the neural border territory (1, 2). In this presentation, novel players in dorsal ectoderm specification into neural border cells and in neural crest induction will be presented, both from experimental approaches and from transcriptomics approaches, using small RNAseq and single cell sequencing (3, 4). The high degree of neural crest multipotency is unique when compared to other ectoderm cells, either neural or non-neural, or to other cell lineages in the embryo in general. However, the molecular mechanisms involved in the acquisition of this potential to form multiple derivatives remain debated (5,6). Our results highlight new mechanisms by which the neural border specifiers control a gene module, necessary and sufficient for the establishment of neural crest multipotency in vivo. When this module is altered, neural crest cells revert to an « ancestral » phenotype, reminiscent of the precursors described in non-vertebrate chordates.

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62 Regional and cell type-specific chromatin state and transcription factor networks during gastrulation. Ann Rose Bright, *Gert Jan C. Veenstra* Molecular Developmental Biology, Radboud University, Nijmegen, The Netherlands.

Histone modifications and their regulation play important roles in embryonic development. How epigenetic mechanisms contribute to cell lineage commitment, however, is not well understood. Secreted factors constitute major inductive signals, affecting many aspects of cellular physiology including transduction of the signals to the nucleus. Within the nucleus, transcription factors activate target genes within the

constraints of chromatin, the state of which is thought to both reflect developmental history and cellular potency. We have used ATAC-sequencing to define open chromatin regions (including promoters and enhancers) in different stages of development and in dissected tissue from early gastrula embryos. Generally we find that, while chromatin accessibility correlates with gene expression, many regulatory regions are accessible in tissue where they are not expressed in the gastrula embryo, reflecting a high degree of multi-lineage potency. To obtain a fine-grained picture of cellular heterogeneity in these tissue explants, we performed small scale single cell RNA-sequencing on animal cap and blastopore tissue. We find many cell clusters, and integrated analysis of motifs in regulatory regions of genes with cell cluster-specific expression identifies many well-known developmental regulators, in addition to transcription factors with unknown developmental function. We have experimentally assessed the effects of these factors in animal caps. Our experiments uncover the gene expression and regulatory factors of cell clusters that emerge during gastrulation, and deconvolute the cell type-specific regulatory networks in animal caps and the organizer.

63 TACC3, a microtubule plus-end tracking protein, facilitates neural crest cell motility. E. Bearce, B. Pratt, *L. Lowery* Boston College, Chestnut Hill, MA.

During development, cells from multiple lineages delaminate from their point of origin to travel through the embryo, providing foundations for the heart, gut, vasculature, and nervous system. Cell motility relies on dynamic coordination of the F-actin and microtubule (MT) cytoskeletons. These systems function together to respond to chemical cues, directionally polarize, and generate force and traction for motility. Therefore, cytoskeletal proteins that impact motility are critical during embryogenesis, and disruption of these genes can give rise to developmental disorders. Our work focuses on how MT plus-end regulators impact migration of cranial neural crest (CNC) cells, a multipotent cell that differentiates to form muscle, cartilage, bone, and nerves of the face. We identified one MT regulator, transforming acidic coiled-coil 3 (TACC3), as a putative effector of CNC motility. We previously showed that TACC3 functions as a MT plus-end tracking protein and regulates MT growth velocities in CNCs. Interestingly, TACC3 is one of five genes deleted in Wolf-Hirschhorn syndrome, a genetic disorder that presents craniofacial defects consistent with disrupted CNC migration. Using in situ hybridization, we show that TACC3 is highly-expressed in motile CNCs in X. laevis. Manipulation of TACC3 protein levels is sufficient to alter CNC velocity in vitro. Overexpression (OE) drives increased single-cell migration velocities and explant dispersion. TACC3 KD results in marked motility and dispersion defects, with fewer cells able to migrate persistently. In order to assess how TACC3 manipulation impacts cell velocity, we use confocal microscopy to examine localization of GFP-TACC3 in live cells, and effects of TACC3 manipulation on MT stabilization, adhesion turnover, and chemotaxis. Finally, KD but not OE of TACC3 significantly impacts pharyngeal arch morphology in vivo. Together, these data support a role for TACC3 in embryonic cell motility.

64 Using the *Xenopus* mucociliary epidermis to study human airway diseases. *P. Walentek* Internal Med. IV & Center for Systems Biological Analysis, University Clinic Freiburg, Freiburg, DE.

The Xenopus embryonic epidermis forms a mucociliary epithelium morphologically and functionally similar to the mammalian airway epithelium. We are using the Xenopus model to investigate the molecular basis for mucociliary development, regeneration and disease. To that end, we investigate the evolutionary conservation of cell types, the conserved roles of signaling factors in mucociliary epithelia as well the functions of airway disease-associated genes. We propose that by taking advantage of the fast development, the great accessibility and ease of manipulation as well as the simplicity of the Xenopus embryonic epidermis, we can quickly generate results to formulate testable hypotheses regarding human airway disease mechanisms. Here, I will present data supporting this idea by providing insights into potential pathophysiological processes underlying common human airway diseases, e.g. cystic fibrosis, chronic obstructive pulmonary disease and airway infections. Specifically, our data suggest that we can employ the Xenopus model to study mucociliary stem and secretory cells in addition to the investigation of multiciliated cells, which has already proven to be extremely successfully. Therefore, our work expands the applicability of the frog system to study human airway diseases at the molecular level.

65 Label-free method to identify cilia proteome in multiciliated cell. Hyojung Shim, Seongmin Yun, Tae Joo Park, *Taejoon Kwon* School of Life Sciences, Ulsan National Institute of Science and Technology (UNIST), Ulsan, KR.

Multiciliated cells (MCCs) on *Xenopus* embryo skin are popular model system to study the process of cilia formation and the function of genes involved in ciliopathy, a human diseases caused by cilia dysfunction. However, proteins comprising the cilia structure in *Xenopus* MCCs are not directly investigated so far. In this study, using improved cilia isolation method for label-free shotgun proteomics, we identified over 2,000 proteins from almost intact cilia structure isolated from *Xenopus* mucociliary epithelia.

Previously reported MCC genes and the gold standard SYSCILA genes are significantly enriched in our data, confirming that our cilia isolation method is successful to collect cilia proteome with minimal contamination. In addition, we compared our data to recently published evolutionary conserved ciliproteome data and proximity-labeling proteomics data of primary cilia, showing that the cilia proteome of *Xenopus* MCCs are highly conserved across taxa. With proximity-labeling proteomics and immunostaining, we proposed that our cilia isolation method can break cilia structures near EVC zone and the transition zone.

Because cilia have important role on signaling, we further investigated the membrane proteins putatively located on the cilia structure. Using in situ hybridization and genome editing with CRISPR, several novel cilia membrane proteins important in cilia function were identified. Our proteomics data and cilia isolation method will be useful to study the mechanism of cilia function in *Xenopus* MCCs in the future.

66 Identification of novel candidate genes associated with SIX1 and Branchiootic and Branchiotorenal syndromes. *Andre Tavares*, Karen Neilson, Himani Majumdar, Sally Moody Anatomy and Regenerative Biology, George Washington University, Washington DC, DC.

According to the CDC, the prevalence of hearing loss in newborns is 1.4 per 1000 babies screened in the United States. Branchiootic (BO) and branchiootorenal (BOR) syndromes are autosomal dominant disorders characterized by hearing loss and branchial cleft fistulas or cysts. BOR

patients also present renal anomalies. Mutations in *SIX* genes and the SIX co-factor *EYA1* gene are present in half of BO/BOR patients. We are using *Xenopus laevis* as a model to discover novel potential genes involved in these two related syndromes. Based on a screening of proteins shown to interact with the *so* gene (*Drosophila* Six-related factor), we identified the genes *sobp*, *zmym2* and *zmym4* as potential Six1 co-factors in *Xenopus*. Previous published expression data shows that these genes are expressed with *six1* during craniofacial development. Furthermore, knockdown and overexpression experiments show that these proteins are required for proper expression of neural border, cranial placode and neural crest markers. Recent experiments using luciferase assay to test if these factors are able to modify Six1 transcriptional activity show that all three proteins seem to directly or indirectly interact with Six1, potentially by competing with Eya1. These results indicate that Sobp, Zmym2 and Zmym4 are required for proper craniofacial development, and that they may interact with Six1 and suggesting their utility as potential candidate genes for BO/BOR. Work funded by NIH R01 DE02265.

67 Identification of drivers of aneuploidy in breast tumors. *Todd Stukenberg*[1], Katherine Pfister[1], Ira Hall[2] 1) Biochemistry and Molecular Genetics, University of Virginia, SOM, Charlottesville, VA; 2) Washington University Genome Center, St Louis MO.

Although aneuploidy is found in the majority of tumors, the degree of aneuploidy varies widely. It is unclear how cancer cells become aneuploid or how highly aneuploid tumors are different from those of more normal ploidy. We developed a simple computational method that measured the degree of aneuploidy or structural rearrangements of large chromosome regions of 522 human breast tumors from The Cancer Genome Atlas (TCGA). Highly aneuploid tumors overexpressed activators of mitotic transcription and the genes encoding proteins that segregate chromosomes. Overexpression of three mitotic transcriptional regulators, E2F1, MYBL2 and FOXM1, was sufficient to increase the rate of lagging anaphase chromosomes in a Xenopus animal caps demonstrating that this event can initiate aneuploidy and establishing the animal cap system as a model organism for Chromosomal Instability. Highly aneuploid human breast tumors were also enriched in TP53 mutations. TP53 mutations co-associated with the overexpression of mitotic transcriptional activators suggesting that these events work together to provide fitness to breast tumors.

68 Rho flares repair local tight junction leaks. R. Stephenson[1], T. Higashi[1,2], I. Erofeev[3], T. Arnold[1], M. Leda[3], A. Goryachev[3], *A. Miller*[1] 1) Molecular, Cellular, & Dev Biology (MCDB), University of Michigan, Ann Arbor, MI, USA; 2) Current address: Department of Basic Pathology, Fukushima Medical University, Fukushima, Japan; 3) Centre for Synthetic and Systems Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK.

Tight junctions contribute to epithelial barrier function by selectively regulating the quantity and type of molecules that cross the paracellular barrier. Experimental approaches to evaluate the effectiveness of tight junctions are typically global, tissue-scale measures. Here, we introduce Zinc-based Ultrasensitive Microscopic Barrier Assay (ZnUMBA), which we used in *Xenopus laevis* embryos to visualize short-lived, local breaches in epithelial barrier function. These breaches, or "leaks", occur as cell boundaries elongate, correspond to visible "breaks" in the tight junction, and are followed by transient localized Rho activation, or "Rho flares". We discovered that Rho flares restore barrier function by driving concentration of tight junction proteins through actin polymerization and ROCK-mediated localized contraction of the cell boundary. We conclude that Rho flares constitute a damage control mechanism that reinstates barrier function when tight junctions become locally compromised due to normally occurring changes in cell shape and tissue tension.

69 Promoters contain two distinct regulatory codes during the egg to embryo transition. *C.D. Reid*, Hui Zhu, Qin Li, Julie C. Baker Genetics, Stanford University, Stanford, CA.

Within the first day of development the amphibian embryo undergoes dramatic cell proliferation, morphological changes, and activates numerous pathways for cell fate specification. Each of these changes is driven by alterations in gene expression, but a comprehensive understanding of the promoters that control gene expression remains elusive. To create a high-resolution map of transcription start sites (TSS) in the early embryo, I generated CAGE-Seq datasets for four critical time points: egg, blastula, gastrula and neurula stages. TSS in the egg and blastula are highly similar, suggesting that maternally specified promoters are maintained during early embryogenesis. In the egg, blastula and gastrula, promoters contain a TATA-binding protein (TBP) motif, suggesting that TBP is driving gene expression during early embryogenesis. Unexpectedly, neurula stage promoters contain the Initiator (INR) element, which binds TFIID family members, demonstrating that tissue specification during neurulation includes a fundamental change in the sequences driving gene expression. I find that a number of consistently expressed promoters shift TSS usage as embryogenesis progresses. Specifically, many genes use an egg promoter containing a TATA box, then shift to a promoter containing the INR element at the neurula stage. This suggests that the basal transcriptional machinery used to drive gene expression is fundamentally changing as the egg transitions to an embryo. The motif for YY1, a transcription factor essential for neurulation, is commonly found near shifting promoters, suggesting YY1 drives a shift from TATA based gene expression to INR based gene expression. This work demonstrates that a shift from maternal to embryonic gene expression involves fundamental changes in basal transcription machinery.

70 Translational mechanisms and the diversification of cell types along the animal-vegetal axis. Megan Dowdle[1], Sookhee Park[1], Melissa Marchal[2], Douglas Houston[2], *Michael Sheets*[1] 1) Univ. of Wisconsin Dept. of Biomolecular Chemistry School of Medicine and Public Health Madison WI; 2) University of Iowa, Department of Biology, Iowa City, IA.

The functions Bicaudal-C (Bicc1) translational repressor functions to both temporally and spatially control the synthesis of maternal proteins that in turn regulate critical developmental events in vertebrate embryos. We are investigating this important repressor on two fronts. First, we have examined the mechanisms by which Bicc1 achieves specificity in recognizing its mRNA target sites. Bicc1 contains multiple KH-domains, known RNA binding modules. Using *in vivo* and *in vitro* assays we have defined amino acid residues within a single KH domain that are critical for RNA recognition, translational repression and control of embryonic cell fates. Second, we have examined how Bicc1 functions to generate asymmetries in developing *Xenopus* embryos. Bicc1 is encoded by a vegetally-localized mRNA. During oocyte maturation this mRNA is released from the vegetal cortex and its translation is activated. As a consequence the Bicc1 protein and its translational repression activity form a vegetal to animal gradient in developing embryos. We previously identified a set of Bicc1 target mRNAs from *Xenopus* embryos and

quantitatively measured Bicc1's translational repression activity with reporter mRNAs containing the 3'UTRs of the targets. We observed that the efficiency of Bicc1 repression varied with different targets; from complete repression to less than 25% repression. The gradient distribution of Bicc1 in conjunction with the variable repression of individual target mRNAs by Bicc1 should create animal-vegetal gradients derived from the synthesis of proteins encoded by the target mRNAs. These secondary gradients are predicted to have different slopes depending upon the efficiency of Bicc1 repression. The existence of such secondary animal-vegetal gradients is supported by experiments monitoring the activity of different reporter mRNAs injected into specific regions of 32-cell embryos. In addition, because many of the target mRNAs encode proteins, such as Wnt11b that function as concentration dependent cell fate regulators the secondary animal-vegetal gradients should endow embryonic cells with distinct protein compositions as well as distinct fates. Our results demonstrate that the Bicc1 is a concentration dependent regulator of translation, embryonic cell fates and diversification of cell types along the animal-vegetal axis.

71 Maternal transcription factors coordinate to pre-mark genomic regulatory regions and activate the endoderm gene regulatory network. *Kitt D. Paraiso*, Masani Coley, Jessica Cheung, Margaret B. Fish, Ira L. Blitz, Ken W.Y. Cho Developmental and Cell Biology, University of California, Irvine, Irvine, CA.

Zygotic gene activation by maternal transcription factors (TFs) is a crucial step in the early differentiation events in metazoan embryos. Maternal TFs activate the gene regulatory networks (GRN) in the three germ layers, triggering the cell differentiation processes that enable the formation of diverse somatic cells. While much is known about the early *Xenopus* endoderm GRN, the precise mechanism of how maternal TFs coordinate to activate the network is unknown. We searched for potential maternal endodermal TFs based on their vegetal-localization in early *Xenopus tropicalis* embryos and identified the crucial role of Otx1 for endoderm specification. Vertebrate Otx1 genes and their invertebrate homologs show conserved temporal (maternal) and spatial (vegetal) expression patterns during deuterostomes development. We show that *Xenopus* Otx1 collaborates with other maternal TFs (Foxh1 and Vegt) to regulate endoderm specification. Chromatin immunoprecipitation reveals that the three maternal TFs bind to the same regions of the genome prior to the zygotic gene activation, and possibly forming a multi-protein complex. The pre-marked regions of the genome are later occupied by co-activator, co-repressor, epigenetic marks and RNA polymerase II. Since these regions gradually accumulate epigenetic marks, we propose that occupancy of these three maternal TFs pre-mark cis-regulatory regions during early cleavage stages for the subsequent gene activation at later stages. Overexpression of a combination of the maternal TFs in animal cap assays shows that the collaboration between Vegt and Otx1 is essential to ensure a robust activation of the endoderm program. Specifically, Otx1 has the ability to work synergistically and antagonistically in a gene-specific manner. LOF analysis shows that Otx1 is required for the inhibition of mesoderm genes in the putative endoderm and that this inhibitory function is attributed to the recruitment of the co-repressor TLE. Lastly, the *Xenopus* Otx1 and the sea urchin spOtx show the high degree of conservation not only in their Otx expression pattern, but also their GRN structures. Overall, our data uncover the essential role of Otx1 as a "core" endoderm regulator and how the embryonic genome is pre-marked by a select group of maternal TFs, which prepare the genome to be fully activated at later developmental stages, thus ensuring the proper activation of the endoderm GRN.

72 Identifying connections between non-canonical Wnt-signaling and morphogenesis with quantitative phospho-proteomics. *E. Van Itallie*[1], J. Kreuzer[2], W. Haas[2], M.W. Kirschner[1] 1) Systems Biology, Harvard Medical School, Boston, MA; 2) Massachusetts General Hospital Cancer Center and Department of Medicine, Harvard Medical School, Charlestown, MA.

The role of non-canonical Wnt signaling in *Xenopus* gastrulation and neurulation has been established over the past 15 years using perturbations to a variety of genes in these pathways. However, the progress in functionally connecting these components to the changes in cytoskeleton dynamics and generation of force necessary for successful anterior-posterior axis extension has been more limited. Based on previous work in the field, we hypothesized that kinases and their targets are a critical way in which the non-canonical Wnt signaling pathway changes cell behavior during this time period. We endeavor to measure how changing the levels of the non-canonical Wnt ligands Wnt11 and Wnt11b changes the phospho-proteome of gastrulating *Xenopus laevis* embryos. In order to do this we are using translation-blocking morpholinos to decrease the levels of both of these proteins robustly in a large number of late gastrula embryos. We observe that the roles of the similar Wnt11 and Wnt11b proteins are different from each other during both gastrulation and neurulation. We are developing a method to validate morpholino-mediated decreases in translation directly that will be widely applicable in situations where antibodies are not available or cannot distinguish between closely related proteins.

73 14-3-3 recruits keratin intermediate filaments to mechanically sensitive cell-cell contacts. *R. Mariani*, G. Weber Biological Sciences, Rutgers University-Newark, Newark, NJ.

Intermediate filament cytoskeletal networks simultaneously support mechanical integrity and influence signal transduction pathways. While intermediate filaments were initially characterized as relatively stable polymers, they have been increasingly recognized to be subject to significant dynamic change. Marked remodeling of the keratin intermediate filament network accompanies collective cellular morphogenetic movements that occur during gastrulation in *Xenopus laevis*. While this reorganization of keratin is initiated by force transduction on cell-cell contacts mediated by C-cadherin, the mechanism by which keratin filament reorganization occurs remains poorly understood. In this work we demonstrate that 14-3-3 proteins regulate keratin reorganization dynamics in *Xenopus* mesendoderm. Utilizing immunofluorescence, we found that 14-3-3 proteins display different expression and sub-cellular localization patterns across the tissues of the gastrulating embryo. 14-3-3 shows strong co-localization with keratin filaments at cell-cell junctions in migrating mesendoderm. Employing LC/MS-MS, we identified Keratin 19 as a novel intermediate filament target of 14-3-3 proteins in the whole embryo and, more specifically, mesendoderm tissue. We subsequently used co-immunoprecipitation of endogenous proteins to demonstrate that 14-3-3 proteins associate with Keratin 19 as well as C-Cadherin. To gain insight into the relationship between 14-3-3 proteins and keratin reorganization, we expressed a genetically encoded 14-3-3 inhibitor and analyzed keratin organization. Performing FRAP experiments with Keratin 19-GFP as the target in migrating mesendoderm explants, we found that inhibition of 14-3-3 results in decreased recovery of fluorescent keratin to bleached zones. Additionally, dissociation and reassembly of explanted mesendoderm tissue demonstrated that 14-3-3 assists in the recruitment of keratin filaments to newly assembled

cell-cell junctions. Synthetically coupling 14-3-3 to Keratin 19 through a unique fusion construct conversely induced the localization of this keratin population to the region of cell-cell contacts. Taken together, these findings indicate that 14-3-3 acts on keratin intermediate filaments and is involved in their reorganization to sites of cell adhesion.

74 Beta-catenin and myosin II differentially regulate optic axonal projections and growth cone filopodia in the optic tract. *Tamira Elul*[1], James Ha[1], Radhika Rawat[2], Kim Vu[1] 1) Touro University California, Vallejo, CA; 2) University of California, Berkeley.

The retino-tectal projection of lower vertebrates is an experimentally amenable neuronal circuit for studying mechanisms of axon pathfinding *in situ*. To establish the retino-tectal projection, optic axons must navigate through the optic tract to their target tissue in the midbrain- the optic tectum. During their extension through the optic tract, optic axons defasciculate or disperse, and display terminal growth cones containing filopodial protrusions. Here, we studied how two essential cellular factors - b-catenin and Myosin II - function to regulate diverse optic axon pathfinding behaviors and growth cone filopodia in the optic tract of whole brains taken from *Xenopus* tadpoles. We expressed a mutant of β-catenin that contains the a-catenin but lacks the Cadherin binding site (b-catNTERM) in, and applied the Myosin II small molecule inhibitor Blebbistatin to, optic axons in the optic tract of intact brains. Expression of β-catNTERM increased dispersion of optic axons in the dorsal half of the optic tract. In contrast, application of Blebbistatin inhibited extension of optic axons through the optic tract of whole brains. In addition, optic axons that expressed b-catNTERM formed growth cones that lacked filopodial protrusions, whereas growth cones of optic axons that were exposed to Blebbistatin displayed increased numbers of filopodial protrusions *in situ*. These results suggest that β-catenin and Myosin II differentially sculpt optic axonal projections, and oppositely modulate growth cone filopodial protrusions in the optic tract. Our data also imply that optic axons may express distinct types of growth cone filopodia that function to regulate different axon pathfinding behaviors such as extension and fasciculation in the optic tract.

75 Importin α partitioning to the plasma membrane regulates intracellular scaling. *Chris Brownlee*, Stephanie Malinich, Rebecca Heald MCB, University of California, Berkeley, Berkeley, CA.

The interphase nucleus and the mitotic spindle scale smaller with cell size through both physical and biochemical mechanisms, but control systems that coordinately scale intracellular structures are unknown. We show that the multipurpose nuclear transport receptor importin α is modified by palmitoylation, which targets it to the plasma membrane and modulates its binding to nuclear localization signal (NLS)-containing proteins that regulate nuclear and spindle size in *Xenopus*. Reconstitution of importin α targeting to the outer membrane of cell-like compartments recapitulated scaling relationships observed during embryogenesis, which were altered by inhibitors that shift levels of importin α palmitoylation. Modulation of importin α palmitoylation in human cultured cells similarly affected nuclear and spindle size. These experiments identify importin α as a cell surface area-to-volume sensor that coordinately scales intracellular structures to cell size.

76 Mitotic spindle length: How complexity arises from molecular interactions. *Simone Reber* IRI Life Sciences, Humboldt-Universität zu Berlin, DE.

The metaphase spindle is an evident example why the size of intracellular organelles matters: The spindle must be large enough and span sufficient distance to physically separate chromosomes into two opposite halves of the cell. While approaching a near to complete proteomic parts lists of the metaphase spindle, mechanisms that control their defined size remain poorly understood. Cytoplasmic extracts prepared from eggs of the African clawed frog Xenopus laevis have proven valuable for studying cellular processes such as spindle assembly and organization. More recently, comparison with extracts of the related frog Xenopus tropicalis allowed the identification of spindle scaling factors. We are interested in how the basic building block of the spindle, αβ-tubulin, contributes to spindle assembly and morphology. Ultimately, we wish to uncover how molecular machines that act at the Angstrom scale are able to create physiological structures in the µm scale that enable faithful chromosome segregation and thus cell proliferation.

77 Context dependent regulation of GSK3 in migrating neural crest cells. S. Gonzalez Malagon[1], A. Lopez Munoz[1], D. Doro[1], T. Bolger[1], E. Poon[2], E. Tucker[2], H. Adel Al-Lami[1], M. Krause[3], C. Phiel[4], L. Chesler[2], *K. Liu*[1] 1) Centre for Craniofacial and Regenerative Biology, King's College London, London, GB; 2) Paediatric Solid Tumour Biology, Institute of Cancer Research/Royal Marsden NHS Trust, Surrey, SM2 5NG United Kingdom; 3) Randall Division of Cell & Molecular Biophysics, King's College London, London, SE1 1UL, United Kingdom; 4) University of Colorado Denver, Department of Integrative Biology, Denver, CO 80204.

Neural crest migration is critical to its physiological and pathophysiological function, and this lineage has contributed to our understanding of cellular behaviours such as contact inhibition of locomotion. Nevertheless, mechanisms controlling *in vivo* neural crest migratory activity are comparatively unknown, in large part due to difficulties in accessing this cell population during vertebrate embryogenesis. Here, we uncover new genetic and biochemical requirements of glycogen synthase kinase 3 (GSK3) in regulating the cytoskeleton during neural crest cell migration. We demonstrate that glycogen synthase kinase 3 (GSK3) is specifically tyrosine phosphorylated (pY) in neural crest cells and that this activation depends on anaplastic lymphoma kinase (ALK), a protein which is associated with metastasis in neuroblastoma. Consistent with this, neuroblastoma cells with pathological increases in ALK express high levels of pY-GSK3 while migration of these cells can be inhibited by GSK3 blockade. In normal neural crest cells, loss of GSK3 function leads to stabilization of the actin cytoskeleton, increased pFAK and misregulation of Rac1 and lamellipodin, key regulators of cell migration. Consequently, genetic reduction of GSK-3 results in catastrophic failure of cranial neural crest migration.

78 Nuclear size scaling with DNA content in *Xenopus*. *Y. Hara*, H. Heijo, S. Shimogama, S. Nakano Yamaguchi University, Yamaguchi city, Yamaguchi, JP.

Genome size generally correlates with nuclear size in the cell as well as the overall cell size across species. Although this intuitive intracellular scaling relationship, which is the size of the nucleus as a container of DNA correlates with the inside DNA content, has long been known, the

underlying mechanism for regulating this relationship has remained unclear. In order to gain insights how DNA content inside the nucleus can regulate the nuclear size, here we investigate the process of nuclear formation by manipulating the DNA content using a reconstruction of nuclei in a cell-free extract of *Xenopus laevis* eggs. First, when reducing in DNA content by inhibiting DNA replication or altering DNA materials from different species with distinct genome size, the speed of nuclear expansion and expected final size are reduced. Both parameters correlate positively with the nuclear DNA content, indicating that the DNA content does not set only the initial size of the nucleus before starting nuclear expansion but also control the dynamics of nuclear expansion. Next, we investigate an involvement of this DNA—content—dependency in the known mechanisms for regulating nuclear expansion dynamics in *Xenopus*. However, even in manipulating the ability of these mechanisms, the DNA—content—dependency of nuclear expansion still could be observed, suggesting that there is a different way to contribute the nuclear size scaling in a DNA—content—dependent manner. Taken together with the known characterizations of DNA inside the nucleus, we would like to provide a new putative role of nuclear DNA in scaling the nuclear size in *Xenopus*.

79 RARγ is required for mesodermal gene expression prior to gastrulation. *Znf703* is a novel RAR target important in mesodermal, neural crest, and otic development. *A.S. Janesick*[1], W. Tang[2], T. Shioda[3], B. Blumberg[4] 1) Otolaryngology Head and Neck Surgery, Stanford University School of Medicine, Stanford, CA; 2) Division of Biology, California Institute of Technology, Pasadena, CA; 3) Center for Cancer Research, Massachusetts General Hospital, Charlestown, MA; 4) Department of Developmental and Cell Biology, 2011 Biological Sciences 3, University of California, Irvine, CA.

The developing embryo is exquisitely sensitive to retinoic acid (RA) concentration, particularly during anteroposterior patterning. In contrast to Nodal and Wnt signaling, RA was not previously considered to be an instructive signal in mesoderm formation during gastrulation. We show that RARγ is indispensable for the expression of early mesoderm markers and is, therefore, an obligatory factor in mesoderm competence and/or maintenance. RARγ1 is required for cellular adhesion, as revealed by cellular dissociation and *N-cam* mRNA depletion in animal caps harvested from RARγ1-knockdown embryos. Mesodermal derivatives such as somites are disrupted by RARγ1 knockdown, which causes loss of MYOD protein in the presomitic mesoderm, but ectopic expression of MYOD protein in the trunk, all at the expense of somite maturation (as indicated by 12/101).

We identified several novel targets up-regulated by RAR signaling in the early gastrula that are expressed in the circumblastoporal ring and linked to mesodermal development, including *Znf703*. *Znf703,* however, is also expressed in the neural plate border and is exclusively absent from the developing otocyst. Overexpression of *Znf703* inhibits *Wnt8* and *Gdf3* at gastrula stage while exhibiting a later effect on neurula stage embryos where *Znf703* promotes anterior *Sox2* expression and inhibits otic and neural crest patterning (as revealed by *Eya1* and *Sox10* expression). Using hormone-inducible *Znf703* constructs, we show that *Znf703*'s effect on early mesoderm is *separable* from its disruption of neural crest and otic patterning. Given that *Znf703* is an inhibitory component of the Wnt signaling pathway, this gene represents an important mediator of RAR-Wnt crosstalk in mesodermal, neural crest, and otic development.

80 Resetting the 3D genome in real time during normal development and in disease models. Kaivalya Shevade[1], Shuyi Nie[3], *Ruchi Bajpai*[1,2] 1) Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, Los Angeles, CA; 2) Department of Biochemistry and Molecular Medicine, Keck School of Medicine, USC, Los Angeles, CA; 3) School of Biology, Georgia Institute of Technology, Atlanta, GA.

As normal cells switch fates during development, respond to stimuli or undergo reprogramming, changes occur in the 3D genomic architecture. However, real time dynamics of this fundamental process has remained a mystery and it is hard to imagine. Here we identified a transient window during Xenopus neural crest cell differentiation where rapidly proliferating immature neural crest cells stop cycling, switch their 3D genome and then re-enter cell division as a distinct, more-mature, stem cell type. We demonstrate that this epigenetic switch is independent of cell division events and asymmetric inheritance of proteins. We have captured the first movies of whole genome dynamics during the process of cell fate switch in normal cells. An initial, rapid increase of 3-5 times in nuclear volume is followed by formation of innumerable lobes separated by infolded nuclear envelop generating progressively smaller spatial domains to compartmentalize genomic re-structuring. These sub-compartments are rapidly resolved in the final stages of the epigenetic switch to reform the familiar spheroid nucleus, but with a new 3D architecture and a new molecular identity or cell fate. Using photo-convertible proteins to tag part of the genome in an unbiased manner we discovered unexpected, yet fundamental rules of genome reorganization that are conserved from frogs to humans. Here we demonstrate that first, massive chromatin redistribution occurs, breaking down the existing 3D architecture without an appreciable change in transposon accessible DNA sites. This is followed by progressively smaller, independent units of localized genomic reorganization. Arrest at different stages of this process in a 'disease-gene' dependent manner, coupled with defective cell fate transition underscores the importance of genome reorganization in human health. It also emphasizes (i) the non-random nature of- and (ii) genetic orchestration of- chromatin dynamics. These discoveries together with two complementary and simple methods to image chromatin dynamics will pave the way for understanding the 3D genome-structure and its real-time reorganization that is key in changing gene-expression during development, regeneration and repair.

81 Histone methyltransferase Dot1L is a coactivator for thyroid hormone receptor during *Xenopus* development. *Liezhen Fu*, Luan Wen, Yun-Bo Shi Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), NIH, Bethesda, MD.

Histone modifications are associated with transcriptional regulation by diverse transcription factors. Genome-wide correlation studies have revealed that histone activation marks and repression marks associated with activated and repressed gene expression, respectively. Among the histone activation marks is histone H3 K79 methylation, which is carried out solely by a single methyltransferase, Dot1L. We have been studying thyroid hormone (T3)-dependent amphibian metamorphosis in the two highly related species, the pseudo-tetraploid *Xenopus laevis* (*X. laevis*) and diploid *Xenopus tropicalis* (*X. tropicalis*), as a model for postembryonic development, a period around birth in mammals that is difficult to study. Using Chromatin Immunoprecipitation (ChIP) assay and gene expression analysis, we show that H3K79 methylation levels are

induced at T3 target genes during natural and T3-induced metamorphosis and that Dot1L itself is a T3 target gene, suggesting that T3 induces Dot1L expression and Dot1L in turn functions as a T3 receptor (TR) coactivator to promote vertebrate development. We show that in co-transfection studies or in the reconstituted frog oocyte *in vivo* transcription system, overexpression of Dot1L enhances gene activation by TR in the presence of T3. Furthermore, making use of the ability to carry out transgenesis in *X. laevis* and gene knockdown in *X. tropicalis,* we demonstrate that endogenous Dot1L is critical for T3-induced activation of endogenous TR target genes while transgenic Dot1L enhances endogenous TR function in premetamorphic tadpoles in the presence of T3. Our studies thus provide, for the first time, complementary gain- and loss-of functional evidence *in vivo* for a cofactor, Dot1L, in gene activation by TR during vertebrate development.

82 Novel roles of *six3* in early eye development revealed by analysis of a *Xenopus tropicalis* CRISPR mutant. *S. Manohar*, T. Nakayama, M. Fisher, R.M. Grainger Department of Biology, University of Virginia, Charlottesville, VA.

Six3, a homolog of *Drosophila optix*, is a homeobox-containing transcription factor critical for early anterior patterning and forebrain/eye formation. In humans, haploinsufficiency of *SIX3* has been associated with holoprosencephaly. *Six3* is a key component of the gene regulatory network (GRN) controlling eye formation along with genes such as *Pax6*, *Rax*, *Lhx2* and *Otx2*. However, the position of *Six3* in the eye GRN is unclear, in part because mouse homozygous *Six3* mutants are headless and conditional mutants targeting *Six3* expression in the eye do not inactivate its expression from the time of its inception. Thus, definitive epistatic relationships are difficult to determine, e.g. with other key genes like *Pax6*.

We have used CRISPR technology to generate a *six3* null mutant line in *X. tropicalis* that has been uniquely valuable in revealing how *Six3* regulates eye development. In contrast to mouse, *Xenopus six3* mutants retain a head but have malformed eyes with missing or undifferentiated lenses and a disorganized retina. We describe three key findings. 1) The rudimentary eyes in the *Xenopus six3* mutant allow us to investigate the role of *six3* from its inception, rather than in conditional knock-outs in mice where this is not the case. We see that *pax6* expression is unaffected in the frog mutant i.e. *pax6* and *six3* work independently in the early eye GRN, and not that *Six3* is upstream of *Pax6* as suggested in conditional mouse knockouts. 2) Examining early retinal patterning genes, we see a marked reduction in *mab21l1, foxe3, vsx1* and *foxn4* expression, consistent with abnormal retinal layering in the mutant. We focused on the function of the nuclear protein *mab21l1* because of lens phenotypes in mouse mutants. Injection of *mab21l1* mRNA into the *six3* mutant yields a partial rescue of the lens, but also rescue of the abnormal layering in the retina as well, suggesting that rescue of the lens may be mediated by the rescued retina, i.e. by restoring an inductive signal from primitive retina to lens. 3) To test the hypothesis that *six3* is upstream of lens-inducing factors we transplanted wildtype presumptive lens ectoderm (PLE) into *six3* mutant embryos where it did not form lenses, unlike positive control transplants (wildtype into wildtype) which always formed a lens. Taken together, our data suggest a revised perspective where *six3* is independent of *pax6* in early eye formation and mediates the lens program through inductive signaling via the early neuroretina.

83 Using *Xenopus laevis* to identify genes involved in successful vs. unsuccessful central nervous system axon regeneration. J.L. Belrose[1], M.A. Sammons[1], K.M. Gibbs[2], *B.G. Szaro*[1] 1) Department of Biological Sciences, State University of New York at Albany, Albany, NY; 2) Department of Biology and Chemistry, Morehead State University, Morehead, KY.

Xenopus occupies a transition point in the phylogenetic loss of regenerative capacity of the central nervous system (CNS) in response to injury. Whereas *Xenopus* successfully regenerates optic axons throughout life, it loses the capacity to regenerate spinal cord axons during metamorphosis. We used this feature and RNA-seq to identify injury-induced genes shared in common between two regenerative CNS tissues [*i.e.*, frog retina after optic nerve injury (ONI) & tadpole hindbrain after spinal cord injury (SCI)] that differed from those induced by injury in a CNS tissue that fails to regenerate axons [*i.e.*, frog hindbrain after SCI]. Overall, the fraction of the genome exhibiting statistically significant (FDR < 0.05) injury-induced expression ranged from 1.4% (3 day ONI eye) to 11.7% (3 day SCI frog hindbrain), with the vast majority of differentially expressed genes being unique to each tissue (>90%). Despite these differences, the overall response, as measured by numbers of differentially expressed genes, was similar between the two regenerative cases, in which it peaked during maximal regenerative axon outgrowth, and markedly different in the non-regenerative case, in which it peaked during the early, post-trauma phase. At the early and late phases of the injury response, a set of injury-induced genes was shared in common between the two regenerative tissues (26 and 15, respectively). These mainly comprised genes associated with modulating wound healing and inflammation, whereas at the peak regenerative phase, the shared set (293) included additional genes associated with axonal outgrowth, transcriptional and post-transcriptional control of gene expression, cell signaling pathways, and epigenetic changes to chromatin structure. Thus, *Xenopus* offers a paradigm for identifying pro-regenerative genes involved in core processes underlying successful CNS axon regeneration. *Supported by NYS DOH SCIRB C30837GG, C32249GG, C32091GG (BGS) and NIH 1R15HD076643-01A1 (KMG).*

84 Identification of a novel nucleosome-remodeling complex relevant to immunodeficiency, centromere instability and facial anomalies (ICF) syndrome using comparative chromatin proteomics in Xenopus egg extracts. *H. Funabiki*[1], C. Jenness[1], M. Unoki[2], H. Sasaki[1] 1) Laboratory of Chromosome and Cell Biology, The Rockefeller University, New York, NY; 2) Division of Epigenomics and Development, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.

Evaluating roles of histones and histone regulators in vivo by genetic manipulations has a major caveat; their manipulation may affect transcription profile of thousands of genes, causing pleiotropic indirect consequences. Taking advantage of Xenopus egg extracts, which can recapitulate a variety of chromatin-dependent events in the absence of active transcription, we established a method to reconstitute physiological functions of nucleosomes using recombinant histones and synthetic DNA templates. This system enabled us to establish pathways that depend on the nucleosome, concomitant with proteomic analysis of chromatin. Combination of comparative chromatin proteomics under variable conditions that affect chromatin functionalities and hierarchical clustering effectively enable identification of protein pathway and protein complexes. Through this strategy, we have discovered that HELLS and CDCA7, whose mutations are linked with the ICF syndrome, form a stoichiometric complex on chromatin, but they dissociate from mitotic chromatin in a manner dependent on Aurora B. We further showed that CDCA7 is essential stimulator of the nucleosome remodeling activity of HELLS, and that ICF patient mutations in CDCA7

caused a defect in recruiting CDCA7 and HELLS to chromatin. Although CDCA7 and HELLS are thought to help the de novo DNA methyltransferase DNMT3b, whose mutation also causes ICF syndrome, underlining mechanisms behind centromere instability and immunodeficiency in the syndrome are not clear. We are currently addressing this question using Xenopus egg extracts, human tissue culture cells and patient cell lines.

85 Spatial regulation of chromosome-derived signals for spindle assembly. *Doogie Oh*, Daniel Needleman Harvard University, Cambridge, MA.

The spindle is a subcellular structure assembled during mitosis for segregating sister chromatids into the two daughter cells. It is composed of dynamic microtubules and associated proteins. Although most of the molecular components have been identified, it is still not well understood what molecules and their modes of interaction are important for assembling the spindle. Xenopus egg extracts provide platforms by which we can perform *in-vitro* assays for examining the effects of individual molecules and their modes of interaction on spindle assembly, architecture, and morphology. One significant finding is the role of GTPase Ran for the metaphase spindle assembly. Ran can be charged with guanosine triphosphate (GTP) by the action of chromatin-localized RCC1, which produces concentration gradient of diffusible Ran-GTP, thereby transmitting the positional information for chromosome location. The Ran-GTP gradient can activate spindle assembly factors (SAFs) for stimulating microtubule nucleation proximal to the chromosomes and self-organization of the spindle around them. I developed a novel fluorescence fluctuation microscopy for measuring the gradients and investigating how such spatial regulations emerge. Using U2OS human cell lines, I examined how Ran and SAFs behave in mitosis and found that the Ran-GTP gradient recruits the SAFs by feedback for amplifying microtubule nucleation near the chromosomes. I applied this idea into slide-and-cluster models, which leads to a hypothesis that the positional signal for chromosome location spatially propagates by microtubule slide and feedback amplification for assembling the spindle. Using RNAi knockdown, I examined whether perturbing the Ran-GTP gradient affects the properties of the spindle. The RNAi of a GTPase-activating protein, RanGAP, decreases the gradient of Ran but increases that of a kinesin-14 motor, HSET and the microtubule density inside the spindle. HSET is a SAF that associates and directs into microtubule minus-end and is known to crosslink and slide parallel microtubules for forming spindle poles. Based on them, I propose that the chromosome signal regulates the spatial distribution of HSET by feedback and control spindle organization. Taken together, my research emphasizes the importance of combining the *in-vitro* assays and cellular genetics for developing general models and elucidating emergent properties in the assembly of subcellular structures.

87 Extreme nuclear morphology in *Xenopus* . *H.E. Arbach*, M. Harland-Dunaway, A.E. Wills Biochemistry, University of Washington, Seattle, WA.

An ellipsoid nuclear shape is highly conserved across cell types and species. Deviations from ellipsoid morphology through modulation of nuclear lamina and other nucleoskeletal components regulate complex cellular properties such as differentiation and tissue elasticity. Most perturbations of nuclear morphology are associated with pathologies, including progeria, cancer, and muscular dystrophy. The dearth of healthy models of nuclear shape variation has limited the understanding of the mechanisms that govern nuclear shape. Here, we introduce nuclear branching in the epidermal fin cells of *Xenopus* as a model for extreme nuclear morphological variation in a healthy, genetically tractable, easily manipulated, and visualized tissue. We find that nuclear branching arises and elaborates during development but diminishes at the onset of metamorphosis. Cells with branched nuclei have active cell cycles and contain marks of active enhancers and heterochromatin throughout the nucleus. Disruption of actin filaments and the nuclear lamina protein, LaminB1 decreases nuclear branching. However, nuclear branching is not dependent on forces sustained by the tissue during swimming. We have also found that LaminB1 is necessary for proper fin formation, suggesting that the loss of nuclear branching compromises the integrity of this highly specialized epithelium. Overall, this study establishes a new model for extreme nuclear morphological variation in a healthy tissue, which may provide insight into the range of structural constraints that can influence nuclear shape, how nuclear morphology can be perturbed, and the how nuclear morphological variation interrelates with other parameters of cell health.

88 Optogenetic platform to probe spindle positioning and spindle positioning and cytokinesis signaling *in vitro*. *Jessica Bermudez*[1], Matthew Good[2] 1) Bioengineering, University of Pennsylvania, Philadelphia, PA; 2) Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

While proper cell division is critical to the development of living cells, the timing and mechanistic details of this dynamic process remain unresolved. To gain fundamental insights on disease and development, we must first understand how intracellular signals that direct cell division are organized in space and time. Here we present a minimal *in vitro* platform to decode the molecular mechanisms that link spindle positioning and cell cycle progression. This simplified system and the perturbations it enables offer benefits over the complexity of living cells and *in vivo* genetic studies which preclude the analysis of short, dynamic processes. Precise spatial regulation of the mitotic spindle – a macromolecular machine that separates chromosomes - is necessary for proper cell division, particularly during the rapid cell cycles present in early embryo development. Once positioned, the spindle initiates other mitotic events such as cytokinesis through cleavage furrow signaling. To decode the molecular mechanisms that link cortical signaling and spindle positioning, we present a minimal *in vitro* platform. Our goal is to reconstitute spindle positioning *in vitro* by encapsulating *Xenopus* extract, which can form spindles, along with engineered proteins within a size-tunable compartment with a patterned synthetic boundary. By doing so it should be possible to investigate how cell geometry affects spindle positioning and the transport and activation of cell cycle signals. We have expressed and purified protein pairs that dimerize in response to illumination by blue light and are also fused to spindle microtubule interacting domains. To achieve a sustained patterning of proteins following illumination, we employ an irreversible optochemical system that consists of a photocaged small molecule and modular proteins that dimerize upon photo-uncaging. By merging optogenetic recruitment with a boundary tethering mechanism, we are poised to pattern a boundary with proteins that interact with *in vitro* reconstituted spindles contained inside the cell-like compartment. We have engineered proteins that can sense activity of key regulators of cell division, such as small GTPase RhoA, and plan to characterize how they are

spatially activated throughout the cell. With this platform, we can determine how the rates of signaling vary in cells with extreme geometries and further examine link between spindle positioning and the molecular pathways associated with cell division.

89 GTPase steering by an enzymatic corral. *Tom Burke*, Nicholas Davenport, William Bement LCMB, The University of Wisconsin at Madison, Madison, WI.

Dynamic arrays of actin filaments and myosin-2 ("actomyosin") drive a broad variety of dynamic biological processes ranging from cell division to wound repair. Such arrays are controlled by the Rho GTPases, proteins that exert their effects on actomyosin by stimulating "effector" proteins when in their active (GTP-bound) state. Traditionally, information flow from the Rho GTPases to the cytoskeleton has been viewed as linear, with GTPase activators (GEFs) stimulating a given GTPase, which then activates effectors which, in turn, modify actomyosin. Subsequently, the process is terminated by inactivation of GTPases by inhibitor proteins (GAPs). However, it is becoming apparent that control of actomyosin arrays entails rapid flux of GTPases from the active to inactive stages that is somehow subject to continual modulation via feedback from the actomyosin itself. Here we test a feedback model in which circular, ring-likewaves of Rho activity that direct cell wound repair in the Xenopus oocyte model are driven forward by a self-organizing "enzymatic corral" that forms at their trailing edge. Preliminary results indicate that 1) trailing edge Rho inactivation depends on an F-actin binding protein known as Cortactin and 2) Cortactin exerts its effects on Rho activity by serving as a binding site for two GAPs (RG1 and RG8, RG1/8). Both Cortactin and RG1/8 localize behind the Rho zone, perfectly situated to facilitate the trailing-edge inactivation. As predicted, Cortactin and RG1/8 functionally affect Rho levels. Over-expression of RG1/8 dramatically diminishes the Rho zone.Expression of a Cortactin truncation missing its SH3 domain (implicated in RG1/8 recruitment) causes increased Rho levels, and expression of the SH3 domain in isolation not only causes an outward spreading of Rho activity, but imparts gaps in the usually contiguous ring of RG1/8. F-actin assembly and turnover likely play a crucial role in corral formation as well. Inhibition of the Arp2/3 complex with the small-molecule inhibitor CK-666 significantly depletes Cortactinfrom behind the Rho zone, and pharmacological stabilization of F-actin with Jasplakinolidedelocalizes Cortactin resulting in an accompanied failure of RG1/8 localization.

90 Investigating nuclear size scaling using microfluidic encapsulation of *Xenopus laevis* embryonic cytoplasm. *P. Chen*[1], K.M. Nelson[2], M. Tomschik[1], J.C. Gatlin[1], J.S. Oakey[2], D.L. Levy[1] 1) Molecular Biology Department, University of Wyoming, Laramie, WY; 2) Chemical & Petroleum Engineering Department,University of Wyoming, Laramie, WY.

How nuclear size is regulated is a fundamental cell biological question, and altered nuclear size is often linked to disease, especially cancer where nuclear enlargement is used diagnostically and prognostically. However, mechanisms of nuclear size regulation remain unclear. We were interested to learn how cytoplasmic volume contributes to nuclear size regulation. To address this question, we made use of early *X. laevis* embryogenesis because there are concomitant reductions in both cell and nuclear volume between fertilization and gastrulation (stage 10.5-12). To test how cytoplasmic volume affects nuclear size, we used microfluidic technology to encapsulate *X. laevis* gastrula stage extract containing endogenous nuclei in droplets of defined volume and shape. Nuclei in droplets expanded to a new steady-state size after ~3 hours. In droplets ranging in volume from 0.02- 0.5 nL, nuclear volume increased by 1.2- to 3-fold, with larger increases occurring in larger droplets. However, in droplets greater than ~0.5 nL, the increase in nuclear volume reached a threshold of ~3-fold. Also, when gastrula-stage nuclei were treated with stage 5 embryo extract, the average nuclear volume increased ~4.5-fold in ~0.08 nL droplets. These findings demonstrate that both cytoplasmic volume and composition contribute to nuclear size scaling during development. Furthermore, nuclei exhibited similar growth trends in spherical and flattened droplets of comparable volume, indicating droplet shape minimally influences nuclear growth.

To identify cytoplasmic components limiting for nuclear growth, we biochemically fractionated *X. laevis* egg extract and identified fractions capable of enhancing nuclear growth in gastrula stage extracts. Histone chaperone nucleoplasmin (Npm2) was identified and verified as a promising candidate, as the nuclear scaling curve shifted upward when droplets were supplemented with Npm2 protein and gastrula stage embryos microinjected with Npm2 exhibited larger nuclei. Having ruled out transcription-dependent effects of Npm2 on nuclear size, our data suggest that the amount of Npm2 influences nuclear size by controlling nuclear histone levels, which may alter the mechanical properties of chromatin in a way that influences nuclear growth.

91 An interaction between Drg1 and Dsh modulates ciliogenesis during *Xenopus* development. M. Lee, J. Yoon, J. Sun, YS. Hwang, *I. Daar* National Cancer Institute, NIH, Frederick, MD.

Cilia are critical structures for proper embryonic development and maintaining homeostasis. Although ciliogenesis has been extensively studied, there are still significant gaps in our understanding of all the proteins involved in regulating and directing ciliogenesis. We identify Drg1 (Developmentally regulated GTP binding protein) as a novel binding partner of Dishevelled (Dsh), a known regulator of ciliogenesis. Using the *Xenopus laevis* embryo as a model system, we show that morpholino-mediated knockdown of Drg1 causes a reduction in the length and number of cilia in the gastrocoel roof plate (GRP) and multiciliated cells (MCCs). While expression of wild type Drg1 rescues these ciliogenesis defects in the morphant, a Drg1 mutant with a reduced ability to interact with Dsh fails to rescue the phenotype. In addition, Drg1 morphants display defective basal body migration and docking to the apical surface of MCCs, as well as abnormal rotational polarity of basal bodies. Moreover, apical actin and RhoA activity are also decreased in Drg1 morphants, suggesting a possible cause for the deficient basal body behaviors. Lastly, Drg1 knockdown results in decreased protein complex formation between Dsh and Daam1, and between Daam1 and RhoA. These results further support the concept that the Drg1/Dsh interaction regulates the nucleation and stability of MCC apical actin. Thus, Drg1 is a newly identified partner of Dsh in regulating ciliogenesis.

92 RhoGDI mediates spatiotemporal patterning of GTPase activity during cell wound repair. *AE Golding*[1], WM Bement[2,3] 1) Graduate Program in Cell and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 2) Department of Zoology, University of Wisconsin-Madison, Madison, WI; 3) Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

The RhoGTPases experience constant flux as they move through the GTPase cycle. GTPases associated with membrane lipids are activated by GEFs and inactivated by GAPs. A third regulator, RhoGDI, is believed to extract inactive GTPase from the membrane and drive it into a soluble form within the cytoplasm. To better understand RhoGDI function *in vivo*, we have employed the *Xenopus laevis* system in which cell damage elicits rapid formation of concentric zones of Rho and Cdc42 activity around wounds. Overexpression of RhoGDI negatively regulates Rho and Cdc42 activity. better understand the spatiotemporal patterning of GTPases, I visualized the accumulation of Rho and Cdc42 around the wound by expressing internally-tagged GTPases. By comparing the localization of GTPase to zones of GTPase activity, I have identified a pool of inactive GTPase that remains associated with the plasma membrane. Additionally, I sought to determine RhoGDI's ability to extract inactive versus active GTPase. RhoGDI is able to extract constitutively-active Cdc42. Finally, I have generated a mutant RhoGDI that is still able to extract inactive GTPase but is unable to extract active GTPase. These data suggest that the amino acids mutated confer nucleotide specificity to RhoGDI. The mutant RhoGDI localizes more strongly to wounds than wild-type and, remarkably, decreases Rho but increases Cdc42 activity. In contrast to the textbook model of RhoGDI function, these results suggest that RhoGDI extracts both active and inactive Cdc42 from membranes. If this is indeed true, RhoGDI can directly control GTPase patterning via extraction of active GTPases independently of GEFs and GAPs.

93 Sendai virus vector-based gene transfection into *Xenopus* embryos and cells. *Y. Haramoto*, Y. Ito, Y. Onuma Biotechnology Research Institute for Drug Discovery (BRD), National Institute of Advanced Industrial Science and Technology (AIST) , Tsukuba, Ibaraki, Japan.

Sendai virus (SeV) is a nonsegmented negative-strand RNA virus and a member of the paramyxovirus family. A fusion protein (F) gene-deleted SeV vector which does not make an infective particle has developed for a gene delivery system. The SeV vector transfects to dividing and non-dividing mammalian cells and tissues. SeV binds to sialic acid on the cell membrane via the hemagglutinin-neuraminidase (HN) protein and causes membrane fusion via the F protein. The vector RNA stays in the cytosol without integrating into the genome DNA of host cells, so it has been applied for gene therapy and producing of induced pluripotent stem cells (iPSCs).
We tested the SeV vector system in *Xenopus* embryos and cells. Embryos were treated with the SeV vector coding Azami-Green (AG) fluorescent protein gene in 0.1 x Steinberg's solution. A few days was sufficient to detect AG fluorescent.
Next, we tried to generate iPSCs from *Xenopus* cultured cells, by using SeV vector with human *OCT3/4*, *SOX2*, *KLF4,* and *c-MYC*. The gene expression changed sequentially in 24 to 72 hours after transfection. The *pou5f3.1* (*oct-91*) and *tdgf1.3* (*frl-1*), orthologues of mammalian pluripotent markers *Oct3/4* and *Cripto/TDGF1*, showed increased expression. The *ventx2.1* and *ventx3.2* also upregulated, among *vent/ventx* family genes that are candidates of Nanog alternative. We propose that SeV vector system is a useful tool for amphibian research.

94 Protein dynamics of the intraflagellar transport (IFT) complex. *Jaime V. K. Hibbard*, John B. Wallingford Molecular Biosciences, The University of Texas at Austin, Austin, TX.

Primary cilia play a crucial role in embryonic patterning as mediators of hedgehog signaling. Defects in the structure or function of primary cilia result in a broad range of developmental disorders, termed ciliopathies. Ciliopathies are characterized by phenotypes such as craniofacial and skeletal defects, polycystic kidneys, retinal degeneration, obesity, and situs inversus, among others. Proper cilia formation and function requires the bi-directional trafficking of protein cargo along the axoneme microtubules in a process called intraflagellar transport (IFT). This process is powered by molecular motors and the conserved IFT protein complex. To function in ciliogenesis, the IFT complex must assemble and accumulate at the basal body, a structure at the base of cilia. However, the mechanisms of complex assembly *in vivo* remain unclear. Adapting tools for live imaging, we are studying the protein dynamics of IFT complex formation and basal body recruitment in *Xenopus* multiciliated cells. Specifically, we are investigating the localization of IFT complex interactions using bimolecular fluorescence complementation and the kinetics of IFT complex turnover using fluorescence recover after photobleaching. These techniques will inform our understanding of cilia assembly and maintenance, processes required for embryonic development.

95 The role of DDX protein family members in Xenopus development. *A. Hirth*[1], E. Fatti[1], A. Svorinic[1,2], C. Cruciat[1,3], S. Perez Acebron[1,4], C. Niehrs[1,5] 1) Division of Molecular Embryology, German Cancer Research Center (DKFZ), Heidelberg, DE; 2) Center for Biochemistry (BZH), University of Heidelberg, DE; 3) Faculty for Applied Sciences, University of Esslingen, DE; 4) Centre for Organismal Studies (COS) Heidelberg, DE; 5) Institute of Molecular Biology (IMB), Mainz, DE.

Wnt/β-catenin signaling plays an important role in *Xenopus* embryonic development. Recently, the DEAD-box RNA helicase DDX3 has been identified as regulator of Wnt signaling. By binding to CK1ε and enhancing its activity, DDX3 is capable to increase Wnt signaling both in human cell lines (HEK293T) and in *Xenopus* embryos.
DDX3 is part of the large protein family of DEAD-box RNA helicases, which comprises more than 30 members, that all share a conserved core, consisting of an ATPase and a RNA binding domain. This conservation prompted the question whether other DEAD-box RNA helicases share the function of DDX3 in activating CK1ε. This ongoing study aims to evaluate whether other members of the DEAD-box RNA helicase family are functioning as regulators of Wnt signaling via similar mechanisms. Preliminary studies on other DDXs indeed indicate a broader role for DDXs in CK1 regulation as shown both *in vitro* and in *Xenopus tropicalis*.

96 Actin flows engage cadherin microclusters to remodel cell junctions during convergent extension. *R.J. Huebner*, S. Weng, C. Lee, O. Papoulas, J.B. Wallingford Molecular Biosciences , University of Texas at Austin, Austin, TX.

Convergent extension (CE) is the conserved process of collective cell movement that drives anterior-posterior axis elongation in animals, but there has been sustained controversy over the cell biological mechanism driving this process. A key step during CE is shrinking of mediolaterally-aligned cell-cell junctions and exchange for new anterior-posterior junctions. This requires actomyosin contractility at sites of junction remodeling, but it is unclear how contractility machinery interacts with the cadherin based cell adhesions that hold these junctions together. Here, based on new data from super-resolution in vivo time-lapse imaging, we propose a novel model for junction remodeling in which discrete actin flows engage cadherin microclusters to shrink subdomains at the lateral edge of cell-cell junctions. Proteomic data

indicate a key role for the poorly defined catenins ARVCF and plakoglobin in this process. These data represent a novel mechanism for junction remodeling and also provides mechanistic insight into the deeply conserved process of convergent extension.

97 Elucidating the function of RAPGEF5 in β-catenin nuclear translocation. *W.Y. Hwang* Genetics, Yale University, New Haven, CT.

Wnt signaling plays critical roles in both embryonic development and adult tissue homeostasis. Wnt activation leads to stabilization of β-catenin, a key effector molecule for Wnt signaling, in the cytoplasm and enables its subsequent nuclear entry and Wnt target activation. To date, a mechanism by which β-catenin translocates into the nucleus is not clearly defined. Previously, in a patient with heterotaxy, a disorder of right-left patterning that can lead to a severe form of CHD, we identified the guanine nucleotide exchange factor, RAPGEF5 as a candidate gene. Our work demonstrates that RAPGEF5 affects LR patterning via regulating the nuclear entry of β-catenin. Furthermore, our results suggest that Rapgef5 regulates the nuclear localization of β-catenin independently of NLS mediated nuclear transport machinery. Based on these data, we **hypothesize** that Rapgef5 maintains nuclear Rap protein(s) in an active GTP bound state, which will preferentially import and/or retain β-catenin in the nucleus. **The overarching goal of my project is to determine if Rapgef5 regulates β-catenin nuclear import and/or export by measuring transport kinetics and to determine specifically which Raps regulate this process.** Aberrant activation of Wnt/ β-catenin signaling is implicated in multiple congenital anomalies including CHD as well as cancer; therefore, a better understanding of the β-catenin nuclear transport mechanism can be exploited to develop novel therapeutics that acts downstream in the Wnt/ β-catenin pathway.

98 Cell responses to mechancial stresses in the superfical and deep layers of the stage 10 *Xenopus laevis* blastocoel roof. *Emma Johns*[1], Georgina Goddard[1], Megan Moruzzi[1], Alexander Nestor-Bergmann[3], Oliver Jensen[2], Sarah Woolner[1] 1) Faculty of Biology, Medicine and Health, University of Manchester, Manchester, GB; 2) School Mathematics, University of Manchester, Manchester, GB; 3) Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, GB.

Cells in living tissue must be able to cope with a variety of forces including tensile (stretching), compressive (pushing) and shearing (a combination of tensile and compressive forces) and it is vital that cells can respond appropriately to these forces. Mechanical regulation of cell division is one clear example of such a response: cells change their division rate and align their division orientation in a manner that helps relieve mechanical stresses and thus maintain tissue homeostasis. Previous research has explored the effects of mechanical forces upon single cultured cells, however, much less is known about the response of cells to mechanical forces in complex multi-layered tissues.

To bridge this gap, we are using *Xenopus laevis* animal caps to investigate how mechanical force regulates cell behaviour in a whole tissue. We dissect tissue at stage 10, when the blastocoel roof of the *Xenopus* embryo consists of three distinct layers of cells: a superficial epithelial layer and two deep layers of mesenchymal-like cells. In previous work, we have shown that the superficial layer responds to tensile force by altering cell division rate and orientation. We are now exploring how the mechno-responses of the deepest layer compare to the superficial layer. The deep layer cells are more motile and, in the embryo, undergo radial intercalation to drive epiboly. In preliminary data, we find that, unlike the superficial cells, the deep cells do not alter cell division orientation upon the application of tensile force. We therefore hypothesise that the superficial and deep layers respond differently to applied force and utilise different approaches to relieve mechanical stress.

We are using a combination of experimental, mathematical and computational techniques to investigate mechano-responses across the cell layers of the animal cap. In order to investigate cell behaviours across the tissue we are developing a novel stretch system for use in a light sheet microscope. This system allows superficial and deep layers to be simultaneously imaged while an external global stretch is applied. We are combining this experimental approach with complementary computational modelling with the aim of creating a model which describes and analyses local and global mechanical forces acting across the whole tissue.

99 Spindle assembly in egg extracts of the Marsabit clawed frog, *Xenopus borealis*. *M. Kitaoka*[1], R. Gibeaux[2], R. Heald[1] 1) Molecular and Cell Biology, University of California, Berkeley, CA; 2) Univ Rennes, CNRS, IDGR (Institute of Genetics and Development of Rennes), Rennes, France.

Egg extracts of the African clawed frog, *Xenopus laevis*, have provided an instrumental *in vitro* cell-free system to elucidate fundamental aspects of the cell cycle, including mechanisms of spindle assembly. Comparative studies with extracts from the related Western clawed frog, *Xenopus tropicalis*, which is smaller at the organismal, cellular, and subcellular levels, have enabled the identification of spindle and organelle size scaling mechanisms. Here, we investigate the Marsabit clawed frog, *Xenopus borealis*, which is intermediate in size but more recently diverged evolutionarily from *X. laevis* than *X. tropicalis*. We developed an *X. borealis* egg extract to provide a new system to further investigate interspecies spindle scaling and morphometric variation. As with *X. laevis* and *X. tropicalis*, spindles formed in extracts prepared from metaphase cytostatic factor (CSF) arrested *X. borealis* eggs appeared very similar to meiosis II spindles *in vivo*. CSF-arrested *X. borealis* formed spindles around various sources of DNA, including sperm and embryo nuclei and chromatin-coated beads, and could be cycled through interphase. Interestingly, the microtubule distribution across the length of *X. borealis* cycled spindles differed noticeably from both *X. laevis* and *X. tropicalis*. Extract mixing experiments revealed that titration of *X. borealis* extract with *X. laevis* extract did not alter spindle size, whereas addition of *X. tropicalis* extract caused *X. borealis* spindles to shrink. This reveals common scaling phenomena across *Xenopus* species, while characterization of spindle factors katanin, TPX2, and Ran indicate that *X. borealis* spindles possess both *X. laevis* and *X. tropicalis* features. Thus, *X. borealis* egg extract provides a third *in vitro* system to investigate interspecies scaling and spindle morphometric variation.

100 Basal body synthesis and patterning is regulated in a positive feedback loop with apical cell size in multiciliated cells. *Saurabh Kulkarni*[1], Lance Davidson[2], Brian Mitchell[3], Mustafa Khokha[1] 1) Yale School of Medicine, New haven, CT; 2) University of Pittsburgh, Pittsburgh, PA; 3) Northwestern University, Feinberg School of Medicine, Chicago, IL.

Within a cell, organelles are spatially organized to provide specialized microenvironments for different cellular functions; however, the

complexity of organization makes the underlying molecular mechanisms challenging to understand. Multiciliated cells (MCCs) of *Xenopus* epidermis provide a powerful opportunity to address this problem. MCCs are epithelial cells that undergo dramatic intracellular patterning of sub-cellular structures to generate hundreds of polarized beating cilia. Specifically, formation of an intricate network of F-actin and hundreds of basal bodies at the apical surface is critical for the MCC assembly. However, how the cell determines the number of basal bodies to synthesize and their patterning is not understood. We discovered that the size of the apical surface is a critical factor in deciding the number of basal bodies. During embryonic development, the apical surface of the MCC expands as the cell intercalates into the superficial epithelium. During this apical expansion, the number of basal bodies synthesized linearly scales with the apical surface area. Further, different *Xenopus* species and Axolotls have dramatically different cell sizes based on their different embryonic sizes. Nevertheless, the number of basal bodies scales with apical surface area despite a six-fold difference. In addition, we also show that genetically or mechanically manipulating apical cell size alters the number of basal bodies. To explain this scaling phenomenon, we show that basal bodies carry actin-remodeling proteins that both form the F-actin lattice necessary for basal body patterning as well as generate the necessary force to expand the apical surface. In summery basal body synthesis and patterning is regulated in a positive feedback loop with apical cell size in MCCs.

101 Microtubule Stabilization is Sufficient to Bypass the RNA Requirement in Spindle Assembly. *T. Lou*, A. Grenfell, R. Heald Department of Molecular Cell Biology, UC Berkeley, Berkeley, CA.

The mitotic spindle is a complex macromolecular structure responsible for the proper segregation of genetic material during cell division. Fortunately, the *in vitro* cell-free system from *Xenopus* egg extracts has allowed us to recapitulate many features of *in vivo* spindles. While research has been focused on understanding the protein components of spindle assembly, recent work has shown RNA to be important for efficient mitotic spindle assembly. Treatment of *Xenopus laevis* egg extracts with RNase A, which removes cytoplasmic RNA, has been shown to severely impact spindle assembly, suggesting that RNAs may be important for this essential process; however, little is known about their contributing roles. In this study, we investigate the specific morphological defects that arise in RNase A-treated extracts. We observed that the spindles formed in RNA-depleted extracts have reduced microtubule density, and that spindle and chromatin morphology was abnormal. In our systematic investigation, we examined the sensitivity of the mitotic spindle to RNase A. We determined that the phenotypic defects resulted specifically from the catalytic activity of RNase A. Furthermore, we found that addition of either glycerol or sucrose—both of which promote microtubule assembly—to RNA-depleted extracts partially suppressed the spindle defects. Our work reveals that RNA is required for spindle assembly by validating that degradation of RNA in egg extracts prevents proper spindle formation. This requirement can be circumvented by artificially stabilizing microtubules, which indicates that RNA may contribute to this process. Overall, we demonstrate the existence of RNA-dependent microtubule stabilization processes, as well as the importance of RNA-dependent spindle and chromatin assembly mechanisms.

102 Mechanisms of spindle assembly and scaling across Pipid frogs. *K.E. Miller*, R Heald UC Berkeley, Berkeley, CA.

The molecular mechanisms by which the mitotic spindle scales in size, as well as how it adapts its morphology to a wide range of genome sizes across different cell types and organisms, is poorly understood. In recent years, our laboratory has utilized two model frog species, the larger *Xenopus laevis* and the smaller *Xenopus tropicalis*, which have correspondingly larger and smaller cells, organelles, and genomes, to identify spindle scaling factors. However, it remains unclear whether these mechanisms are conserved across different species, or whether novel ones exist. To this end, we have developed an egg extract system using the tiny related Pipid frog *Hymenochirus Boettgeri* which recapitulates spindle assembly in vitro and enables identification and functional testing of potential scaling factors. Interestingly, mixing egg extracts of *X. laevis* with those from *H. boettgeri* results in spindles of intermediate size in a dose-dependent manner, as it does for mixing extracts of *X. laevis* and *X. tropicalis*, indicating that cytoplasmic factors are responsible for regulating spindle size. However, although spindles formed in *H. boettgeri* extracts are statistically similar in length to those of *X. tropicalis*, mechanisms of spindle scaling appear to be different. Unlike *X. tropicalis*, *H. boettgeri* egg extracts have low microtubule severing activity and spindle microtubules recruit reduced amounts of previously identified scaling factors, such as TPX2 and Eg5. Interestingly, the microtubule depolymerizing kinesin Kif2a is enriched on *H. boettgeri* egg extract spindles compared to those in *Xenopus*, highlighting a novel mechanism for spindle scaling across species. Additionally, we are starting to examine mechanisms of spindle scaling in response to very large genome size using the rare dodecaploid frog *Xenopus longipes*.

103 Mechanics of epithelial cell renewal in vivo. *Jakub Sedzinski*[1], Edouard Hannezo[4], Fan Tu[2], Maté Biro[3], John Wallingford[2] 1) Danish Stem Cell Center DanStem, University of Copenhagen, Copenhagen N, DK; 2) Department of Molecular Biosciences, Center for Systems and Synthetic Biology, and Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712, USA; 3) Centenary Institute of Cancer Medicine and Cell Biology, Locked Bag 6, Newtown, NSW 2042, Australia; Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia; 4) Institute of Science and Technology Austria, Am Campus 1, A-3400, Klosterneuburg, Austria.

Epithelial sheets are crucial components of all metazoan animals, enclosing organs and protecting the animal from its environment. Epithelial homeostasis poses unique challenges, as the addition of new cells and loss of old cells must be achieved without disrupting the fluid-tight barrier and apicobasal polarity of the epithelium. Several studies have identified cell biological mechanisms underlying extrusion of cells from epithelia, but far less is known of the converse mechanism by which new cells are added. Here, we combine molecular, pharmacological, and laser-dissection experiments with theoretical modeling to characterize forces driving the emergence of an apical surface as single nascent cells are added to a vertebrate epithelium in vivo. We find that this process involves the interplay between cell-autonomous actin-generated pushing forces in the emerging cell and mechanical properties of neighboring cells. Our findings define the forces driving this cell behavior, contributing to a more comprehensive understanding of epithelial homeostasis.

104 Exploring Congenital Heart Defects in Patients with Wolf-Hirschhorn Syndrome. *E. Snow* Boston College, Chestnut Hill, MA, MA.

Wolf-Hirschhorn Syndrome (WHS) is a neurodevelopmental disorder characterized by intellectual disabilities, characteristic craniofacial dysmorphism, congenital heart defects, seizures, and other developmental malformations. Previous studies have shown that patients with WHS typically exhibit genetic irregularities on the short arm of chromosome 4, such as microdeletions and microduplications. Most mutations occur in a critical genomic region, containing the genes WHSC1 and WHSC2. Other WHS-associated genes such as TACC3 and LETM1 are located telomeric to this critical region, and their mutations exacerbate the characteristic phenotype. WHS patients present with a variety of heart defects involving the ventricles, atriums, and septum; however, the mechanism behind these defects has not been well studied in the past. Additionally, our lab has previously shown that WHS-associated genes have been implicated in the migration of neural crest cells (NCCs), a type of multipotent stem cell that develops into the parts of the heart. We have hypothesized that the inability of NCCs to properly migrate plays a role in WHS defective heart formation. Using Xenopus laevis as a model organism, we have explored the effects of WHS related gene knockdown on embryonic cardiogenesis and tadpole cardiac morphology. Embryonic cardiogenesis has been measured by immunofluorescence of specific cardiac markers, and tadpole cardiac morphology was investigated using optical coherence tomography and high-resolution microscopy. Quantitative and qualitative analysis of these results has further elucidated the genotype-phenotype relation between a WHS critical region deletion and the associated congenital heart defects.

105 Creation of F0 homozygous mutant embryos by using *Xenopus* cultured cell lines and nuclear transfer. *H. Yoshida*[1], G. Gorbsky[2], M.E. Horb[1] 1) Bell Center for Regenerative Biology and Tissue Engineering and National Xenopus Resource, MBL, Woods Hole, MA; 2) Cell Cycle and Cancer Biology Research Program, OMRF, Oklahoma City, OK.

Producing homozygous mutant embryos is important for the analysis of gene function to better understand embryogenesis, regeneration and disease. The ideal test for gene function is to generate homozygous mutant animals. New gene editing technologies, such as CRISPR-Cas, have made it possible to create mutations in *Xenopus g*enomic DNA, but the F0 embryos contain mosaic mutations; to generate homozygous mutant animals requires breeding to the F2 generation. In addition, much breeding space is required to keep any F0-F2 generated mutant animals. Therefore, the time required to generate homozygous mutant animals combined with the need for extra space are major problems in generating homozygous knock-out animals. We are working on developing a more rapid method for generating homozygous mutant *Xenopus* animals, and thus overcome some of these obstacles. This method relies on the generation of F0 animals through nuclear transfer from euploid *Xenopus* cell lines. Homozygous mutations can be easily selected for in the cell lines, prior to nuclear transfer. The added benefits of such an approach include the ability to freeze the mutant cell lines, creation of homozygous mutant animals at any time and the fact of embryonic lethality is less problematic. In this poster, I present our recent work on improving nuclear transfer.

106 Uncovering the molecular determinants of chromosome scaling during *Xenopus* development. *C.Y. Zhou*[1], Mingxuan Sun[1], Bastiaan Dekker[2], Job Dekker[2], Rebecca Heald[1] 1) Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Program in Systems Biology, University of Massachusetts Medical School, Worchester, MA.

Life in all vertebrates begins with the fertilization of an egg, which is followed by rapid and successive cell division events in the absence of cell growth. As a result, the dimensions of the cell, along with its components, decrease over time, providing a unique opportunity to study the mechanisms that regulate biological scaling of the cell division machinery relative to cell size. Using the vertebrate model system *Xenopus laevis*, we and others have discovered a number of molecules, which we term scaling factors, that regulate the dimensions of the mitotic spindle. However, very little is known about how the mitotic chromosomes, which are densely packaged to fit the dimensions of the mitotic spindle, scale during embryonic development. Using the egg extract system, the Heald Lab previously demonstrated that mitotic chromosomes formed using G2-arrested nuclei isolated from stage 20 embryos are 50% shorter than those isolated using stage 8 embryo nuclei. These results suggest that these G2 nuclei contain a set of epigenetic factors which define chromosome size at a specific developmental stage. Our overall goal is to identify these chromosome scaling factors and test their roles in regulating faithful cell cycle pathways during embryogenesis. Toward this goal, we have begun developing Hi-C as a tool for mapping architectural changes that occur on chromosomes as they shrink during embryogenesis. We have shown that this technique works well in *Xenopus* egg extracts and are currently analyzing samples from stage 8 and stage 20 embryos. To identify potential scaling factors that re-size chromosomes during development, we will perform mass spectrometry on G2-arrested nuclei from various developmental stages. Eventually, we will deplete these candidate scaling factors from egg extract and embryos and assess their effect on cell cycle progression. The results from work will have an immediate impact on our understanding of how chromosome architecture is coordinated with the cell cycle and during development, with broader implications for how chromosome dimensions are potentially tuned across evolution.

107 The congenital heart disease candidate gene MYRF plays an unexpected role in left-right patterning. *S. Amalraj*, E. Mis, M. Khokha Pediatric Genomics Discovery Progam, Department of Pediatrics and Genetics, Yale School of Medicine, New Haven, CT.

Congenital heart disease (CHD) is the most common major birth defect, affecting nearly 3% of children, and is the leading cause of infant mortality. Heterotaxy (Htx) is a disorder of left-right (LR) patterning, in which organs, including the heart, are mispatterned relative to the LR axis. Htx is associated with severe forms of CHD, but its genetic causes remain largely undefined. A recent genetic analysis of Htx/CHD patients identified numerous candidate genes, including the transcription factor Myelin Regulatory Factor (MYRF). MYRF emerged as a candidate gene in three patients, who all carried mutations in highly conserved regions of the DNA binding domain. This is intriguing, as MYRF has an identified role in the generation and maintenance of myelin in the central nervous system, but no known function in cardiac development or LR patterning. Here, we show that depletion of *myrf* using CRISPR based gene modification in *Xenopus tropicalis* embryos results in midline heart looping defects, phenocopying our patients. We then analyzed global LR patterning markers and found abnormal bilateral expression of *pitx2*, but normal expression of *coco* in *myrf* depleted embryos. Because nodal signaling occurs between *coco* and *pitx2* in the LR patterning cascade, we then examined *nodal* expression and observed that although *nodal* was properly expressed on the left side of *myrf* depleted embryos, *nodal* expression intensity was increased in *myrf* depleted embryos compared to control embryos. Additionally, we depleted *myrf* in

one cell of a two-cell embryo and observed that left-sided depleted embryos resulted in heart looping defects, abnormal bilateral *pitx2* expression, and normal *coco* expression. Conversely, right-sided depleted embryos had no LR patterning defects. Together, our data suggests *MYRF* plays a role in LR patterning, possibly by acting as a midline protein regulating transcription of *nodal*. Loss of *myrf* may allow nodal protein to diffuse into the right side of the embryo, leading to bilateral *pitx2* expression and heart looping defects. We conclude that patient driven gene discovery can provide new insights into the molecular mechanisms that drive cardiac patterning and LR axis formation.

108 Caveolin 1 is required for *Xenopus* muscular function. *M. Breuer*, H. Berger, M. Staps, A. Borchers Philipps Universität Marburg, Marburg, DE.

Muscle cells are subjected to severe mechanical stress through an increase in membrane tension caused by muscle contraction. Caveolae, specialized invaginations of the plasma membrane, have been shown to play an important role during mechanoprotection in muscle cells by acting as stretch sensors and membrane reservoirs during mechanical stress. Loss of caveolae formation by knockdown of the cytoplasmic caveolae-coat protein Cavin 1, leads to locomotion-induced lesions in the zebrafish notochord as well as loss of sarcolemma and T-tubule organization in mouse muscle cells. In this study we analyzed the function of Caveolin 1 – an essential structural component of Caveolae – in the development of *Xenopus laevis*. Caveolin 1 is mainly expressed in the *Xenopus* notochord and the epidermis throughout developmental stages. Morpholino-mediated knockdown of Caveolin 1 results in a striking swimming defect, characterized by paralysis of the morphants. Muscle differentiation and maturation are unaffected by Caveolin 1 knockdown. However, phalloidin staining as well as transmission electron microscopy of muscle cells revealed aberrant sarcomeric structures and disorganized actin fibers organization. Additionally, we could also show that axonal outgrowth of motorneurons as well as acetylcholine receptor clustering was disrupted by Caveolin 1 knockdown. Thus, these findings suggest a novel function of Caveolin 1 in the locomotor system of *Xenopus laevis*.

109 Novel vectors for functional interrogation of *Xenopus* ORFeome cDNAs. Z.R. Sterner[1], S.A. Ranking[2], J. Choi[1,3], M. Wlizla[2,4], A.M. Zorn[2], *D.R. Buchholz*[1] 1) Biological Sciences, University of Cincinnati, Cincinnati, OH; 2) Division of Developmental Biology, Cincinnati Children's Research Foundation and Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio; 3) Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; 4) National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA.

The recently available *Xenopus laevis* ORFeome v1.0 contains 7444 validated, full-length cDNA sequences cloned into Gateway-compatible entry vectors. To increase the utility of the ORFeome, we have constructed the Gateway-compatible destination vectors pDXTP and pDXTR, which in combination can be used to control the spatial and temporal expression of any ORF of interest. These vectors can be integrated into the genome via well-established microinjection-based SceI, tol2, or phi-C31 transgenesis procedures. pDXTP receives a promoter / enhancer of interest; this promoter / enhancer controls spatial expression of a doxycycline-inducible transcription factor rtTA. pDXTR receives an ORF of interest, and it contains a tetracycline response element upstream of the ORF, which enables temporal control of the ORF simply by addition of doxycycline at any desired time point to the embryo culture buffer. pDXTP and pDXTR contain green and red fluorescence reporters, respectively, to readily confirm transgenic integration by screening the eyes of embryos for fluorescence. In addition, when an ORF is cloned into pDXTR via Gateway cloning, it becomes part of a poly-cistronic message encoding both the ORF of interest and an mCherry-histone H2B product, which allows for cell-autonomous verification of ORF expression via red nuclear fluorescence. Function of all essential features of pDXTP and pDXTR has been experimentally validated. pDXTP and pDXTR provide flexible molecular cloning and transgenesis options to accomplish tissue-specific inducible control of ORF expression in transgenic *Xenopus*.

110 MUSA1 Depletion During *Xenopus laevis* Development Alters Somite Shape and Structure. *B. Cadiz-Rivera*[1,2], J.C. Velázquez[1,2], S.A. Lamboy[1], T. Zbinden[1], N. Flores[1], T.M. Lozada[1], J. Ramirez[2], C. Domingo[2], E. Traverso[1] 1) University of Puerto Rico, Humacao, PR; 2) San Francisco State University, California, USA.

The study of the regulation of muscle mass has taken importance during the last couple of decades due to the loss of muscle mass in aging and disease. One of the key players in muscle mass regulation in adult mice is the Muscle Ubiquitin ligase of the SCF complex in Atrophy-1 (MUSA1). MUSA1 is involved in the targeting of proteins for proteasome degradation. We are interested in the study of MUSA1 in *Xenopus laevis*, specifically its role during development. We have detected MUSA1 expression, of both homeologs L and S, in the somites by *in situ* hybridization. In addition, MUSA1 mRNA is present in the animal pole of the egg, and its expression stays high until gastrula stages, when it decreases. Then, its expression increases at the onset of organogenesis (as shown by RT-PCR and *in situ* hybridization). The timing and localization of MUSA1 expression during organogenesis implies a role in somitogenesis, and most likely muscle development. Somites are transitory blocks formed during the development of vertebrates, which give rise to muscle cells, among other tissues. A pair of somites forms every 50 minutes in the dorsal part of the embryos starting at the neurula stages, continuing through the tailbud stages. We have performed immunofluorescence with confocal microscopy as a technique to better describe somite structure and shape during development, and use this to assess the phenotype caused by MUSA1 depletion during development. We performed MUSA1 knockdown using a morpholino against both homeologs, followed by the characterization of the morphant's phenotype. The study of somite shape was done by immunostaining of Laminin and Fibronectin, as these proteins are located in the extracellular matrix (ECM) that surrounds the blocks. In addition, we also used the muscle specific marker 12/101 to identify the cells within the somite, and β-integrin to delineate the cell membranes. In our analysis, MUSA1 morphants showed curvatures along the A/P axis, elongated muscle fibers, and alterations in the somite structure. Our data indicates that MUSA1 plays an important role for proper somitogenesis to occur during development. We will further test this by using other morpholinos and including other perspectives of the tri-dimensionality of the somite.

111 The dynamic interplay of Sox transcription factors regulating the activity of endoderm specific *cis*-regulatory modules. *Jin Cho*[1], Kitt Paraiso[1], Jessica Cheung[1], Margaret Fish[1], Ira Blitz[1], Aaron Zorn[2], Ken Cho[1] 1) Developmental and cell biology, University of California, Irvine, Irvine, CA; 2) Division of developmental biology, the department of pediatrics, college of medicine, University of Cincinnati, Cincinnati, OH.

Sox (SRY-related high motility group (HMG)-box) family transcription factors (TFs) have critical roles in both maintaining the pluripotency of embryonic cells and regulating the lineage-specific differentiation. What is unclear is how such conserved TFs are involved in so many different tissue specifications, while defining diverse cell fates. Among various Sox TFs, we examined three different Sox TFs that are important in mediating endoderm specification - maternally expressed Sox3 and Sox7, and zygotically expressed Sox17. Sox17 is a conserved endodermal core TF. Sox7 is an endodermally expressed factor, but its function has been poorly understood. Sox3 is required for a neuroectodermal development. However maternal function of Sox3 has not been investigated although it was shown to act as a repressor of nodal5. Since maternal Sox3 is ubiquitously expressed in cleavage stage embryos, we examined the DNA binding ability of Sox3, Sox7 and Sox17 in blastula and early gastrula stage embryos. We found a large overlap in the binding of Sox3 to early gastrula endodermal cis-regulatory modules (CRMs) that also bind Sox17, and these have less overlap with the binding of Sox7. We also compare the interaction of the SOX factors with other TFs and epigenetic markers to understand the regulatory mode of the SOX TFs with partner TFs during germ layer specification. Our results suggest that Sox3 may pre-mark Sox17 binding regions before the onset of zygotic Sox17 acts on the endodermal CRMs.

112 Characterizing the Role of Mink1 in Congenital Heart Disease. *V Colleluori*, E Mis, M Khokha Genetics, Yale University, New Haven.

Congenital Heart Disease (CHD) is the most common birth defect, affecting approximately 1% of all live births and is a of the leading causes of infant mortality globally. A recent genetic analysis of CHD patients identified a novel candidate gene, *mink1*. *Mink1* encodes a serine-threonine germinal-center kinase with known functions in the JNK and PCP/Wnt signaling pathways. However, it has no known role in LR patterning or cardiac development. CRISPR knockout strategies to deplete *mink1* in the high-throughput human disease model, *Xenopus*, resulted in malformation of the cardiac outflow tract, recapitulating the patient phenotype, and LR patterning defects. Additionally, novel defects in formation of multiciliated cells were observed. **The overall goal of this project is to investigate the molecular mechanism by which *mink1*affects LR patterning, heart development, and multiciliated cell formation in the *Xenopus* (frog) model system.** Loss of function experiments were employed to determine the earliest time point of *mink1* activity during the LR patterning cascade by testing molecular markers *pitx2* and *coco*. Observation of the structure of the left-right organizer tissue supports the hypothesis that early patterning defects during gastrulation of the embryo cause downstream defects in left-right axis patterning and organogenesis. Mechanistic hypotheses will be guided by an analysis of temporal and spatial expression of *mink1* in the whole embryo and the left-right organizer. On the multiciliated epidermal surface, loss of function experiments have confirmed a regulatory role for *mink1* during formation of multi-ciliated cells of the *Xenopus* epidermis. Focused hypotheses will for formed based on the role of *mink1* in the Notch signaling pathway, which will be tested by rescue of the multiciliated cell phenotype. Finally, the role of each *mink1* domain, as well as the nature of patient mutations, will be determined using multiple functional assays in *Xenopus*.

113 Chibby: a Regulator of Ciliogenesis and Kidney Development. *Mark E. Corkins*[1], Hannah Hanania[2], Melanie Carroll[1], Michael Klymkowsky[3], Rachel Miller[1,4,5,6] 1) Pediatrics, UTHealth McGovern Medical School, Houston, TX; 2) Program in Biochemistry and Cell Biology, Rice University, Houston TX; 3) Department of Molecular, Cellular, & Developmental Biology, University of Colorado, Boulder; 4) Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, Texas; 5) The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Program in Genetics & Epigenetics, Houston, Texas; 6) The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Program in Biochemistry & Cell Biology, Houston, Texas.

Genetic disorders that affect the development of cilia (ciliopathies) result in a variety of diseases. One of the most common clinical features of ciliopathies is the development of cysts within the kidney. Cystic diseases of the kidney (CDKs) occur in ~1/800 births, making them the most common life-threatening hereditary disorder. Specific manifestations of each CDK depend on the gene affected. How ciliopathies result in CDKs is largely unknown. However, Wnt signaling may play a role in the development of CDKs, given that it is closely related with cilia. Loss of cilia in multiple models results in increased Wnt signaling, and artificially increased Wnt signaling in mouse results in the development of cysts within the kidney. How the Wnt pathway is regulated by cilia is largely unknown, but current models point to pools of the Wnt protein, β-catenin, found in the transition zone of the cilia. β-catenin is the central transcription factor involved in canonical Wnt signaling, and it functions to activate genes bound by the Tcf/Lef transcriptional repressors. A protein called Chibby has been shown to bind β-catenin and shuttle it from the nucleus into the cytoplasm. Additionally, Chibby is found in the transition zone of cilia along with β-catenin. Though loss of Chibby results in malformed cilia, the function of Chibby at the cilia and within the kidney itself is largely unknown. We have found that loss and overexpression of Chibby results in kidney abnormalities. The current study uses *Xenopus laevis* and tissue culture to elucidate the roles of Chibby and β-catenin in the development of the kidney and cilia

114 Role of Lbh during Cranial Neural Crest migration and gastrulation. *Helene Cousin*, Emma Weir Vet. & Animal Sciences Department, University of Massachusetts at Amherst, Amherst, MA.

The cranial neural crest (CNC) is a key contributor to craniofacial development. These cells originate from the border of the future brain during neurulation, undergo extensive anterior and ventral migration and differentiate in the many tissues composing the face and the cranium. Changes in migration or differentiation of these CNC are at the origin of the diversity of the neurocranium and viscerocranium among vertebrates. While this plasticity is key to evolution, it also makes craniofacial structures prone to developmental defects such as cleft lip and palate or micrognathia.

We previously characterized a SNP in the gene *limb bud and heart homolog* (*lbh*), between the long-jawed pelagic *Maylandia zebra* (MZ)and the short-jawed benthic *Labeotropheus fuelleborni* (LF)which contributes to adaptive variation in the jaw of cichlid fishes (Powder et al., MBE 2014). *lbh*encode a small putative transcriptional co-factor whose function during development is understudied. In*Xenopus laevis,* Lbh protein

is maternally expressed and is found in embryos throughout early tailbud stages.*In situ* hybridizationshowed that *lbh*transcripts are enriched in the CNC before and during their migration. The morpholino-based knock down of Lbh leads to the significant decrease of protein after stage 19 and results in the inhibition of CNC migration *in vivo*. However, it does neither perturb CNC induction nor their migration *in vitro*. In this case, Lbh function is exerted in the cytoplasm and that two of its domains are critical for this function. Using LC-MS/MS, we are currently identifying binding partners that dock specifically to these domains site and investigating the functional relationship between these proteins during CNC migration.
We started to investigate the function of maternal Lbh using the recently developed Trim-Away knock down technology (Clift et al. Cell 2017). The global loss of Lbh protein leads to severe gastrulation defects. The targeted loss of the protein in the precursor cells of the CNC leads to embryos that develop properly until tail bud stage. However, their CNC failed to migrate which validates the results obtained by morpholino knock down.

115 Leptin: A novel role as a nutritional modulator of development and regeneration. *Marietta Easterling*, Jennifer Cundiff, Kayla Titialii, Audrey Parks, Erica Crespi School of Biological Sciences, Washington State University, Pullman, WA.

Leptin is a fat-secreted hormone that relates the nutritional state to modulate energy balance to multiple physiological systems. Because the timing and size of limb development is known to be nutritionally dependent, we tested the hypothesis that leptin is a nutritional modulator of morphogenesis by investigating its role in limb development in *Xenopus laevis*. First, we showed that administration of recombinant Xenopus leptin (rX-leptin), via both intraperitoneal (IP) and intracerebroventricular (ICV) injections accelerated developmental timing and stimulated cell proliferation, as shown by increased number of phosphorylated histone H3-ir cells in limb buds, relative to those of saline-injected tadpoles. We also used in situ hybridization and immunocytohistochemistry to map expression of leptin mRNA and protein and the long-form of the leptin receptor in developing limbs, and showed that leptin is dynamically expressed through Nieuwkoop-Faber stages 50-54 limbs. Expression of both leptin and leptin receptor was expressed throughout the early limb bud, but was then localized in the ectoderm and blood vessels during later stages. Furthermore, leptin protein was co-expressed with Xen-1 expression, a marker for neurons during development and regeneration suggesting it could be a neurosecretory factor. We also show that the rate of regeneration is nutritionally modulated in X. laevis, and administration of rX-leptin at the time of amputation via IP and ICV injections accelerates limb regeneration. We see leptin protein at the site of amputation as early as 6 hr post amputation (hpa), but as previous studies have shown leptin mRNA is not upregulated at the site until 12 hpa, likely reflecting endocrine sources of leptin. Leptin mRNA and protein are expressed in the blastema, as is leptin receptor, which becomes localized to the ectoderm much like the developing limb. These studies show for the first time that leptin signaling has pleiotropic actions on both limb development and regeneration, via paracrine, endocrine, and possibly neurosecretory sources by stimulating cell proliferation and angiogenesis in the developing limb, and by promoting wound healing and blastema formation in the regenerating limb.

116 Genetic variability in the robustness of retinoic acid signaling during early embryogenesis. *Abraham Fainsod*[1], Madhur Parihar[2], Liat Bendelac[1], Michal Gur[1], Abha Belorkar[2], Keren Kinberg[1], Rajanikanth Vadigepalli[2] 1) Developmental Biology and Cancer Research, IMRIC, Hebrew University, Jerusalem, IL; 2) Department of Pathology, Thomas Jefferson University, Philadelphia, PA.

Retinoic acid (RA), a biologically active derivative of vitamin A (retinol), signals through the nuclear receptors, RAR's and RXR's. RA is an early and important regulatory signal of numerous embryonic processes. RA biosynthesis begins with the onset of gastrulation. The dependence on sources of retinoids (retinol) or carotenoids, makes RA signaling susceptible to environmental effects as in Vitamin A Deficiency syndrome. RA levels in the embryo are tightly regulated to prevent the teratogenic effects of deviations during embryogenesis. RA biosynthesis proceeds through two sequential oxidation reactions from retinol to retinaldehyde to RA, and it is modulated by enzymes that reduce retinaldehyde back to retinol, or enzymes that modify RA into inactive forms tagged for degradation. Manipulation of embryonic RA levels has shown that inhibition or degradation at individual steps results in relatively mild developmental malformations. In contrast, combinatorial manipulation of RA levels results in severe defects, suggesting that the mild malformations of individual treatments are the result of partial compensation, i.e. robustness. To directly test the RA signaling robustness, embryos were treated with RA or inhibition of RALDH activity for 2 hours from late blastula to early gastrula and subsequently washed. Samples were collected at different time points thereafter. RA target genes, like the *Hox* genes, returned to normal expression relatively quickly, while RA metabolic enzymes took longer to go back to normal expression levels. We conclude that RA metabolism adapts to bring RA levels back to normal. In parallel, time series experiments were analyzed by RNAseq. Interestingly, soon after the end of the 2 hour treatment, relatively few RA target genes exhibited abnormal expression, further evidence of the robustness of this pathway following environmental disturbances. Computational analysis is being pursued to determine the kinetic characteristics of the robustness. Our initial results from an unbiased pattern analysis suggest that the components of the RA biosynthesis pathway constituted the majority of the gene expression changes that were induced by RA treatment and by RALDH inhibition. Furthermore, the six biological repeats, embryo clutches, exhibited different response levels and kinetics. These observations suggest that genetic polymorphisms greatly affect RA robustness. We show a model system to study the robustness of the RA signaling pathway.

117 Rusc proteins regulate muscle development via Hedgehog signaling. *J. Fu*, Z. Jin, J. Yang Comparative Biosciences, University of Illinois at Urbana-Champaign, Champaign, IL.

The Hedgehog (Hh) signaling pathway is an evolutionarily conserved pathway that regulates cell fate and differentiation in embryos. The members of the RUN and SH3 domain-containing (Rusc) family of proteins were found to physically interact with Sufu and inhibit Hh signaling. Since Hh signaling plays major roles in both somite patterning and myoblast differentiation during embryonic development, we hypothesize that Rusc proteins, as the inhibitors of Hh signaling, are involved in the regulation of muscle development. We approach this question by examining the expression pattern and the role of Rusc1 and Rusc2 both in vivo and in vitro. Here we report that maternal Rusc1 is predominantly expressed in Xenopus embryos. Loss of Rusc1 resulted in severe muscle developmental defects in Day 6 embryo, including disorganized somite and lack of hypaxial muscle, which can be rescued by Hh signaling downregulation. On the other hand, we show that the expression of Rusc2 is predominant and increases during the differentiation of C2C12 mouse myoblasts. Rusc2 knockdown by lentiviral shRNA

lead to delay in myoblast differentiation. This work thus demonstrates an essential role of Rusc proteins during vertebrate muscle development.

118 Determining Target Genes Regulated by miR-206 and miR-1 during Early Skeletal Muscle Development in *Xenopus laevis*. *J.Q. Garcia*, M. Lopez-Pazmino, J. Ramirez, C. Domingo Biology, San Francisco State University, San Francisco, CA.

MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level by interacting with mRNA targets. Muscle Specific miRNA, myomiRs, regulate skeletal muscle proliferation, differentiation and regeneration. Our laboratory recently demonstrated that knock down of miR-206 affects formation and proper differentiation of muscle in *Xenopus laevis*. Our aim is to better understand the complex signaling pathways regulated by miR-206 and miR-1, two closely related myomiRs that recognize the same binding sequences on mRNA targets. Preliminary miRNA target screens of *X. laevis* cDNA libraries identified several putative mRNA targets regulating proliferation, protein trafficking, adhesion and maintenance of muscle cell fate. However, it is unclear whether these mRNAs are true targets of miR-206/miR-1. We have adapted a fluorescent (GFP/RFP) reporter assay in *X. laevis* that measures the ability of miRNAs to downregulate the signal of GFP fused to the 3′ UTR sequences bearing putative miR-206/miR-1 sites. In our reporter system, we co-inject, at the one-cell stage, a GFP RNA bearing putative miR-206/miR-1 binding sites, and an RFP RNA to normalize the relative GFP/RFP levels. As a proof of concept, we demonstrate that a GFP fused to the 3′UTR of *pax7*, a gene expressed in muscle satellite cells and a known target of miR-206/miR-1, is downregulated by both miR-206/miR-1 duplexes. Control miRNA duplexes did not affect the fluorescent signal of GFP-pax7 3′UTR. Our bioinformatic screen identified *serum deprivation-response protein* (*sdpr*), a gene involved in cell migration and proliferation, as a miR-206/miR-1 target. We show that in the presence of either miR-206 or miR-1 duplexes the GFP-sdpr 3′UTR expression is down regulated by a one-fold difference compared to controls. Furthermore, we use qRT-PCR analysis to demonstrate that *sdpr* is up-regulated when miR-206 levels are knocked down compared to wild type and control RNA samples. These results confirmed that *sdpr* is a miR-206/miR-1 target and likely plays a role in early skeletal muscle development. Together, these results will lead to a better understanding of the myomiR signaling pathway underlying muscle development and maintenance.

119 Trim29, a E3-ligase involved in neural tube closure and neural crest specification. *Thomas Hollemann*, Astrid Vess, Jie Ding, Herbert Neuhaus Institute for Physiological Chemistry, Martin-Luther University Halle-Wittenberg, Halle, Saxony-Anhalt, DE.

Tripartite motif protein 29 (TRIM29) was identified as an AT group D-complementing gene (ATDC) due to its ability to complement the radiosensitivity defect of AT fibroblast cell lines. The TRIM protein family of RING-type E3 ligase is characterized by the presence of a tripartite motif composed of a RING domain, one or two B-box domains and a coiled-coil domain. E3 ubiquitin ligases are enzymes that function as scaffold proteins mediating the interaction between E2 ubiquitin-conjugating enzyme and the substrate. TRIM29 is an untypical member of the family since it lacks the RING domain. TRIM29 has been reported to be overexpressed in several cancers including esophageal, breast, lung, bladder, colorectal and pancreatic cancer. During Xenopus larval development, *trim29* is expressed exclusively in non-neural ectoderm including the region of which neural crest cells emerge. Suppression of *trim29* function led to severe neural tube closure defects accompanied by a loss of neural crest specification. Interestingly, those cells that are supposed to contribute to the closure of the neural tube, delaminate from the embryo and rest in the vitelline membrane as shown by live cell imaging. On the other hand, remaining cells in the injected side of *trim29* morphants are characterized by a low number of mitotic (pH3) and apoptotic cells (tunel) as well forming a hyperplastic or hypertrophic structure of yet unknown specification. Ongoing work in the lab is focused on the identification of interaction partners and substrates for Trim29 by mass spectrometry-based approaches using embryos and cell culture.

120 Examining the role of Bromodomain and Extra-Terminal motif (BET) protein function in the maintenance of pluripotency and Neural Crest formation. *P. Huber*, C. LaBonne Northwestern University, Evanston, IL.

Neural Crest Cells (NCCs) are a stem cell population unique to vertebrate embryos. NCCs reside in the ectoderm at the neural plate border, and retain their broad multi-germ layer developmental potential through neurulation. Much remains to be learned about the genetic and epigenetic mechanisms that control the retention of pluripotency in NCCs at stages when neighboring cells are undergoing lineage restriction. Here we report that the activity of epigenetic readers of the BET (Bromodomain and Extra Terminal) family is essential for both maintenance of pluripotency in naïve blastula cells, and the formation of NCCs. Blastula cells from embryos treated with pharmacological inhibitors of BET factors BRD2, BRD3, and BRD4 lose pluripotency, as evidenced by down-regulated expression of key components of the pluripotency GRN, and are unable to respond to lineage instructing cues. Similarly, treatment of embryos with these inhibitors leads to decreased expression of NC markers at neurula stages. Our data suggest that the key BET family member targeted by these inhibitors is BRD4. We show that morpholino-mediated depletion, or CRISPR/Cas9-mediated mutation, of BRD4 phenocopies the effects of the inhibitors. Interestingly, gain of function studies point to distinct activities of BRD4 and BRD2 /BRD3 in modulating NCC formation, which may provide insights into the unique roles played by these important acetylation readers. Together these findings advance our understanding of the epigenetic control of pluripotency and the formation of the vertebrate neural crest.

121 Possible involvement of immune antigen Ouroboros in disappearance of brachial sac skin during *Xenopus* metamorphosis. *I. Ishimori*, Y. Izutsu Department of Biology, Faculty of Science, Niigata University, Niigata, JP.

In anuran amphibian, hindlimbs of the tadpoles can be visible at the early developmental period in the lateral side of the trunk bodies. On the other hand, forelimbs begin to develop under the dermis of the trunk skin during metamorphosis. Then, the forelimbs are finally released through the hole in the trunk skin, which is formed by perforations with histolysis. The tissue/organs including perforating skin is named as "brachial sac". The brachial sac skin disappears when the forelimbs appeared from the hole in the skin. However, the mechanism underlining disappearance of the brachial sac skin remains unresolved. Previously, we have reported that Ouroboros (Ouro1 and Ouro2) proteins and adult T cells are involved in the process of the tail disappearance during metamorphosis (Mukaigasa *et al*.....Izutsu, 2009). In this study, to investigate whether or not Ouro proteins and T cells are involved in disappearance of the brachial sac skin during perforation, the immunohistochemical

analysis was performed. Moreover, we performed knockdown experiments of the *ouro1* genes using a transgenic tadpole F2 line. When the *ouro1* gene was knockdown upon the brachial sac skin, a part of the skin remained even after perforation. From these results, we consider that Ouro proteins play a role not only in the tail, but also in the brachial sac during metamorphic tissue disappearance.

122 GJA1 depletion causes ciliary defects and abnormal laterality. *D. Jang*, T. Park School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Korea.

Gap junction protein alpha 1 (GJA1), also known as Connexin 43 (CX43), is the most common and a major subunit of the gap junction complex. In the cytoplasm, the C-terminal domain of GJA1 protein regulates the cytoskeletal network, including actin and tubulin for cell protrusions, migration, and polarity. But the mechanisms and roles of GJA1 protein in the formation and function of cilia is yet to be determined. Cilia are a microtubule-based cellular organelle and play crucial roles in embryonic development and physiological maintenance of human body. Disruption of cilia formation and function is known to cause syndromic disorder, ciliopathy, in human. In this study, we examined knockdown phenotypes in *Xenopus* and human RPE1 cells and found that, by regulating ciliogenesis, *GJA1* gene is critical during early development. GJA1 protein is localized not only at the gap junction, but also at the epithelial ciliary axoneme of multiciliated cells in *Xenopus* embryos and the pericentriolar matrix around primary cilium of human RPE1 cells. Dominant negative mutant-mediated dysfunction of gja1 protein caused severe malformation of motile cilia formation while the basal bodies are normally formed and apically localized. Further analysis revealed that, morpholino mediated-knockdown of *gja1* disrupted normal ciliogenesis in *Xenopus* multiciliated cells and siRNA mediated-knockdown of *GJA1* decreased the number of primary cilium in human RPE1 cells. Moreover, knockdown of *gja1* in gastrocoel roof plate (GRP) showed decreased GRP cilium length, which caused reversed or disrupted left-right asymmetry in embryonic development. These finding suggest that *GJA1* is necessary for proper ciliogenesis as it affects ciliary axoneme formation and assembly.

123 The Role of DNA Methylation in the Maintenance of Pluripotency. *K. Johnson*, C. LaBonne Molecular Biosciences, Northwestern University, Evanston, IL.

Embryonic development can be characterized as a progressive restriction of cell potential, and cells that possess pluripotency do so only transiently. Understanding the genetic and epigenetic mechanisms that control maintenance pluripotency and the onset of lineage restriction is a problem of central importance. The neural crest is a fascinating cell type in which to investivate these mechanisms, as these cells retain their broad develomental potenial through neurulation before ultimately contributing a large number of features to the vertebrate body plan. Here we investigate the role of DNA methylation in the retention of blastula pluripotency and the genesis of neural crest. We find that inhibition of DNA methylation at the two cell stage leads to dramatically perturbed expression of key pluripotency factors by blastula stages. Further, inhibiting DNA methylation in naïve blastula cells interferes with the ability of these cells to transist to epidermal, mesodermal and endodermal states. These findings demonstrate a central role for DNA methylation in early lineage diversification. Treatment with methylation inhibitors during gastrulation leads to reduced expression of neural crest markers, further highlighting the similarities between these cells and naïve blastula cells. Finally, we show that upregulating the activity of the demethylase enzyme, tet3, leads to patterning defects at the neural plate border. Together, these findings provide novel insights into the epigentic control of pluripotency and the genesis of the nerual crest.

124 Islet-1 expressing cells contribute to the creation of new cardiomyocytes at the initial stage of heart regeneration. *M. Kanagawa*, S. Umezawa, T. Kinoshita Department of Life Science, College of Science, Rikkyo University, Tokyo, JP.

Cell proliferation of cardiomyocytes leads to mammalian heart growth prior to birth. Soon after birth, the cardiomyocytes exit the cell cycle and further increase in myocardial volume occurs only as a result of cardiomyocyte hypertrophy. As cardiomyocytes show no proliferation in adult mammals, regeneration of adult mammalian heart cells is thought not to occur. In contrast, our previous research revealed that heart regeneration can occur in adult *Xenopus laevis*, although the mechanism was unclear. Among the molecular markers for cardiac stem cells, Islet-1 has been used to identify cardiac progenitor cells. In mammalian adult hearts, Islet-1 expressing (Islet-1+) cells have been reported to be quiescent and non-proliferative. However, our previous results showed that Islet-1+ cells are proliferative in *Xenopus* adult hearts, suggesting that Islet-1 may play an important role in the regeneration process (18th Int. Cong. DB). In this research, we aimed to clarify the role of Islet-1+ cells in *Xenopus* heart regeneration. A histological analysis showed that a blood clot appeared in the resected surface of the myocardium 1 day post amputation (dpa). The blood clot was gradually absorbed during 1 month after amputation, and the resected myocardial margin was covered with tropomyosin positive cells from 1 month after amputation. RT-PCR analysis of the gene expression showed strong transient gene activation of Islet-1 in the resected myocardium at 1 dpa. In addition, immunohistochemical analysis showed that Islet-1+ cells began to accumulate in the resected margin of myocardium after 1 dpa. Co-staining with Islet-1 and BrdU antibodies showed that a significant number of Islet-1+ cells entered into the cell division cycle at 3 dpa. Immunohistochemical analysis also revealed that the expression level of tropomyosin in cardiomyocytes near the resected surface varied considerably, suggesting the degeneration of tropomyosin in some cardiomyosytes. These results suggest the possibility of cardiomyocyte dedifferentiation to Islet-1+ cells, which proliferate leading to regeneration of the *Xenopus* heart. Together these results suggest that Islet-1+ cells contribute to the creation of cardiomyocytes at the initial stage of heart regeneration.

125 *Xenopus* Tmem150b is required for neural tube formation by regulating nodal signaling. *B. Keum*[1], I. Yeo[1], H. Lee[1,2], J.K. Han[1] 1) Department of Life Sciences, Pohang University of Science and Technology, Pohang, KR; 2) Current address: Division of Molecular Embryology, DKFZ-ZMBH Alliance, 69120 Heidelberg, GER.

The notochord is a mesoderm-derived tissue known to produce signals, such as Nodal and Sonic Hedgehog (SHH), for proper neural tube closure. It is also known that nodal signaling is essential for notochord formation. However, the basis of the fine molecular mechanism explaining notochord function for neural tube formation remains to be elucidated. Here, we found a novel gene, *Xenopus* Transmembrane protein 150b (Tmem150b), which is directly induced by nodal signaling, and is specifically expressed in the chordamesoderm and notochord. Knockdown of Tmem150b in *Xenopus* results in neural tube closure-defective phenotypes, and interestingly, these defective phenotypes may

be caused by the disrupted expression of SHH at the ventral neural tube. Moreover, both knockdown and overexpression of Tmem150b affect some nodal target genes, such as chordin, goosecoid, and mixer, suggesting that Tmem150b may function as a novel membrane factor for nodal signaling pathway. Considering a putative regulatory domain of Tmem150b for GPI-anchored protein, Tmem150b possibly functions with membrane factors of nodal signaling, such as EGF-CFC factors. Remarkably, Tmem150b turns out to interact with XCR2, one of the *Xenopus* EGF-CFC factors, which shows a similar expression pattern to Tmem150b. These key findings serve as a foundation for the discovery of a novel factor of nodal signaling pathway important for neural tube formation, and to identify its underlying molecular mechanisms.

126 Identifying the role of T-type Calcium Channels during neural tube closure. *S.M. Khairallah*[1], P. Salas [1], E. Mis [2], W.C. Smith [1] 1) Molecular, Cellular, and Developmental Biology, University of California Santa Barbara, Santa Barbara, California, USA; 2) Pediatric Genomics Discovery Program, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut, USA.

Neural tube morphogenesis is a complex process that involves the elevation, folding, and fusion of the neural tube along the dorsal midline. Defects in neural tube closure are among the most common human birth defects, present in as many as 1 in every 1000 births. Our lab previously identified a developmental mutant in the primitive chordate *Ciona*, *bugeye*, which contains a mutation in the promoter region of a T-type Ca^{2+} Channel gene, *cav3*. This mutation leads to fusion failure of the anterior neural tube and results in open anterior neural tube. We found that T-type Ca^{2+} are required for proper gene regulation during neural tube closure as a repulsion protein, Ephrin-Ad, was found to be overexpressed in the *bugeye* mutant. Furthermore, overexpression of Ephrin-Ad was sufficient to cause the open brain phenotype in wildtype embryos. Moreover, a requirement for T-type Ca^{2+} channels appears to be conserved among chordates, as we have found that morpholino knockdown and Crispr of *cav3* orthologs in *Xenopus* (cav3.2 and 3.3). Although, the neural tube closure defects observed in *Xenopus* are much more extensive than in *Ciona*, and include a delay in neural tube closure, loss of anterior bottle cells, craniofacial defects, and anterior-to-posterior open neural tubes. Interestingly these phenotypes are similar to neural tube defects seen with disruption of the PCP and actomyosin pathways, including knockdown of Calpain2 and Myosin IIB. Currently, we are investigating the hypothesis that Cav3.3 is downstream of the PCP pathway to maintain and regulate the folding and fusion of the neural folds and that knockdown of Cav3.3 leads to the misregulation of the PCP pathway, thus leading to neural tube closure defects.

127 Peroxiredoxin5 controls vertebrate ciliogenesis via modulating mitochondrial reactive oxygen species. *Chowon Kim*, Youni Kim, Hyun-Shik Lee School of lifescience, BK21 Plus KNU CreativeBioResearch Group, College of Natural Sciences, Kyungpook National University, Daegu,.

Prdx5 is widely studied for its antioxidant properties and its protective roles in neurological and cardiovascular disorders are reported previously. But the information about the developmental roles of Prdx5 is lacking and in-depth analysis is required to establish the functional significance of Prdx5 during vertebrate development. In this study, we investigated the biological functions of Prdx5 on mitochondria and ciliogenesis in developing embryos. We have found that the several of Prdx genes are strongly expressed in the multiciliated cells in developing Xenopus embryos and its peroxidatic function is crucial for normal cilia development. The depletion of Prdx5 increased the level of cellular ROS that consequently led to the mitochondrial dysfunctions. Our proteomic and transcriptomic approaches revealed that excessive ROS upon Prdx5 depletion subsequently reduced levels of pyruvate kinase (PK), a key metabolic enzyme in energy production. We confirmed that the promotor activity of PK was significantly reduced upon Prdx5 depletion. Furthermore, the reduction of PK in turn led to ciliary defects observed in Prdx5 depleted cells. Our data revealed the relationship between excessive ROS level as a result of Prdx5 depletion and ciliogenesis suggesting cilia as another potent target of ROS.These finding indicates that Prdx5 protects mitochondria from the excessive ROS, which is in turn critical for normal ciliogenesis.

128 A Toll-like receptor signaling cascade activates Nedd4l to reign in Nodal signaling during vertebrate gastrulation. *Hyungseok Kim*, Isabelle Cooperstein, Jan Christian Neurobiology and Anatomy, University of Utah, Salt Lake City, UT.

During gastrulation, nodal ligands induce mesoderm and specify anterior fates. We identified Tril (Toll-like receptor 4 interactor with leucine-rich repeats) as a novel transmembrane protein that is required to dampen nodal signaling during *Xenopus* gastrulation. When expression of Tril is knocked down, nodal signaling is enhanced and this leads to defects in gastrulation and head development. Tril was originally identified as a co-receptor for Toll-like receptor3 (Tlr3) and Tlr4, which activate a signaling cascade leading to NF-kB dependent induction of cytokines during innate immunity. However, Tlrs are generally believed to be dispensable for early vertebrate development. Thus, how Tril signals are transduced from the membrane to dampen responses downstream of nodal pathway activation was a mystery. Using an in vivo reporter assay, we showed that NF-kB activity is unchanged in embryos in which expression of Tril is reduced. We then used a yeast two hybrid screen to identify the ubiquitin ligase Pellino2 as a protein that binds to the cytoplasmic tail of Tril. Pellino2 is known to function downstream of Tlrs, raising the possibility that Tril activates an NF-kB independent Tlr signaling cascade to dampen nodal signaling. Consistent with this, we show that nodal activity is elevated in embryos expressing a dominant mutant form of Tlr3, Tlr4 or the downstream kinase, IRAK1, which is known to phosphorylate, and activate pellino2. Finally, we show that pellino2 binds to, stabilizes and activates the ubiquitin ligase Nedd4l. Nedd4l normally dampens the ability of nodal to activate downstream signaling by targeting nodal receptorss for degradation, and also terminates signaling by targeting phosphorylated Smad2 for degradation. Thus, our current data suggest that Tril functions as a Tlr3/4 co-receptor during early development to activate a signaling cascade culminating in activation of Nedd4l, which negative regulates several components of the nodal signal transduction pathway. Tlrs are known to play non-immune roles in developmental patterning and morphogenesis in insects, and our studies provide evidence that Tlr-dependent signaling is also required for embryonic patterning in vertebrates.

129 Loss-of-function and rescue analyses revealed that the immune T cells are necessary for tail tissue degeneration of *Xenopus* via Ouro proteins. *H. Kobayashi*, Y. Izutsu Department of Biology, Faculty of Science, Niigata University, Niigata, JP.

We have previously reported two novel keratin-related proteins, Ouro1 and Ouro2, function in the process of tail regression in *Xenopus* metamorphosis. (Mukaigasa *et al*.....Izutsu, 2009). These proteins have been isolated as candidates for the target antigens against *Xenopus* immune T cells. In this study, we investigated whether or not the effector *Xenopus* T cells are necessary for the tail regression via Ouro proteins. To remove the T cells from the pre-metamorphic tadpoles, a mouse monoclonal antibody against *Xenopus* T cells, XT-1 was injected in peritoneal cavity. We used transgenic and J strain hybrid F5 lines, carrying an expression plasmid for *ouro1* and *ouro2* genes under the *Xenopus* heat shock promoter. After treatment with the monoclonal antibody, degeneration of Ouro-overexpressing tails was significantly inhibited. The rescue experiment was performed by transfer of T cells obtained from the J strain frogs. It was succeeded in providing degenerated tails in those T cell-transferred tadpoles. From these results, it was suggested that the T cells are necessary for regression of Ouro-expressing tails at metamorphosis.

130 Critical roles of lysine demethylase Kdm3a in craniofacial and neural development during *Xenopus* embryogenesis. *Hyun-Kyung Lee*, Na-Young Lee, Hyun-Shik Lee School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, College of Natural Sciences, Kyungpook National University, Daegu, South Korea.

Kdm3a is a histone demethylase that is specific for mono- and dimethyl H3K9 residue via dioxygenase reaction. Kdm3a is also known as jhdm2a, jmjd1a and testis-specific gene A (Tsga), which has jmjc-domain that mediates catalytic activity by binding to α-ketoglutarate and LXXLL motif, a nuclear hormone receptor interacting sequence. It has been reported that Kdm3a is critical for spermatogenesis and metabolism by regulating expression of genes that are important in sperm nuclear condensation as well as in fat metabolic activities. Kdm3a is also involved in various cellular processes such as sex determination and androgen receptor-mediated transcription as well as differentiation of embryonic carcinoma cells. Therefore, we evaluated the developmental role of kdm3a in Xenopus laevis. Our results show that Kdm3a is expressed during early embryonic stages ranging from egg to tadpole, but high expression was observed at neurula stage. Kdm3a mRNA expression was also observed in neural plate and retina at neurula stage, whereas in anterior region including forebrain and branchial arches at tadpole stage. Knockdown of Kdm3a using anti-sense morpholino leads to defective craniofacial formation. Whole mount in situ hybridization with neural marker and neural crest markers confirmed that Kdm3a is involved in neurogenesis and neural crest development. Based on these results, it might be possible that histone demethylase Kdm3a regulates craniofacial and neural development through modulating transcriptional activities of neural specific genes.

131 The role of macrophages in *Xenopus* tadpole tail regeneration. *L. Loh*, A. Wills University of Washington, Seattle, WA.

Macrophages play a critical role in the balance between scarring and regeneration. When macrophages are removed from regenerative species such as zebrafish, axolotls, or spiny mice, they lose their regenerative capacity. More detailed comparisons of the innate immune response in regenerative animals and their non-regenerative counterparts have revealed crucial differences in the acute response of macrophages to injury. In non-regenerative species the majority of the macrophages recruited to the wound are pro-inflammatory macrophages that act to fight pathogens by secreting proteases and pro-inflammatory mediators. Comparatively, regenerative species show higher levels of IL-4 and IL-13 activated macrophages that act to suppress the immune response and initiate tissue growth. Despite the emerging significance of the innate immune system in regenerative versus non-regenerative healing, there are multiple unknowns about how macrophages are affecting regeneration. The role of macrophages in *Xenopus* regeneration has not yet been described, but this is an excellent model system for this research area. *Xenopus* can regenerate their limb buds and tails as tadpoles but lose this ability as adults, which allows for within-species controls between regenerative and non-regenerative stages. To date, we have established a reliable macrophage depletion protocol in *Xenopus* tadpoles and shown that *Xenopus* tadpoles require macrophages for proper tail regeneration. Now we are exploring how macropahge depletion affects gene expression in signaling pathways such as wnt, bmp and fgf that are known to be critical for proper regeneration following tail amputation. This research may help clarify why *Xenopus* are strong but stage-specific regenerators and allow us to better understand the role macrophages play in regeneration.

132 Mitochondrial energetics define cardiac patterning and early signaling in vertebrate development. *A. MacColl Garfinkel*, E. Mis, M. Khokha Genetics & Pediatrics, Yale University, New Haven.

Congenital heart disease is the leading cause of birth defects and infant mortality in the United States, affecting 9 of every 1000 children born each year. One especially severe form of CHD is heterotaxy, or the rearrangement of internal organs along the left-right axis. Through recent genetic analysis of affected patients, novel candidate genes for CHD have been identified and analysis of their underlying mechanisms is ongoing. One such gene is LRPPRC, which encodes a mitochondrially-localized mRNA binding protein that regulates mitochondrial gene expression and OXPHOS, critical for aerobic metabolism. LRPPRC, although implicated in human developmental disease, has not yet been studied in a developmental context. The molecular mechanism of LRPPRC's involvement in CHD and the specific tempero-spatial requirements of aerobic metabolism in organ patterning are unknown. Through the use of Xenopus troplicalis, a powerful genetic model for human cardiac disease, and the use of modern gene editing tools such as CRISPR/Cas9 we have begun to elucidate the role of LRPPRC in embryonic patterning. We have identified a potential role for LRPPRC and aerobic metabolism in the regulation of Wnt signaling during gastrulation, required for proper patterning of the left-right axis and organ morphogenesis. This work was supported by NIH training grant 5T32GM007223-42 to AMG.

133 How miRNAs and bicC contribute to the dand5 regulation in the GRP during left-right determination in Xenopus laevis. *M. Maerker*[1], M. Getwan[2], A. Schweickert[1], M. Blum[1] 1) Institute of Zoology, University of Hohenheim, Stuttgart, DE; 2) Centre for Clinical Research (ZKF), Freiburg, DE.

While vertebrates are from the outside bilaterally symmetric in regards to the left-right-axis, they show a widespread asymmetric organisation of the inner organs. A disturbance of this arrangement can result in illness or even lethality during embryogenesis. Responsible for the correct breakage of asymmetry is a left-right organizer. This transient cilliary structure produces a fluid flow, which results in the asymmetric nodal signalling cascade. In Xenopus laevis (X. laevis) this structure is called gastrocoel roof plate (GRP) and consists of the notochordal flow producing cells and the somitic sensing cells. In order to release nodal on the left side of the GRP, the nodal repressor dand5 has to be degraded in the somitic cells, which is triggered by the fluid flow.

The downregulation of dicer, a miRNA processing endonuclease, via antisense oligo nucleotids (morpholinos), results in a partial inability of the fluid flow to degrade dand5 mRNA on the left side. This results in the absence of pitx2, an asymmetric morphogenic marker which is necessary for correct organogenesis. Loss of function experiments of bicaudal C (bicC), a RNA binding protein, also leads to absence of pitx2 later on. However, bicC seems to regulate the translational level of dand5 regulation rather than the degradation of dand5 itself. Epistasis experiments with these two regulatory mechanisms showed that both mechanisms are necessary for the regulation of dand5. An overexpression of bicC deletion constructs showed that the protein as well as the RNA binding domains are necessary for the correct regulation of dand5. These experiments were all validated by the use of a dand5 3'UTR reporter, which showed the translational site of the dand5 regulation.

The results show that dand5 is regulated by miRNAs and bicC alike after the flow event in X. laevis.

134 Regulatory mechanisms of collective cell migration in early development of *Xenopus laevis*. *R. R. Malinda*, N. Ueno Division of Morphogenesis, National Institute for Basic Biology, Okazaki, Aichi, JP.

During early development of vertebrates, the cellular migration is one of the most important cell behaviors that ensures proper positioning of tissues and organs. It is a fundamental but rather complex process, and therefore thought to be achieved by a highly coordinated and cooperative manner, often called collective cell migration. Furthermore, collective cell migration, partly depends on mechanical properties generated during collective movement of cells which are coordinating with extracellular matrix in surrounding. In *Xenopus laevis*, gastrulation in early development is well studied event for understanding this phenomenon where cells massively migrate towards the anterior side of embryos spreading a mesodermal sheet on the inner side of embryo. However, underlying regulatory mechanisms of the coordinated cell movement are still not clear.

To understand these highly complex cellular dynamics, we approach to study directional migration of the leading edge of mesodermal cells (LEM) by the Traction Force Microscopy (TFM). To analyze the traction force in LEM migration, we are currently using *ex vivo* assay in which ectodermal explants expressing SDF1α is placed as the source of chemoattractant, and LEM explants expressing membrane-GFP or Histone H2B-GFP to visualize cell shapes and nuclei, respectively, are allowed to migrate towards the SDF1α explants in a directed manner. We confirmed that recapturing the gastrulation, LEM tissues migrate towards SDF1α on fibronectin coated gel in which small fluorescent beads are embedded, which thereafter, be used to quantify the generated traction force by the explants.

We observed that GFP labeled LEM cells directionally migrate to the end of SDF1α serving as the chemoattractant in our experimental design. Furthermore, measurement of traction force was analyzed by the Particle Image Velocimetry (PIV) for the LEM tissue during migration. However, current method is still challenging to quantify results in all other related aspects, therefore we are working to optimize this explant assay by introducing microfluidic chamber for LEM migration in a more efficient way, where high throughput results are expected. Along with, cadherin family plays an important role in cell-cell junctions where it interfaces with the coordinated behavior of this collective migration and the mechanical properties of the cells, and we aim to show the importance of cadherins during the collective movement in LEM migration. We are currently investigating the mechanisms regulating the collective behavior of LEM in *Xenopus* focusing on the spatiotemporal change of force filed, change in the relative position of the cells, formation of cell protrusion, etc. within the migrating tissue.

135 *EMC1* a Candidate Gene for a Diverse Array of Congenital Diseases is Important for Neural Crest Cell Development. *J. Marquez*, J. Criscione, E. Mis, M. Khokha Pediatric Genomics Discovery Program, Department of Pediatrics and Genetics, Yale University School of Medicine, New Haven, CT.

The endoplasmic reticulum (ER) membrane protein complex (EMC) is a conglomerate of 10 subunits in *Homo sapiens* (EMC1-10). This complex is essential for the synthesis, folding, and localization of multi-pass transmembrane proteins. Genetic studies have provided evidence for the role of mutations in *EMC1* in a variety of childhood diseases. Investigating mechanisms of dysfunction will help us understand disease in the context of *EMC1* mutations. *Xenopus tropicalis* provides a model that has allowed us to observe the severity of Emc1 loss of function in the neural crest cell (NCC) lineage and has provided insight into how mutations in *EMC1* may contribute to disease. To investigate developmental phenotypes in an Emc1 loss of function model, we used morpholino oligos (MOs) to deplete Emc1 in whole embryos by injecting at the one cell stage or in one-half of the embryo by injecting one cell at the two-cell stage to maintain the contralateral side as an internal control. This loss of function model results in various developmental anomalies. Pigment cells, cardiac outflow tracts, and craniofacial cartilage display morphological abnormalities. Label free quantitative mass spectrometry indicated that proteins involved in signaling pathways important for neural cret cells (NCCs) were particularly affected by knockdown of Emc1. Given the importance of NCCs in the development of tissues involved in the observed phenotypes, we investigated this population of cells. Markers of neural crest specification and migration are abnormally distributed or lost in the injected half of embryos. Given the importance of the EMC for multi-pass transmembrane proteins, we assessed possible connections between this function and NCC development. A prominent class of multi-pass transmembrane proteins important for NCC development is the Frizzled proteins that serve as part of the WNT signaling pathway. WNT signaling is one of the drivers of the NCC GRN. Indeed, Fzd7 decreases over time in Emc1 depleted embryos. These findings point to a potential mechanism for the dysfunction seen in NCC derived tissues of patients with mutations in *EMC1*.

136 Neural crest induction by Wnt signalling requires fhl3. M. Alkobtawi[1,2], P. Pla[1,2], *A.H. Monsoro Burq*[1,2,3] 1) Univ. Paris Sud, Université Paris Saclay, CNRS UMR 3347, INSERM U1021, F-91405, Orsay, ; 2) Institut Curie, CNRS UMR 3347, INSERM U1021, F-91405, Orsay, France; 3) Institut Universitaire de France, 75005 Paris.

A complex interplay of signalling pathways and transcription factors activates the Neural Crest (NC) gene regulatory network (GRN) that drives NC induction in the neural border territory, followed by epithelial to mesenchymal transition, migration and differentiation into multiple derivatives. This network is enriched in factors altered in numerous human congenital disorders named neurocristopathies among which, many affect cranio-facial development.

Using X. laevis frog embryos, we show that *fhl3,* a gene encoding a four-and-a-half LIM domain protein, is expressed in the early paraxial mesoderm and in NC cells, in addition to its known expression during muscle development. *Fhl3* morphant embryos fail to activate the neural border and neural crest specifiers and show defects in NC induction and migration. This results into severe cranio-facial malformations at tadpole stages. We demonstrate that *fhl3* is necessary in the paraxial and intermediate mesoderm for their neural crest-inducing activity in the ectoderm, and that efficient Wnt signalling from the mesoderm may depend upon *fhl3* in this context. Altogether, our results introduce Fhl3 as a novel and essential player in the early steps of activation of the NC GRN.

137 Embryonic regeneration by relocalization of the Spemann organizer during twinning in Xenopus. *Y. Moriyama*[1], A. Fukui[1], E De robertis[2,3] 1) Faculty of Science and Engineering , Chuo University, Tokyo, JAPAN; 2) Howard Hughes Medical Institute, University of California, Los Angeles, CA; 3) Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, CA.

The formation of identical twins from a single egg has fascinated developmental biologists for a very long time. Previous work had shown that Xenopus blastulae bisected along the dorsal–ventral (D-V) midline (i.e., the sagittal plane) could generate twins but at very low frequencies. Here, we have improved this method by using an eyelash knife and changing saline solutions, reaching frequencies of twinning of 50% or more. This allowed mechanistic analysis of the twinning process. We unexpectedly observed that the epidermis of the resulting twins was asymmetrically pigmented at the tailbud stage of regenerating tadpoles. This pigment was entirely of maternal (oocyte) origin. Bisecting the embryo generated a large wound, which closed from all directions within 60 minutes, bringing cells normally fated to become Spemann organizer in direct contact with predicted ventral-most cells. Lineage-tracing analyses at the four-cell stage showed that in regenerating embryos midline tissues originated from the dorsal half, while the epidermis was entirely of ventral origin. Labeling of D-V segments at the 16-cell stage showed that the more pigmented epidermis originated from the ventral-most cells, while the less-pigmented epidermis arose from the adjoining ventral segment. This suggested a displacement of the organizer by 90°. Studies with the marker Chordin and phospho-Smad1/5/8 showed that in half embryos a new D-V gradient is intercalated at the site of the missing half. The displacement of self-organizing morphogen gradients uncovered here may help us understand not only twin formation in amphibians, but also rare cases of polyembryony.

138 The role of AP-1 family genes in the caudal stem zone and tissue regeneration in *Xenopus tropicalis*. *M. Nakamura*[1], H. Yoshida[2], M. Horb[2], K. Takebayashi-Suzuki[1], A. Suzuki[1] 1) Amphibian Research Center, Graduate School of Science, Hiroshima University, JP; 2) National Xenopus Resource, Woods Hole, USA.

The caudal stem zone is essential for the formation of neural tissues and somites, and is regulated by morphogen signals such as BMP, Wnt and FGF. Recent studies have shown that neuromesodermal progenitors (NMps), which comprise the progenitor pool of mesodermal and neuronal cells, are important for the formation of caudal structures; however, the molecular mechanisms that integrate morphogen signals in NMps remain unclear. Previously, we showed that *junb*, an AP-1 family gene, might be involved in tail formation by regulating multiple morphogen signals. Here, we assessed whether JunB is required for the maintenance and formation of NMps. We found that morpholino-mediated knock-down of *junb* delays tail elongation. In addition, AP-1 inhibitor treatment resulted in a short tail phenotype. Inhibition of JunB suppressed the expression of *brachyury* and *wnt8*, which are involved in NMps maintenance and formation during tailbud stages. Thus, JunB is important for mesodermal determination during tail elongation. The *Xenopus* tadpole tail, which contains spinal cord, muscle, and notochord, can regenerate after amputation via the regenerating bud, which forms at the amputation site and contains the progenitor pool for axial structures. Because JunB is involved in tail elongation, we analyzed the expression of *junb* during tail regeneration. *junb* was expressed from early to late phases of tail regeneration. Moreover, knock-down and knock-out of *junb* delayed regeneration. Interestingly, the expression of genes involved in the synthesis of reactive oxygen species was upregulated in the regenerating bud of *junb* morphants. Collectively, these results suggest that JunB is likely to be important for both tail elongation and regeneration.

139 Assessment of maternal transcript sub-localization within the animal-vegetal pole of the *Xenopus laevis* egg. *R. Naraine*[1,2], P. Abaffy[1,2], O. Smolik[1,2], R. Sindelka[1] 1) Laboratory of Gene Expression, Institute of Biotechnology, Czech Republic; 2) Faculty of Science, Charles University, Czech Republic.

Xenopus laevis has been one of the fundamental tetrapod model organism used to study early development. The large size of its oocyte and its easily identifiable animal and vegetal axis have made it suitable for studies aimed at detecting asymmetrical maternal transcripts and their role during embryogenesis. Many previous researches involving the elucidation of the maternal transcripts associated with the animal and vegetal poles within this model organism have been limited to low throughput analyses utilizing either qPCR or microarray analysis. More recent techniques like RNASeq, have been used successfully to produce high throughput data, but they have still been limited for the most part to a bilateral partitioning of the animal vegetal axis and therefore have not assessed the possibility of transcript sub-localization.

In this research, Tomo-seq is utilized on the egg of *Xenopus laevis*, to create multiple (five) sections of the egg, separating it along the animal-vegetal axis. The maternal transcripts from each section were then extracted, ribo-depleted and then sequenced using Illumina HiSeq instrument. The sequences were then aligned to the reference genome and the proportion of genes within each section assessed using DESeq2. Overabundant genes within a given section were then assessed for the enrichment of short conserved sequences that may serve as localization motifs.

Four different sub-localization categories were detected within the egg of *X. laevis* and were denoted as extremely animal, animal, vegetal and

extremely vegetal. No significantly enriched motifs were detected within the *X. laevis* animally enriched genes while eight major motifs were identified within the UTR region of the genes enriched within the *X. laevis* vegetal pole. It appears that there is a substantial level of maternal transcript asymmetry within the egg with several preferential sub-localization patterns observed. The partitioning to the animal pole is still unclear since no potential localization motifs were identified, but it is possible that the mechanism may be encoded within the structural component of the RNA. Our future aim is to assess more model organisms to quantify the similarity of transcript distribution and motif conservation.

140 Wip1 functions as a Smad4 phosphatase to terminate TGF-β signaling pathways. *D-S Park*, S-C Choi Department of Biomedical Sciences, University of Ulsan College of Medicine, Pungnap-Dong, Songpa-Gu, Seoul, Republic of Korea.

Members of Smad protein family function as key mediators of TGF-β signaling to control a broad range of cellular responses in metazoan, including cell proliferation, differentiation, and embryonic development. While the phosphorylation of Smads is necessary to both convey the TGF-β signals initiated at the cell surface to nucleus and activate transcriptional responses, it also plays roles in impairing the function of these proteins. The dephosphorylation of Smads functions to terminate TGF-β signaling, which leads to recycling of these mediators for subsequent signaling, and also to attenuate their turnovers. Therefore, the phosphorylation and dephosphorylation cycle of the Smad proteins is crucial for controlling the strength and persistence of the TGF-β signals. Key kinases responsible for the phosphorylation of Smads have been identified, but specific phosphatases involved in the dephosphorylation of these proteins remain largely unknown. In this study, we demonstrate that Wip1 acts as a Smad4 phosphatase to regulate negatively TGF-β signaling pathways. Wip1, encoded by the *PPM1D* gene, is a member of the PP2C family of serine/threonine phosphatases. The gain-of-function of *Wip1* repressed gene expression induced by Activin/Nodal or BMP signals, which required its phosphatase activity. In contrast, knockdown of *Wip1* enhanced the ability of Activin/Nodal signals to induce the expression of target genes and interfered with neural induction by a BMP antagonist, Noggin. Consistently, epidermal differentiation was promoted at the expense of neural development in *Wip1*-depleted ectodermal cells. In addition, Wip1 was found to associate with Smad4 in a signal-dependent manner. We also found that Wip1 dephosphorylates Smad4 in the linker region at Thr277, thereby affecting its nuclear retention and/or export. Thus, Wip1 suppression of expression of mesodermal markers could be rescued by co-injection of wild-type Smad4 but not by a Smad4-T277A mutant. Taking together, we suggest that Wip1 functions to regulate the strength and/or duration of TGF-β signaling through dephosphorylation of Smad4.

141 Novel Cellular Compartments Transiently Utilized Prior to the Refractory Period of *Xenopus* Regeneration. *J.H. Patel*, H.E. Arbach, A.E. Wills Department of Biochemistry, University of Washington, Seattle, WA.

Many vertebrates lack the ability to effectively regenerate tissues in response to wounding, though embryonic tissues retain some degree of regenerative capacity. *Xenopus* tadpoles are capable of full, scarless tail regeneration, but gradually lose regenerative competency through metamorphosis. During a brief stage of development, known as the refractory period, tadpoles temporarily lose regenerative competency. The refractory period is also concurrent with changes in nutrient acquisition, from maternally deposited yolk to feeding, suggesting that metabolic shifts could be responsible for the loss of regenerative capacity; however, the link between metabolism and the refractory period is poorly understood. Here, we utilize the regenerating fin margin epithelia to probe this relationship by characterizing microtubule-associated cellular compartments identified in the developing tail fin. Microtubules appear to surround these compartments and disruption of tubulin polymerization via nocodazole leads to significant changes in the size of these structures, suggesting an important role for these cytoskeletal elements in regulation of these compartments. Further, these compartments rapidly decline in abundance following tail amputation. We propose that they may be nutrient storage compartments which are utilized to facilitate the increased resource demand during regeneration. Indeed, preliminary evidence suggests a role in glycogen storage for these compartments. Further studies into the composition and mechanism of utilization for these compartments will enable us to probe the metabolic requirements for regeneration.

142 Role of calcium activity during early neural development. *Sudip Paudel*[1], Atiqur Rahman[1], Eileen Ablondi[2], Morgan Sehdev[2], Peter Kemper[1], Margaret Saha[1] 1) College of William and Mary, Williamsburgs, VA; 2) Harvard Medical School, Harvard University.

Calcium signaling regulates a wide array of cellular processes, including cell division, apoptosis, cell motility, and neural induction. Previous literature has demonstrated the importance of calcium activity during various stages of embryonic development including gastrulation and neurulation. However, relatively little is known about spatiotemporal calcium dynamics, at a single cell level, *in vivo* during early neural development. We used calcium imaging and a post-imaging cellular-phenotype identification approach to understand the pattern, molecular-cellular basis, and regularity of this activity both *in vitro* and *in vivo* during early neural development in *Xenopus laevis*. Using an *in vitro* approach, we have observed that the differentiated (but prior to synapse formation) neurons that express neural beta tubulin (NBT), in comparison with other cell types present in dissected neural plate, exhibited more predictable and persistent calcium dynamics. In our *in vivo* approach, we used a genetically encoded calcium marker (GCaMP) to monitor calcium activity of neural plate of whole mount embryos. We provide a quantitative description of spatiotemporal dynamics of calcium activity at a single cell resolution during neurulation. Similar to our *in vitro* results, our preliminary analysis suggest that cells correlating with NBT expression, compared to other cell types in the neural plate, exhibit more predictable and persistent calcium activity, which is characterized by more number of high amplitude spikes that span longer duration. These findings provide more detailed insights into the roles of spontaneous calcium activity during early neural development.

143 Comparison of CRISPRi and morpholino effectiveness in the *Xenopus* endoderm. *G. Payne*[1], M. Wlizla[1,2], M. Horb[1,2] 1) Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA; 2) National Xenopus Resource (NXR) Center, Marine Biological Laboratory, Woods Hole, MA.

The introduction of antisense morpholine oligonucleotides (MOs) nearly two decades ago has allowed for important advances in the study of embryonic development. However, in recent years, the issue of nonspecific effects of MOs has arisen and thus, so has the call for a potential alternative method of creating F0 knockdowns. One such alternative is CRISPR interference (CRISPRi). CRISPRi utilizes a catalytically inactive

dead Cas9 (dCas9) which lacks the ability to cleave DNA. Instead, dCas9, without alteration to the genomic DNA, binds to the target site and functions as a steric block to transcription, preventing either transcription initiation or elongation depending on the location of the target sgRNA. In this poster, the effectiveness of CRISPRi and morpholinos will be compared by employing both methods in the knockdown of the gene delta-like 4 (dll4), part of the Neurog3 gene regulatory network in the *Xenopus* endoderm. As the efficacy of CRISPRi has not yet been well tested in *Xenopus*, this study will explore whether CRISPRi could be useful alternative to MOs.

144 The role of neural specific Src splice variants in neuronal specification and differentiation. *A.R. Pizzey*, G.J.O. Evans, H.V. Isaacs University of York, York, GB.

C-Src is a ubiquitously expressed non-receptor tyrosine kinase with roles in cell growth, adhesion and differentiation. An alternative spliced form of C-Src - N1-Src - is highly conserved in vertebrates. In Xenopus, N1-Src contains a 5 amino acid insert within the Src SH3 domain. Another Src splice variant, N2-Src has been identified exclusively in mammals. High expression levels of N-Srcs are associated with a favourable prognosis in neuroblastoma, a rare childhood cancer, leading to spontaneous differentiation and resolution of the cancer. Both N1- and N2-Src have been found to be enriched in neural tissue during mouse development. We have shown that in Xenopus development, N1-Src expression is seen in neural stages, confined to the neural plate. The aim of my project is to understand the role that N1-Src plays in regulating neuronal specification and differentiation in the developing Xenopus nervous system. Specifically, I am focusing on how N1-Src activates a transcriptional programme in early neural development. We have shown N1-Src drives differentiation in mammalian cell culture and is essential for neuronal differentiation in the Xenopus model system. N1-Src microexon splice blocking antisense morpholinos mediate N1-Src knockdown, inhibiting neuronal differentiation in the developing embryo. In situ hybridisation and RT-PCR analysis on N1-Src knockdown embryos shows that knockdown has no effect on expression of neural prepattern genes, such as sox2. However, loss of N1-src expression leads to decreases in expression of neuroD and Myt1, along with regional specificity markers Nkx6.1, Gsx2 and Msx1. I am currently investigating the signalling pathways that are regulated by N1-Src. Overexpression work shows that N1-Src is able to upregulate MAP Kinase phosphorylation. I am also using RNAseq to investigate effects of N1-Src knock down on the transcriptome.

145 Comparative Analysis of the Response to Notch Signaling Perturbation across *Xenopus* Species during Embryonic Development. *Mark Pownall*, Andrew Halleran, Ronald Cutler, Caroline Golino, Margaret Saha Biology, College of William and Mary, Williamsburg, VA.

Embryonic development is a dynamic and responsive process in which developing organisms are sometimes able to compensate for genetic perturbations. We have demonstrated that *X. laevis* demonstrate a compensatory response to Notch signaling perturbation through analysis of marker gene expression. Subsequent RNA-seq of Notch perturbed *X. laevis* embryos indicated that homeologs respond differentially following Notch pathway hyperactivation or impediment. The observed changes in homeolog expression raised a major question: how is homeolog expression regulated? To address this question, we have compared the transcriptional response to Notch perturbation in *X. laevis* with that of *X. borealis*, an allotetraploid species that is closely related to *X. laevis*. Based on marker gene expression and qualitative analysis of morphology, *X. borealis* appear more severely perturbed than *X. laevis* following Notch pathway hyperactivation. RNA-seq of Notch perturbed *X. borealis* and *X. laevis* shows that both species have more differentially expressed homeologs than non-homeologs in response to this perturbation. *X. borealis* and *X. laevis* have relatively few differentially expressed genes in common following Notch perturbation. This appears consistent with observed morphological differences between the two species. To expand the comparisons across species, we have produced preliminary data using another tetraploid species, *X. muelleri*. We have complemented RNA-seq analysis with approaches to visualize homeolog-specific expression *in situ*.

146 Keller explant open-face development recapitulates natural morphogenesis of dorsal marginal zone. *Ian Randolph* ICB, UFRJ, Rio de Janeiro, Rio de Janeiro, BR.

During *Xenopus laevis* gastrulation, dorsal involuting marginal zone (DIMZ) converge and extends into notochord and dorsal non-involuting marginal zone (DNIMZ) converge and extends into neural plate. The autonomous capacity of *Xenopus laevis* dorsal marginal zone to remodel itself has been attested, and extensively studied, through 'Keller sandwich explant' procedure. Which consists of two explanted dorsal marginal zone cultured together face to face and provide a unique spatial-temporal view of the notochord and the neural plate being shaped. On the other hand, 'Keller explant open-face' (KE-OF), which consists of an explanted dorsal marginal zone cultured alone, is thought to provide a scenario in which the notochord is shaped by convergent extension, but the neural plate is not. We challenged this characterization by performing in situ hybridizations in order to distinguish endoderm, mesoderm and ectoderm. Our molecular results point out for a novel interpretation upon KE-OF development: 1. DIMZ cells do converge and extend into a notochord like structure, but they do it as they roll towards the ventral side of the explant in an involution like behavior; 2. DNIMZ cells also take part in the convergent extension cell sorting behavior and a neural plate like structure is shaped. In contrast with the previous interpretation, it is shown that the explanted dorsal marginal zone recapitulates both involution and convergent extension morphogenetic movements.

147 Histone Deacetylase activity plays an essential role in establishing and maintaining the vertebrate neural crest. *A. Rao*, C. LaBonne Molecular Biosciences, Northwestern University, Evanston, IL.

Neural Crest cells retain the broad developmental potential of the blastula cells they are derived from, even as neighboring cells undergo lineage-restriction. The mechanisms that enable these cells to preserve their developmental potential remain poorly understood, however. Here we explore the role that Histone Deacetylase (HDAC) activity plays in this process. We show that HDAC activity is essential for formation of neural crest, as well as for proper patterning of the early ectoderm. The requirement for HDAC activity initiates in naïve blastula cells; HDAC inhibition causes loss of pluripotency gene expression, and blocks the ability of blastula stem cells to contribute to lineages of the three embryonic germ layers. We find that pluripotent naïve blastula cells and neural crest cells are both characterized by low levels of histone acetylation, further highlighting the similarities between these cell populations. Importantly, we show that increasing HDAC1 levels enhances

the ability of blastula cells to be reprogrammed to a neural crest state. Together, these findings elucidate a previously uncharacterized role for HDAC activity in establishing the neural crest state, and provide novel insights into the evolution of vertebrates.

148 Leptin expression and downstream signaling in immune organs of *Xenopus* tadpoles and juveniles. *R. E. Reeve*, E. J. Crespi School of Biological Sciences, Washington State University, Pullman, WA.

Leptin, a pro-inflammatory adipokine hormone that regulates food intake and energy balance, but also modulates adaptive and innate immune responses and wound healing in mammals. However, leptin-immune system interactions in non-mammalian vertebrates is not well understood. We conducted a suite of experiments to test the hypothesis that leptin is an immunomodulator in amphibians across life history stages. To characterize the role of leptin signaling in immune function in *Xenopus laevis* larvae, we used antibody staining, qRT-PCR, and *in situ* hybridization to show that leptin mRNA and protein are expressed in the liver, thymus, gut, and skin, but not in the spleen. We also showed that leptin receptor mRNA is expressed in thymus, spleen, liver, and skin, and phosphorylated STAT3, a transcription factor activated by leptin receptor signaling, is upregulated in some of these tissues by injection of recombinant *Xenopus* leptin (rX-leptin). Lastly, administration of rX-leptin rescued larvae from mortality and non-lethal effects of gram-negative bacterial infections and enhances wound healing of tail injury, suggesting that leptin has enhancing effects on immunity. In juvenile *X. laevis*, leptin mRNA and protein is also expressed in liver, thymus, gut and skin, but expression is much lower in the thymus than in larvae. Leptin receptor is expressed and is activated by leptin in the spleen. Peripheral administration of rX-leptin has pro-inflammatory effects after injury, and increases circulating activated lymphocytes and monocytes, and stimulates proliferation of splenocytes. Taken together this research supports the hypothesis that leptin signaling, either through paracrine and endocrine signaling, has enhancing effects on adaptive and innate immune responses in both larvae and post-metamorphic frogs, although future research is needed to characterize specific roles of leptin signaling in immune responses to pathogens or injury, and whether leptin modulates nutrition-dependent immunity.

149 Defining the Neurog3 pancreatic beta cell gene regulatory network. *A. Rodriguez-Vargas*, M. Wlizla, M. Horb Eugene Bell Center for Regenerative Biology and Tissue Engineering and National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA.

Diabetes is a metabolic disorder that can result from impaired function or absence of insulin producing pancreatic beta-cell, which play an essential role in glucose metabolism. Understanding the transcriptional network that allows the development of pancreatic beta cells has the potential of generating the tools necessary to manipulate cell fate decisions. All pancreatic endocrine cells arise from a common progenitor that transiently expresses the basic helix-loop-helix (bHLH) transcription factor Neurogenin 3 (Neurog3). Neurog3 has been the focus in exploring what factors are involved in the regulatory network downstream that determines endocrine cell fates. Particularly, our current study attempts to decipher the molecular lineage that stimulates endocrine progenitor cells to generate beta cells over other endocrine cell types in *Xenopus*. Our lab previously showed that activation of Neurog3 for one or four hours at stage 12 results in increased insulin and somatostatin expression. Using this phenotype, gene expression changes were examined at hourly intervals for the first eight hours revealing dynamic changes. Many of these targets have not been previously shown to play a role in pancreas development. In this poster, I present our efforts to elucidate the function of Neurog3 target genes using gain and loss-of-function experiments, including the generation of CRISPR-mediated knockouts. In the end, our goal is to define the Neurog3 gene regulatory network and to use this information to identify methods to reprogram other cell types into pancreatic beta cells.

150 The formation of cilia tufts on the larval skin is coordinated by Histone H4K20 Methylation. *R. Rupp*, A. Angerilli, J. Berges Molecular Biology, Biomedical Center, LMU, Planegg-Martinsried, DE.

Covalent histone modifications reflect the transcriptional state of genes and convey epigenetic memory to developmental gene expression programs. Methylation of Histone H4 Lysine 20 occurs in a cell cycle dependent manner, with KMT5A (PR-Set7) writing the mono-methylated state on newly incorporated histones during S-phase, which in the G1-phase is converted by KMT5B (Suv4-20h1) and KMT5C (Suv4-20h2) into di- and trimethylated states, respectively. We have shown before that the H4K20me3 mark, typically found in constitutive heterochromatin, is required in the neuroectoderm to downregulate Oct25/Oct91 transcription during the transition from pluripotent to neural ground state. Embryos with reduced H4K20me3 levels maintain Oct25 expression in the neural plate and are severely impaired in primary neuroblast formation (Nicetto et al., PMID: 23382689). Here we report on a second, unpublished phenotype in H4K20me3-depleted embryos, which affects the forming epidermis. It is characterized by an upregulation of Delta-1, a selective increase in multiciliated cell precursors, coupled to a severe reduction in motile cilia formation. Confocal microscopy in mosaic mutant ectoderm reveals a defect in basal body trafficking, axoneme formation and apical cytoskeleton. RNA-Seq analysis of Suv4-20h morphant animal caps reveals that a large fraction of ciliogenic genes are downregulated in H4K20me3-depleted ectoderm, many of which are associated with ciliopathies. The ciliogenic defect can be ameliorated by restoring H4K20me3 levels in the embryo, but not by overexpressing foxj1 or multicilin. This indicates that the phenotype is specific, but occurs downstream of the master regulators of multiciliogenesis, possibly directly on the misregulated genes. We propose a model to explain the coordination of ciliogenic gene expression by H4K20me states. Our observations raise the possibility that impaired seeding of H4K20 methylation could contribute to ciliopathies.

151 Asymmetric cell division breaks pluripotency *in vivo*. *Pierluigi Scerbo*[1], Laurent Kodjabachian[2], Anne-Hélène Monsoro-Burq[1] 1) Institut Curie, Univ. Paris Saclay, CNRS UMR 3347, INSERM U1021, Orsay, France; 2) Institut de Biologie du Développement de Marseille, Aix Marseille Univ., CNRS UMR7288, Marseille, France.

How embryonic pluripotent cells can maintain an uncommitted state as well as an unrestricted potential for multi-lineage commitment is a key and unresolved question in developmental and stem cell biology. Using *Xenopus laevis*, we demonstrated the importance of asymmetric cell division (ACD) and developmentally-regulated proteolysis for the transition of embryonic cells from pluripotent to committed states (1). Using confocal imaging, we demonstrated that the pluripotency factor *Ventx2* is asymmetrically distributed in dividing pluripotent embryonic cells. This process is under the control of the kinase MEK1, which is required for asymmetric degradation of *Ventx2* during mitosis of

pluripotent cells, as well as for global clearance of Ventx2 at gastrulation. In absence of MEK1 activity, Ventx2 protein is stabilized and symmetrically distributed in pluripotent cells and maintains high expression of the zygotic pluripotency gene *Pou5f3/Oct4*. MEK1-deficient cells exit pluripotency and resume differentiation, when Ventx2 is also knocked down. Although many studies described the relevance of ACD in cell fate instruction, mainly using invertebrate models, this study is the first to demonstrate that vertebrate pluripotent cells divide asymmetrically *in vivo*. Ventx2 represents the first pluripotency factor shown to be asymmetrically inherited during embryonic cell division. Considering that human *VENTX* is one of the most specific marker of naïve pluripotency in human epiblast cells (2; 3), our study further supports that the frog Xenopus laevis is a relevant model to identify conserved fundamental principles of pluripotency. Further phylogenetic and functional analyses highlight the ontogenetic and evolutionary relevance of *Ventx* for vertebrate origin and radiation by endowing neural crest cell with multipotency.

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152 Gene regulatory networks for cranial placode development up- and downstream of Six1 and Eya1. S.K. Maharana, N. Riddiford, *G. Schlosser* School of Natural Science and Regenerative Medicine Institute (REMEDI), National University of Ireland Galway, Galway, Galway, IE.
Cranial placodes give rise to many sensory organs and ganglia of the vertebrate head. All placodes arise from a common preplacodal ectodermal domain located around the anterior neural plate and defined by the expression of the transcription factors Six1 and its coactivator Eya1. Six1 and Eya1 continue to be expressed in most placodes and play essential roles for multiple aspects of placode development, including the development of neurons and sensory cells.

To get insights into the gene regulatory network (GRN) downstream of Six1 and Eya1 in cranial placodes, we used RNA-Seq to screen for direct placodal target genes of Six1 and Eya1 in *Xenopus laevis* by overexpressing hormone-inducible constructs of Six1 and Eya1 in pre-placodal explants, and blocking protein synthesis before hormone-inducing nuclear translocation of Six1 or Eya1. Comparing the transcriptome of explants with non-induced controls, we identified hundreds of novel Eya1/Six1 target genes including transcriptional regulators of progenitor fates (e.g. *Sox2*, *Hes8*) and neuronal/sensory differentiation (e.g. *Ngn1*, *Atoh1*, *Pou4f1*, *Gfi1*). Gain and loss of function studies showed that Eya1/Six1 (1) are required for placodal expression of these genes and (2) repress/delay neuronal differentiation by directly activating *Hes8* independent of Notch signaling.

In parallel, we have begun to elucidate the GRN upstream of Six1 and Eya1. The expression of most ectodermal transcription factors becomes restricted to either ventral (non-neural) or dorsal (neural) parts during gastrulation (due to activation or repression by BMP, respectively). In loss of function studies we found that genes encoding both ventrally restricted (*AP2*, *Msx1*, *FoxI1*, *Vent2*, *Dlx3*, *GATA2*) and dorsally restricted (*Pax3*, *Hairy2b*, *Zic1*) transcription factors are required for Eya1 and Six1 expression in the preplacodal ectoderm, while conversely Eya1 and Six1 crossregulate these upstream genes.

Our findings provide pioneering insights into the GRN establishing Eya1 and Six1 expression in the preplacodal ectoderm as well as the GRN regulating placodal neurogenesis downstream of Six1 and Eya1.

153 Differential roles for SoxB1 and SoxE factors in the pluripotent blastula and neural crest during development. *E.N. Schock*, E. Buitrago-Delgado, C. LaBonne Molecular Biosciences, Northwestern University, Evanston, IL.
The neural crest (NC) is an embryonic population of cells that uniquely gives rise to both ectodermal and mesodermal derivatives. Historically, the NC has defied models of lineage restriction, as these cells seemingly regain developmental potential; however, previous work from our lab suggested that the remarkable plasticity of the NC could be explained by the retention of blastula-like pluripotency within the NC. While the biological implications of this expanded embryonic potential in terms of lineage decisions are apparent, the mechanisms by which NC cells retain blastula-like pluripotency remain unknown. Sox family transcription factors are key regulators of many aspects of developmental, including maintaining pluripotency and promoting lineage restriction decisions. Interestingly, two Sox family subclasses, SoxB1 and SoxE undergo dynamic shifts in expression as the embryo transitions from a pluripotent state to early lineage restriction. In the blastula, SoxB1 factors are highly expressed in pluripotent cells, but become restricted to the medial neural plate during neurulation. In contrast, SoxE factor expression is absent from the pluripotent blastula, but turns on laterally at the neural plate border (NPB) in NCCs. Effectively, domains of pluripotency in the blastula are defined by SoxB1 expression while the pluripotent NC are defined by SoxE expression. Using *Xenopus laevis*, we investigate the differential implementation of SoxB1 and SoxE factors in the pluripotent blastula and NC using gain of function, loss of function, and molecular replacement experiments. Furthermore, we explore how post-translational modification of Sox factors impacts the abilities of these factors to maintain a pluripotent state in the early embryo and, later, direct lineage decisions. Our findings provide evidence for both functional similarities and differences between SoxB1 and SoxE factors in the regulation of pluripotency in the embryo.

154 The Bicaudal-C post-transcriptional network; control of embryonic cell fates through temporal and spatially regulated mRNA translation. Sookhee Park[1], Megan Dowdle[1], Chao Yang[2], Paul Huber[2], *Michael Sheets*[1] 1) Univ. of Wisconsin, Dept. of Biomolecular Chemistry School of Medicine and Public Health, Madison, WI; 2) Univ. of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame IN.

Our work demonstrates that the Bicaudal-C (Bicc1) translational repressor is a key and central component of a maternal mRNA network. This network temporally and spatially controls the synthesis of cell fate regulatory proteins that guide critical developmental events in embryos. We have defined four parts of the Bicc1 network. The initial part precisely controls the distribution of the Bicc1 mRNA. In *Xenopus* embryos Bicc1 mRNA and protein are present in gradient along the vegetal-animal axis. The gradient has its beginnings in the vegetal localization of the Bicc1 mRNA during oogenesis. Specific mRNAs are localized through sequences in their 3'UTRs that are bound by proteins, such as VgRBP and anchor the mRNAs to the vegetal cortex. We defined a sub-region of the Bicc1 3'UTR that is sufficient for vegetal localization by analyzing fluorescent RNA fragments in growing oocytes. The second part of the network controls the temporal expression of the Bicc1 protein. Localized mRNAs are translationally repressed until oocyte maturation. Using reporter mRNAs we have defined a region of the 3'UTR responsible for translational control of the Bicc1 mRNA. This region contains multiple binding sites for cytoplasmic polyadenylation binding protein (CPEB1), a polyadenylation factor activated during maturation. Our results indicate that CPEB1 mediates the polyadenylation and translational activation of the Bicc1 mRNA during oocyte maturation. The third component of the Bicc1 network is the set of maternal target mRNAs whose translation is repressed by Bicc1. Focusing on the Cripto1 mRNA, we have defined features of the Bicc1 protein required for RNA binding and defined a minimal Bicc1 binding site within the Cripto1 3'UTR. The fourth component of the Bicc1 network is the set of mRNAs regulated by the proteins encoded by Bicc1 target mRNAs. In particular, some Bicc1 target mRNAs, such as ZFP36L2 encode RNA binding proteins. To extend the Bicc1 network we are defining the mRNA targets of these Bicc1 regulated RNA binding proteins. Our results demonstrate that the Bicc1 network is a highly orchestrated series of mRNA-protein interactions that establish a gradient of Bicc1 protein and translational repression activity during oogenesis and oocyte maturation. Subsequent action of the Bicc1 repression gradient during early embryonic development creates secondary gradients of regulatory proteins that direct and guide cell fates in *Xenopus* embryos.

155 Cass4 positively modulate Activin/Nodal Signaling to regulate Mesoderm formation during *Xenopus* Embryonic Development. *T. Sidrat*, J.K. Han Department of Life Sciences, Pohang University of Science and Technology, Pohang, KR.

Cass4 is the multimeric scaffolding protein that influences cell adhesion and movement, also involved in cell cycle control, cell differentiation and many growth factor signaling. However, its physiological role during vertebrate development is rarely studied. Here we show that Cass4, which is intensively expressed in the organizer tissue of the *Xenopus* gastrula embryo is highly involved in mesoderm specification and patterning. Mesoderm induction and patterning are regulated primarily by the activity of locally expressed members of TGF-beta superfamily. In this study, we show that Cass4 is upregulated in response to Activin/nodal signaling and dorsalizes the early mesoderm by regulating the expression of dorsal mesodermal specification markers. *Xenopus* Cass4 is specifically enriched in the dorsal region of the gastrula embryo and loss or gain-of Cass4 function causes shortened body axis and defective gastrulation movement. These phenotypes are reminiscent of those embryos which showed malformation of mesoderm. Our data show that depletion of Cass4 remarkably decreases the expression of mesoderm markers which could be effectively restored by co-injection of Cass4 mRNA as well as Smad2 mRNA. Moreover blocking the function of Cass4 by its specific morpholino inhibit the activin/nodal signaling by interfering with Activin-mediated activation of luciferase activity. These observations suggest that Cass4 acts as a positive modulator of the Activin/nodal signaling required for downstream activation of activin targeted genes expression for proper formation and patterning of mesoderm.

156 Regulation of integrin signaling in chondrogenesis and osteoarthritis development. *E. Song*[1,2], J. Jeon[3,4], S. Ruiz[5], T. Kwon[1], S. Yang[3], T. Park[1,2] 1) School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Korea; 2) Center for Genomic Integrity, Institute for Basic Science, Ulsan, Korea; 3) Department of Pharmacology, Ajou University School of Medicine, Suwon, Korea; 4) Department of Biomedical Sciences, Graduate School, Ajou University, Suwon, Korea; 5) Department of Molecular and Cell Biology, University of California, Berkeley, CA, United States.

Basic research and clinical trials have investigated the function of integrin signaling in chondrocyte differentiation and cartilage disorders, although the exact functions of integrin signaling during chondrogenesis are not well understood. Integrin signaling is necessary for cartilage development, as the loss of known mediators of integrin signaling causes abnormal cartilage and endochondral bone formation. In contrast, integrin-extracellular matrix (ECM) contacts promote the dedifferentiation of cultured primary chondrocytes, and several studies suggest that integrin signaling serves different roles depending on the chondrogenic stage. Furthermore, integrin signaling is a key source of the inflammatory reactions responsible for joint destruction. Given the ECM-rich environment and the expression of multiple integrin subunits, it is challenging for chondrocytes to minimize integrin-ECM interactions to allow chondrogenic differentiation to proceed and also maintain chondrogenic properties and protect from dedifferentiation or destructive signals. Here, we found a secreted integrin modulator expressed in prechondrocytes and promoting chondrogenesis in vertebrate. Integrin signaling is not only involved in cartilage disorders, but also contributes to various other human disorders such as inflammatory bowel disease, cardiovascular disorders, and cancers. Our discovery on a unique secreted integrin modulator should attract attention from researchers in many fields of biomedical science and will lead to new approaches for treating integrin-related human diseases, including destructive cartilage disorders.

157 Sprouty2-Vangl2 interaction regulates positioning of retinal progenitors within the eye field. *J. Sun*, YS. Hwang, J. Yoon, M. Lee, I. Daar National Cancer Institute, Frederick, MD.

The retinal progenitors of the eye field are specified by transcription factors that promote retinal fate and control cell movement. The positioning of progenitors within the eye field where they receive the local environmental signals that will direct their fate is an important step for eye formation. Recent evidence shows Dishevelled (Dsh) mediates ephrinb1 signaling in the eye field through planar cell polarity pathway (PCP). Sprouty2 (Spry2), a negative regulator of receptor tyrosine kinase signalinghas been reported to inhibit PCP pathway

mediated convergent extension movements during *Xenopus* gastrulation, but the molecular signaling pathways that regulate these movements are largely undefined. Here, we explored the role of Xspry2 in positioning of retinal progenitors within the eye field. Knockdown of Xspry2 prevents retinal progeny from entering the eye field, similarly to the loss of Xdsh or vangl2 which is a PCP core protein. Interestingly, overexpression of vangl2 but not Xdsh could rescue the phenotype induced by loss of Xspry2. This rescue is dependent on the physical interaction between XSpry2 and Vangl2. These results indicate Xspry2 regulates positioning of retinal progenitors within eye field through interaction with vangl2.

158 Mechanical regulation of closing movement and morphology of *Xenopus* neural tube. *M. Suzuki*, N. Yasue, N. Ueno National Institute for Basic Biology, Okazaki, JP.

Neural tube closure (NTC) is an important morphogenetic process during which an anlage of central nervous system is formed from a flat epithelial sheet called neural plate (NP). The NTC involves shape changes of neuroepithelial cells (apical constriction and cell elongation) and mediolateral cell-cell intercalation that function to narrow and bend the NP. In addition, dorsally biased movement of non-neural ectoderm is required for the completion of the NTC. These tissue-autonomous and non-tissue-autonomous cellular movements must generate physical forces and modulate mechanical properties of the NP, but how developmental changes of mechanical forces/properties are integrated for the NT morphogenesis is still poorly understood. We previously constructed the mathematical model explaining the mechanical regulation of the NTC, and found the unexpected role of the cell elongation in shaping the final morphology of the NT. From the prediction of the simulations we also proposed that the surface elasticities of the neural plate/surrounding tissues affect the NT morphology, and that the cell elongation-mediated tissue deformation functions to ensure the robustness of the NT morphology. In order to examine these hypotheses, we developed an atomic force microscopy (AFM) system suitable for measuring mechanical properties of *Xenopus* embryo. We found that the surface elasticity of the apical side of the NP increased during the NTC and showed the gradient along mediolateral axis. The stiffening of the apical surface was not observed in the non-neural ectoderm and required F-actin and non-muscle myosin II, suggesting that the actomyosin-dependent contractility is the source of the stiffening. We also measured the surface elasticity of the basal side of the NT and surrounding mesodermal tissues, and confirmed the highest elasticity of the apical side of the NP. The mechanical properties revealed here should change in time and space ranging from intracellular to tissue levels, and contribute to the large-scale morphogenetic movement of the NP.

159 Mechanisms which regulate the dorsal-ventral patterning of the developing pharynx in *Xenopus laevis*. *A.C. Szpak*[1,2], K. Fan[1,2], T.A. Drysdale[1,2] 1) Western University, London, Ontario; 2) Children's Health Research Institute, London, Ontario.

The pharynx is crucial to the survival of all vertebrates since the pharynx facilitates respiration by connecting the nasal and oral cavity to the larynx and connecting the oral cavity to the esophagus allowing for digestive functions. Even though the developing pharynx displays dorsal-ventral patterning and much is known about the morphogenesis and anterior-posterior patterning of the developing pharynx, currently there is little information identifying the underlying mechanisms that regulate the dorsal-ventral patterning. This is in part due to the complexity of the developing pharynx that requires contributions from all three germ layers: the endoderm, mesoderm and ectoderm, along with neural crest cells. The expression profiles of Sonic Hedgehog (*Shh*) and Bone morphogenetic Protein 4 (*Bmp4*) adjacent to the developing pharynx are reminiscent of their expression around the neural tube where they regulate dorsal-ventral patterning. Therefore, I hypothesize that *Shh* and *Bmp4* are the signaling molecules that regulate the dorsal-ventral patterning of the developing pharynx. *Xenopus laevis* embryos were treated with small molecular activator/inhibitors during developmental stages 13 to 35. At stage 35 the embryos were fixed and the effects on the dorsal-ventral patterning of the developing pharynx were assessed by whole mount *in situ* hybridization for mRNAs that are expressed in the ventral, intermediate and dorsal regions of the developing pharynx using antisense mRNA probes. The hypothesis was supported with respect to the cyclopamine, DMH1 and dorsomorphin treatments in all three pharyngeal arch regions. The inhibition of Shh by exposure to cyclopamine resulted in the predicted dorsal shift of the dorsal-ventral pattern. The inhibition of Bmp4 through exposure to DMH1 and dorsomorphin lead to the predicted ventral shift of the dorsal-ventral pattern of the developing pharynx. These results indicates that Shh and Bmp4 play a role in the regulation of the dorsal-ventral patterning of the developing pharynx.

160 Gαi2/Ric-8A a novel signaling pathway that regulates cranial neural crest cell migration in *Xenopus*. S. Villaseca, J.I. Leal, A. Beyer, *M. Torrejon* Laboratory of Signaling and Development, Department of Biochemistry and Molecular Biology, University of Concepcion, Chile.

The neural crest (NC) is a transient embryonic cell population induced at the border of the neural plate that migrates extensively in order to differentiate in other tissues during development. Ric-8A, a GEF for Gα subunits has been shown to be involved during cranial NC cells migration in *Xenopus*. On the other hand, the heterotrimeric G protein through its Gα subunit has been found participating in migration processes in several cell types and Gαi2 specifically has been described controlling the distribution and chemotaxis of immune cells. Therefore, our purpose is to study the relationship between Gαi2 and Ric-8A on the mechanism that controls the migration of cranial NC cells. For this, we evaluated the effect of loss and gain of function of Gαi2 and the rescue of Ric-8A morphant phenotype over cranial NC cells migration in *Xenopus* by *in situ* hybridization, chemotaxis and dispersion assays. The interaction of Ric-8A and Gαi2 was analyzed by Co-IP and subcellular localization by immunostaining. Cell morphology was evaluated by changes in the actin and tubulin cytoskeleton and integrin localization. We found that loss of function of Gαi2 inhibits cranial NC cell migration *in vivo* and *in vitro*. Furthermore, Gαi2 overexpression was able to rescue the cranial NC cells migration in Ric-8A morphant embryos. In addition, Ric-8A and Gαi2 morphant cells show changes on cell morphology due to changes on the actin cytoskeleton, specifically on the protrusions length and tubulin cytoskeleton. Ric-8A morphant cells show a decrease in the number a size of focal adhesion compared to control cells. However, Gαi2 morphant cells show longer focal adhesions than control cells and collective cell polarity defects evaluated by the localization of Par3 and Rac1 activity. Together these results suggest that Ric-8A, acting through Gαi2 pathway, is required for cranial NC cell migration, probably regulating cell adhesion disassembly and polarity.

161 The Effects of Bisphenol A on *Xenopus laevis* embryonic development relative to the somatic and germline zygotic genome activation. *A. Turgeon*[1], Y. Ge[1], H. Afreen[1], T. Tolson[2], J. Flaws[1], J. Yang[1] 1) Department of Comparative Biosciences, University of Illinois, Urbana-Champaign, Urbana, IL; 2) Department of Chemistry, Tuskegee University, Tuskegee, Alabama.

Bisphenol A (BPA) is a popular plasticizer used in the manufacturing of consumer products and is found ubiquitously in the ground water supply. BPA exposure to humans occurs from ingestion due to plastic food storage containers, inhalation from BPA air pollution, and absorption via mucus membrane and dermal contact with BPA containing products. BPA is a well-known endocrine disruptor. A large body of animal studies has revealed that BPA exposure during embryonic development can lead to many adverse effects. Interestingly, the majority of these studies assess the effects of BPA exposure on organogenesis or the development after birth. It remains largely unclear how exposure to BPA during the earliest stage of development may affect embryonic development. Using an amphibian model, the effect of BPA exposure during early embryogenesis was explored. *Xenopus* embryos can tolerate BPA treatment reasonably during later stage exposures. However, prior to the maternal-to-zygotic transition (MZT), embryos are hypersensitive to BPA exposure. Treating embryos with BPA before the beginning of the zygotic genome activation increases the expression of p53 protein and activates caspase 3, leading to the apoptosis of embryo. In addition, we assessed the effect of BPA exposure on primordial germ cells (PGCs), in which the zygotic genome is activated much later than that in somatic cells. Dosing embryos with a low, non-lethal concentration of BPA was able to significantly reduce the number of primordial germ cells (PGCs) in *Xenopus* embryos. Our results thus demonstrate that during the earliest stage of embryogenesis when the development is solely controlled by maternal factors, both somatic tissues and germline are hypersensitive to BPA exposure.

162 Toward Identification of signals responsible for gliogenesis in *Xenopus laevis*. *C.H. Ulrich*[1], V.V. Shah[2], A.K. Sater[1] 1) Biology and Biochemistry, University of Houston, Houston, TX; 2) MD Anderson Cancer Center, Houston, TX.

Glia are supportive cells in the vertebrate nervous system, consisting primarily of astrocytes, oligodendrocytes, and radial glia. While glial function is well studied, the signals required for initiation of glial development have yet to be elucidated in *Xenopus laevis*. Our preliminary studies indicate that glial differentiation begins between stages 20-24 (post-neural tube closure). To identify the signals essential for initiation of glial development, we isolated explants consisting of neural plate with or without the underlying mesoderm from mid-gastrula embryos and animal caps overexpressing the BMP inhibitor Noggin, which leads to neural specification. All three explant sets were grown until stage 28 and collected for quantitative RT-PCR. In isolated neural plates and neural plates plus dorsal mesoderm, expression of glia-associated genes was similar to that observed in sibling whole embryos. In contrast, in Noggin-injected animal caps (NogAC), expression of glial genes, such as oligodendrocyte-associated genes *olig3* and *sox10* and astrocyte-associated genes *slc1a3* and *slc1a2*, was significantly reduced. These findings suggest that (1) inhibition of BMP signaling alone is insufficient to induce gliogenesis; and (2) signals from the dorsal mesoderm during gastrulation are required for the initiation of gliogenesis at later stages. Expression of glial-associated genes in Noggin-injected neural plates remained similar to their whole embryo siblings, indicating that inhibition of BMP signaling does not prevent gliogenesis. We have performed pair-wise comparisons between the transcriptomes of mid-gastrula neural plates and NogACs to elucidate differences that might underly differences in glial specification. Functional annotation of the differentially expressed genes revealed pioneering transcription factors such as *ascl1/2* and members of Wnt and FGF signaling pathways were differentially expressed in neural plates. Our results indicate that signals important for gliogenesis are active in the *Xenopus* embryo as early as midgastrulation.

163 *neural specific kinase* (*nsk*) promotes early neural development in *Xenopus* embryos. *R.P. Virgirinia*[1], N. Jahan[1], M. Okada[1], K. Takebayashi-Suzuki[1], H. Yoshida[1,4], M. Nakamura[1], H. Akao[1], F. Fatchiyah[2], N. Ueno[3], A. Suzuki[1] 1) Biological Science, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan; 2) Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia; 3) Division of Morphogenesis, National Institute for Basic Biology, Japan; 4) National Xenopus Resource, Woods Hole, USA.

Neural induction and patterning in vertebrates is regulated during early development by several morphogens, such as bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs). Ventral ectoderm differentiates into epidermis in response to BMPs, whereas BMP signaling is tightly inhibited in the dorsal ectoderm which develops into neural tissues. Here, we show that *neural specific kinase* (*nsk*) promotes early neural development and inhibits epidermis differentiation in *Xenopus* embryos. *nsk* is specifically expressed in neural tissues along the anterior-posterior axis during early *Xenopus* embryogenesis. When overexpressed in the ectodermal explants, *nsk* induces the expression of both anterior and posterior neural marker genes. In agreement with this observation, overexpression of *nsk* in whole embryos expands the neural plate at the expense of epidermal ectoderm. Interestingly, the neural-inducing activity of *nsk* was increased in combination with BMP inhibition and with activation of the FGF signaling pathway. Inhibition Nsk protein caused defects in neural development with a significant reduction in the expression of neural marker genes. Overall, our study indicates that Nsk plays an important role in neural induction and patterning during early *Xenopus* embryogenesis possibly via modulation of morphogen signals such as the BMP and FGF pathways.

164 Tbx2 is required for the suppression of mesendoderm during early *Xenopus* development. *D. Weinstein*, S. Teegala, R. Chauhan, E. Lei Biology, Queens College, CUNY, Flushing, NY.

T-box family proteins are DNA-binding transcriptional regulators that play crucial roles during germ layer formation in the early vertebrate embryo. Well-characterized members of this family, including the transcriptional activators Brachyury and VegT, are essential for the proper formation of mesoderm and endoderm, respectively. To date, T-box proteins have not been shown to play a role in the promotion of the third primary germ layer, ectoderm. We report here that the T-box factor Tbx2 is both sufficient and necessary for ectodermal differentiation in the frog *Xenopus laevis*. Tbx2 is expressed zygotically in the presumptive ectoderm, during blastula and gastrula stages. Ectopic expression of Tbx2 represses mesoderm and endoderm, while loss of Tbx2 leads to inappropriate expression of mesoderm- and endoderm-specific genes in the region fated to give rise to ectoderm. Our studies demonstrate that Tbx2 functions as a transcriptional repressor during germ layer formation, and suggest that this activity is mediated in part through repression of target genes that are stimulated, in the mesendoderm, by transactivating T-box proteins. Taken together, our results point to a critical role for Tbx2 in limiting the potency of blastula-stage progenitor cells during vertebrate germ layer differentiation.

165 Muscle development in the frog, *Xenopus tropicalis*. *Johnson Yingcong yang* San Francisco State University.

For decades researchers have used *Xenopus laevis* to understand vertebrate embryogenesis and human diseases. However, its tetraploid genome is a barrier to understanding molecular pathways regulating embryological processes. In contrast, a closely related species, *Xenopus tropicalis,* is diploid and provides a more tractable genetic system to investigate how cells acquire their specific cell fate during early vertebrae development. Thus, using what we have learned in *X. laevis*, we have turned our attention to examine the cell behaviors that underlie muscle formation in X. *tropicalis.* Using immunohistochemistry and confocal microscopy, we have analyzed individual cell shapes as they transition for the presomitic mesoderm to forming somites comprised primarily of elongated myotome fibers. We examined the distribution of b-integrin, laminin, fibronectin and b-dystroglycan in *X. tropicalis* and compared it to the distribution in *X. laevis*. We confirm that the general sequence of cell behaviors observed in *X. tropicalis* is similar to that observed in *X. laevis*. In particular, somite formation in *X. tropicalis* involves a 90-degree rotation to form aligned muscle fibers much like that observed in *X. laevis*. These results suggest that similar cell behavior and signaling pathway are likely driving muscle formation in both species. Thus, we can use *X. tropicalis* as a model to identify gene networks that would be difficult to pursue in *X. laevis.*

166 Ripply-mediated zygotic Wnt inhibition is required for proper head development. *I. Yeo*, S. Yun, J. Han POSTECH, Pohang, KR.

Spemann's Organizer induces dorsal and anterior fate to nearby tissues and make head and body axis in *Xenopus* embryos. Various molecules have been discovered to mediate this organizing activity. We found Ripply as a noble organizer gene through microarray analysis. Ripply has been reported as a somitogenesis regulating factor. Ripply binds to Tbx6 and transforms Tbx6 to a transcriptional repressor in developing somites by recruiting Groucho/TLE co-repressors. However, Ripply was also expressed in dorsal mesendoderm of early gastrula and in head mesoderm of late gastrula. Ectopic expression of Ripply in ventral region induced additional head structure, while knockdown of Ripply induced truncated head. We examined the relation between Ripply and Wnt signaling. Loss of head structure by zygotic Wnt could be rescued by Ripply, and zygotic Wnt over-expression and Ripply knock-down showed synergistic effect. In addition, zygotic Wnt target genes, such as Cdx and Hox, were inhibited by Ripply over-expression. These results imply that Ripply maintains head mesoderm fate by inhibiting zygotic Wnt activity, and that Ripply is required for proper head development.

167 Expression and phenotypic analysis of GTPBP10, a member of the OBG family of proteins, and its potential role in notochord and pronephric development. R. Toossi-Jerry, *M. Yoder* Penn State Brandywine, Media, PA.

Early embryonic development requires a highly coordinated balance between cell migration and cell differentiation in order to properly pattern the axes and germ layers. Gastrulation is a critical phase of embryonic development, which relies heavily on cell sorting mechanisms and leads to the formation of the three primary germ layers: ectoderm, mesoderm, and endoderm. As these tissues further develop, cell sorting continues to play essential roles in the formation of new tissues and tissue boundaries, such as the separation of axial and paraxial mesoderm from a common population of mesodermal cells. Axial mesoderm gives rise to the notochord, which is an essential signaling center and also helps to elongate the anterior-posterior axis and serves as a flexible skeletal rod that supports the body of embryonic chordate animals; while paraxial mesoderm gives rise to somites, the precursors to muscle, bone, and dermal tissues. Proper notochord morphogenesis is absolutely required for early embryonic development in chordates, as abnormalities in the process can result in severe developmental defects, including axis truncation and loss of neural tube patterning. The purpose of this study was to identify novel regulators of notochordal morphogenesis in *Xenopus laevis*. As a result of previously performed screen, we found GTP-binding protein 10 (GTPBP10) as a potential effector of notochord formation. The GTPBP10 protein contains a number of conserved features, which places it in the Obg family of G-proteins. Obg proteins are a large group of GTP-binding proteins that are conserved from bacteria to mammals, and are vital in many organisms for cellular growth. RT-PCR shows that GTPBP10 is expressed throughout gastrulation until at least the tail bud stage. Tissue specific expression cannot be determined for gastrula through neurula staged embryos, but later tail-bud stages show strong GTPBP10 expression in the notochord, pronephros, and various anterior structures. Additionally, morpholino knockdown of GTPBP10 predominately induces an edemic phenotype, with a low percentage of axis-truncation observed. Overall, we determined that GTPBP10 is a member of the Obg family of G-proteins and, due to the spatio-temporal expression and loss of function phenotype, is likely not involved with initial notochordal morphogenesis, but perhaps plays a role in the maturation or maintenance of the notochord. The edemic phenotype, coupled with the pronephric expression suggest that GTPBP10 may be required for proper development of the embryonic kidney.

168 Foxj3 regulates early neural development via control of *Msx1* expression in *Xenopus* embryos. *G.-H. Yoon*, S.-C. Choi Biomedical Sciences, University of Ulsan College of Medicine, Seoul, KR.

Members of forkhead box (fox) protein family are transcriptional factors conserved from human to fly. Foxj proteins, isoforms of Foxj subfamily, have been known to regulate left-right asymmetry, muscle fiber formation, cell proliferation, motility, migration, and invasion during early development and adult tissue homeostasis. Interestingly, in analyzing the relevance of Foxj3 isoform to TGF-β signaling, we found its critical role in *Xenopus* early neural development. *Foxj3* showed both maternal and zygotic transcription in *Xenopus* embryogenesis. Spatially, its transcripts were detectable in the animal and marginal regions of embryo from the cleavage to gastrula stages and in the dorsal trunk and head, later. Gain-of-function of *Foxj3* interfered with the ectopic and *in vivo* expression of several key mesodermal markers induced by TGF-β signals including Activin/Nodal and BMPs. Of note, among the markers tested, only *Msx1* showed enhanced expression in embryos overexpressing *Foxj3*. Consistently, the morpholino-mediated knockdown of *Foxj3* led to down-regulation of *Msx1* expression. Early activation of Foxj3, using its stage-specific inducible construct, caused microcephaly, shortened body axis and dorsally kinked phenotype in embryos. In contrast, its later activation after the onset of neurulation had no effect on the overall body axis of embryos but up- or down-regulated the expression of neural crest markers in its dose-dependent manner. Knockdown of *Foxj3* promoted neural differentiation at the expense of epidermal development. Moreover, neural crest formation was inhibited in embryos depleted of *Foxj3*, which could be reverted by co-expression of *Msx1*. Finally, Foxj3 was found to interact with Smad1 physically. Taking together, we suggest that *Foxj3* regulates neural induction and neural crest formation via modulation of *Msx1* expression in *Xenopus* early development.

169 Targeted search for scaling-responsible genes: a secreted proteinase regulates scaling of the embryonic axial structures by modulating degradation of chordin. *A. Zaraisky*[1], E. Orlov[1], A. Nesterenko[1,2], N. Martynova[1], D. Korotkova[1] 1) Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia ; 2) Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia.

How the embryo scales its patterning in dependence of the overall body size is one of the most challenging problems in developmental biology. We have supposed that there should be some specific genes, which we called scalers, whose expression is sensitive to the overall body size. Using the concentration of scalers protein products as size-sensors, the embryo may scale its patterning by adjusting values of parameters responsible for the establishment of the morphogen gradients that pattern the embryo. To verify this prediction, we have compared the data of transcriptomes profiling of the late gastrula stage normal and half-sized embryos obtained by separation of the right and left blastomeres of the *Xenopus laevis* embryo at two-cells stage. As a result, we have revealed several genes whose expression changes in several times in half-sized embryos. Among them, we studied more thoroughly the role of secreted proteinase (SP), which expression was decreased in five times. We have demonstrated that down-regulation of SP in the normal embryos elicited at least some traits of patterning characteristic to the half-sized embryos, in particular, diminishing of the neural plate and somitic mesoderm. Furthermore, we have shown that this SP inhibits degradation of chordin by anothe secreted proteinase, Xolloid. These findings explain diminishing of axial structures both in the half-sized embryos as well as in those with the inhibited SP. Thus, we have revealed for the first time a gene which may play a role of the scaler because its expression is dependent of the overall size of the embryo while its protein regulates the activity of the key dorsalizing factor chordin. In turn, our findings provide proof of principle of how scalers could be identified in embryos of any species.

170 Retinoic acid signaling is required for normal head development. *L. Bendelac*, M. Gur, Y. Shabtai, G. Pillemer , A. Fainsod Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel-Canada,Faculty of Medicine, Hebrew University of Jerusalem, Israel.

Retinoic acid (RA) is essential for normal vertebrate embryogenesis and abnormal RA levels induce multiple developmental defects. Children exposed to alcohol as a result of maternal drinking during pregnancy, suffer from Fetal Alcohol Spectrum Disorder (FASD). Severely affected FASD individuals exhibit heart, kidney and hearing problems, microcephaly, craniofacial malformations and other developmental malformations, in addition to mental, functional and behavioral disabilities. We established a *Xenopus* model of the severe form of FASD. Taking advantage of this experimental model we could show that a major etiological event in the induction of FASD is the competition between ethanol detoxification and the biosynthesis of RA. Enzymatically, acetaldehyde, the intermediate in ethanol clearance, is a favored substrate of retinaldehyde dehydrogenase 2 (RALDH2) reducing the production of RA to teratogenic levels.
In early gastrula embryos, RA biosynthesis is finalized by expression of *Raldh2* in Spemann's organizer which oxidizes retinaldehyde to RA. RALDH2 activity is supported by expression of four enzymes previously linked to the metabolism of RA, DHRS3, RDH10, ADHFe1 and RALDH3. Later in gastrulation, two RA biosynthetic centers are evident, one in the head-inducing prechordal mesoderm (RALDH3) and in the trunk region (RALDH2). We show that reduction of RA levels with ethanol, DEAB or CYP26A1 causes head malformations, microcephaly, by targeting the endogenous organizer or in secondary axis assays. RALDH2 or RALDH3 RNA injection rescued the head malformations induced by RA reduction by CYP26A1 overexpression. RALDH3 and RALDH2 knock-down affected the expression of gastrula genes previously linked to the induction and formation of the head. In secondary axis assays, knock-down of RALDH3 but not RALDH2 activity affected the formation of anterior head regions. These results identify a second localized source of RA in the prechordal mesoderm and suggest a novel function for RA signaling in the process of head induction and formation during early embryogenesis. Microcephaly is a developmental malformation common to syndromes whose etiology has been linked to reduced RA signaling like FASD, Vitamin A Deficiency syndrome and others.

171 *In vivo* functional validation of candidate congenital hydrocephalus genes in *Xenopus* using CRISPR/Cas9 and optical coherence tomography. *P. Date*[1], P. Ackermann[1,2], C. Furey[1], L. Jeffries[1], M.K. Khokha[1], K.T. Kahle[1], E. Deniz[1] 1) Yale School of Medicine, New Haven, CT, US; 2) RWTH Aachen Univeristy, Aachen, Germany.

Congenital hydrocephalus (CH) is a common disease suboptimally treated with morbid surgical CSF shunting. A poor understanding of CH pathophysiology, including its genetic determinants and molecular mechanisms, has hindered the development of targeted therapeutics. A rapid, genetically tractable small animal model system is needed to functionally validate potential disease genes discovered by next-generation sequencing efforts. Here, we have coupled CRISPR /Cas9 gene editing and optical coherence tomography (OCT) microscale imaging in live, transparent *Xenopus tropicalis* tadpoles to assess the impact of established and candidate human CH mutations on brain ventricular morphology, ependymal cilia function, and CSF flow dynamics. We discovered that *Xenopus* tadpoles have five distinct and directionally polarized "CSF flow fields" generated by ependymal cilia that line the ventricular surface. Using our system, we recapitulated the core phenotypes of human X-linked aqueductal stenosis and hydrocephalus caused by recessive *L1CAM* mutation (OMIM #307000), the most common cause of inherited CH. We also tested a novel candidate CH gene mutation in *Crb2*, discovered using whole exome sequencing, in a sporadic case of aqueductal stenosis and non-communicating ventriculomegaly. Genetic disruption of *Xenopus crb2* recapitulated the human disease phenotypes, and identified cilia function as a potential mechanism for disease pathogenesis. Our results demonstrate the successful use of *Xenopus* to rapidly screen and functionally validate specific, patient-derived alleles of candidate disease genes and further evaluate hydrocephalus phenotypes *in vivo*. This approach could foster the development of precision medicine approaches for CH and other diseases associated with impaired CSF homeostasis.

172 The role of CFAP45 in congenital heart disease and ciliogenesis. *Engin Deniz*[1], Emily Sempou[1], Livija Medne[2], Ian Krantz[2], Mustafa Khokha[1] 1) Pediatrics, Yale University, New Haven, CT; 2) Pediatrics, Division of Human Genetics,Children's Hospital Of Philadelphia,Philadelphia, PA.

Congenital Heart Disease (CHD) is a leading cause of death in newborns. In a consanguineous family, we identified a recessive missense mutation in CFAP45 gene in two siblings that had symptoms of congenital heart disease and heterotaxy (Htx), where left-right patterning of

body axis is compromised.CFAP45 is a member of the coiled-coil domain containing- protein family and has never been implicated in CHD/Htx previously nor does CFAP45 have an identified function; therefore, a pathogenesis mechanism for heterotaxy and CHD is undefined. The homozygous variant of uncertain significance (VUS) is `probably damaging` based on multiple in-silico programs, but to test this functionally and understand the pathogenesis mechanism, we used the *Xenopus* model system. In this work, we seek to describe the role of CFAP45 in early development, more specifically in cardiogenesis, left-right patterning and ciliogenesis.
When we knocked down CFAP45 by morpholino oligonucleotides or CRISPR/CAS9 in *Xenopus*, we detect abnormal cardiac looping, loss of multicilia in the embryonic epidermis, as well as loss of monocilia found in the left-right organizer where monocilia generate and sense leftward fluid flow to break bilateral symmetry and establish laterality that is essential for proper organogenesis such as the heart. Therefore, we can recapitulate the left-right phenotype of the patient. Interestingly, we find that in both multiciliated and monociliated cells CFAP45 is localized to the base of the cilia and to the ciliary axoneme suggesting a unifying structural role for CFAP45 in cilia structure. Finally, we find that the CFAP45 depletion phenotype (loss of cilia) can be rescued using human CFAP45 mRNA but cannot be rescued with the patient mutation bolstering the evidence that CFAP45 is disease causing.
In summary, our preliminary data shows that CFAP45 is localized to the cilia in two different cell types with discrete roles in development and when lost in both types, leads to similar defective ciliogenesis. These findings suggest a fundamental role of CFAP45 in sustaining proper ciliary architecture and eventually left-right and cardiac patterning.
The genetics of congenital heart disease is an area of intense research. The disease currently affects 40,000 newborns annually yet our understanding of the underlying causes remains poor. Our work revealed a novel gene CFAP45 critical for proper cilia formation and leading to heterotaxy further strengthening the link between cardiogenesis and ciliogenesis, potentially identifying a novel target for regenerative medicine.

173 Identifying phenotypic convergence among Tourette Disorder risk genes. *C.R.T. Exner*[1], H.R. Willsey[1], Y. Xu[1,2], N. Sun[3], S. Wang[3], R.M. Harland[2], A.J. Willsey[3], M.W. State[3] 1) Department of Psychiatry, Weill Institute for Neurosciences, University of California, San Francisco; 2) Department of Molecular & Cell Biology, University of California, Berkeley; 3) Institute for Neurodegenerative Diseases, Weill Institute for Neurosciences, University of California, San Francisco.

Tourette Disorder (TD) is a psychiatric disorder characterized by involuntary, repetitive behaviors and with unclear etiology. Recent whole exome sequencing studies conducted by our group have identified, for the first time, high confidence TD risk genes. However, the functions of these genes in brain development and the mechanisms by which their disruption may contribute to TD are unknown. We seek to identify loss-of-function phenotypes common to many of these genes, as shared phenotypes are likely to be the most relevant to the pathology of the disorder. Interestingly, TD risk genes are enriched as a group for involvement in cell polarity, suggesting that dysfunctions in polarity may contribute to TD. Here we leverage *Xenopus tropicalis* and CRISPR/Cas9 genome editing to investigate, in parallel, the neurodevelopmental roles of TD risk genes. We present evidence that top TD risk genes are expressed in the developing brain at stages during which highly polarized cell behaviors are critical in this tissue. We also show that loss of risk gene function causes classic planar cell polarity phenotypes, consistent with a role for these genes in planar polarity. Ongoing work will characterize anatomical and molecular brain phenotypes of mutant embryos with the goal of identifying convergent phenotypes of TD risk genes.

174 Early *Xenopus* neural development as a platform to understanding the etiology and mechanism of human neural genetic disease in Bainbridge-Ropers syndrome. Hava Lichtig, *Dale Frank* Biochemistry, Rappaport Faculty of Medicine , Technion - Israel Institute of Technology, Haiufa, IL.

In humans, the mutant *ASXL3* gene was identified as causing severe developmental defects, microcephaly and craniofacial defects that have recently been defined as Bainbridge–Ropers (BR) syndrome. Certain ASXL3 protein haplotypes are also associated with autism spectrum disorder. ASXL3 protein seems to regulate early stages of neural patterning and development. Understanding ASXL3 protein function in early vertebrate development will shed light on the etiological mechanisms of neuro-developmental disorder causing BP syndrome. Our lab investigates the interactions between transcription factors and signaling pathways during early nervous system formation. Craniofacial defects and microcephaly can result from a perturbation of these transcription factor/signaling pathway interactions. Importantly, we can knock down endogenous *Xenopus* ASXL3 protein activity by translation-blocking antisense morpholino oligonucleotides in embryos and explants to examine how various human wild type and mutant isoforms of the ASXL3 protein alter embryo phenotype when re-expressed on the “null” background. We have shown that the knockdown of ASXL3 protein causes a phenotype that is typical for embryos losing posterior neural structure. Neural folding is inhibited and neural plate elongation is perturbed. These embryos have neural tube defects triggering microcephalic-like phenotypes. In ASXL3 morphant embryos, we find that hindbrain, neural crest, and primary neuron marker expression is severely reduced, but spinal cord marker expression is not significantly perturbed. We have also shown the wild-type human ASXL3 protein functions in *Xenopus*. Ectopic expression of human full-length ASXL3 encoding RNA rescued neural marker expression in ASXL3 knockdown embryos, suggesting that ASXL3 functions in the earliest gene network regulating posterior neural tissue specification. We have also ectopically expressed both wild-type and mutant ASXL3 proteins in explants posteriorized by the Meis3 protein. Both the mutant and wild-type forms of the ASXL3 protein strongly modify expression of posterior neural markers in these explants. We hope that by understanding the correct temporal role of ASXL3 protein in early neural development, we will elucidate the mechanisms underlying both BR and autism syndromes caused by ASXL3 perturbation in early human development.

175 Analysis of novel candidates for Short Rib Thoracic Dysplasia (SRTD) associated genes. *Maike Getwan*, Rebecca Diehl, Florian Heeg, Sophie Schroda, Weiting Song, Soeren Lienkamp University Medical Center Freiburg (Nephrology), Freiburg im Breisgau, Germany.

Short Rib Thoracic Dysplasia (SRTD) belongs to a class of diseases termed skeletal related ciliopathies. Patients suffer especially from shortenings of ribs, long bones of arms and legs as well as polydactyly. Further a nephronophthesis-like phenotype (NPHP), retinopathy and laterality defects can occur. All so far identified SRTD mutations revealed genes that function in a ciliary context and influence Sonic hedgehog signaling, explaining the polydactyly phenotype. However, until now mutations have only been identified in a subset of patients, suggesting that additional disease genes still remained unknown. To shed light on the molecular pathogenesis of SRTD, its connection to NPHP and to expand our knowledge about the role of ciliary proteins during skeletal and renal development, we attempted to identify putative SRTD candidate genes.

We used an *in silico* analysis to find published screens in which known SRTD-genes were significantly enriched. Within these screens we searched for further genes that were also highly significantly enriched – our candidate genes. We identified putative disease genes by expression, phenotype and marker analyses. Expression analyses were done with *Xenopus laevis* whole mount embryos and limb buds. It could be detected in ciliated tissues, especially the pronephros but also the branchial arches, the eye and in some cases also in the limbs. Functional analyses in *Xenopus tropicalis* focused on two SRTD-genes (*ift80* and *ift172*) and one candidate gene. Phenotypic analysis after loss of function experiments using the CRISPR/Cas system displayed again similar defects for SRTD genes and the candidate gene. Both resulted in edema and in craniofacial defects. *ift80*-LOF tadpoles had impaired limb develpment resembling the patient phenotype. Marker analyses showed an influence of SRTD- as well as candidate genes on neural crest cell development but not on other markers tested so far. RNAseq of *ift80* and *ift 172* CRISPR targeted embryos identified dysregulated genes, which will be analyzed in future experiments.

176 Screening Congenital Heart Disease de Novo Mutations via CRISPR Mosaic Knockout in Xenopus Tropicalis. *D. Gonzalez*, Emily Mis, Emily Sempou, Priya Date, Mustafa Khokha Genetics, Yale University, New Haven, CT.

Congenital heart disease (CHD) impacts 1% of live births. Previous whole exome sequencing analysis of patient-parent trios identified de novo mutations in over 300 genes that may be responsible for CHD. 94 of these candidate genes were knocked out in X. tropicalis embryos via CRISPR microinjections at the single-cell stage, with 35% of them causing cardiac looping defects. As many of the genes came from patients with heterotaxy (improper left-right organization of the heart), further investigation of early left-right patterning within these mosaic knockout embryos will be conducted to identify potential pathways that are required for proper left right patterning. Discovering the disease-causing mechanisms of these genes will heighten our understanding of proper heart development in vertebrates.

177 *RPSA*, a candidate gene for isolated congenital asplenia, is required for pre-rRNA processing and spleen formation in *Xenopus*

. *J.N. Griffin*[1,2], Samuel Sondalle[1], Andrew Robson[1], Emily Mis[1], Gerald Griffin[1], Engin Deniz[1], Susan Baserga[1], Mustafa Khokha[1] 1) Yale School of Medicine; 2) King's College London.

As the molecular machines responsible for producing proteins, ribosomes are ubiquitously essential for life. However, despite this universal requirement for ribosome function, a growing number of surprisingly tissue specific and phenotypically heterogenous inherited disorders are associated with impaired ribosome biogenesis. Recently, mutations in RPSA, a protein component of the small ribosomal subunit (SSU), were discovered to underlie approximately half of all isolated congenital asplenia cases. However, the mechanisms by which mutations in this ribosome biogenesis factor lead specifically to spleen agenesis remain to be elucidated in part due to the lack of a suitable animal model for study. Here we reveal that RPSA is required for normal spleen development in *Xenopus*. Depletion of RPSA disrupts pre-ribosomal RNA (rRNA) processing and ribosome biogenesis, and preferentially impairs expression of the key spleen patterning genes *nkx2.5* and *bapx1* in the spleen anlage. Importantly, we also show that while injection of human RPSA mRNA can rescue both pre-rRNA processing and spleen patterning, human mRNA bearing a common disease-associated mutation cannot. Together, we present the first animal model of RPSA-mediated isolated congenital asplenia and reveal a crucial requirement for RPSA in pre-rRNA processing and molecular patterning during early development of the spleen.

178 Targets of Sf3b4 in Neural Crest Development: Insights into the Craniofacial Defects Associated with Nager syndrome. *Santosh Maharana*, JP Saint-Jeannet Department of Basic Science and Craniofacial Biology, New York University, College of Dentistry, New York, NY - USA.

Mandibulofacial dysostosis (MFD) is a human developmental disorder characterized by defects in neural crest-derived craniofacial tissues and bones. Acrofacial dysostosis combines the features of MFD with a variety of limb anomalies. The most common form of acrofacial dysostosis is Nager syndrome. Mutations in *SF3B4* (splicing factor 3b, subunit 4) gene, which encodes a component of the pre-mRNA spliceosomal complex, were recently identified as a major cause of Nager syndrome. It is very intriguing that disruption of a global process, like pre-mRNA processing, may result in such a restricted and specific phenotype, affecting primarily craniofacial development. Interestingly, mutations in genes encoding other components of the spliceosome, *EFTUD2*, *SNRPB* and *TXNL4A* also cause MFD in three related but distinct syndromes, suggesting that interference with the spliceosomal machinery may underlie the etiology of MFD. We have recently developed an animal model for Nager type MFD by specifically knocking down *Sf3b4* function in *Xenopus laevis* embryos. We have shown that in these embryos neural crest progenitors formation is affected through a mechanism that involves increased apoptosis, resulting in hypoplastic craniofacial skeletal structures at the tadpole stage. To understand how Sf3b4 mediates its activity during neural crest formation we are analyzing the impact of Sf3b4 knockdown on pre-mRNA processing using RNA sequencing. Here, we are comparing the transcript profile of two types of samples: (i) cranial neural crest explants isolated from wild-type and Sf3b4-depleted stage 15 embryos; and (ii) wild-type vs. Sf3b4-depleted animal cap explants expressing Noggin and Wnt8, a combination of factors that promote neural crest fate. (iii) In both sets of samples RNA-Seq was performed with and without rRNA depletion (Nugen Ovation Trio Low Input RNA) for comparison. We will present the validation of these samples, the results of the RNA-Seq profiling analysis and the preliminary characterization of a subset of Sf3b4 targets.

179 *De novo* Pathogenic Variants in Neuronal Differentiation Factor 2 (*NEUROD2*) Cause A Form of Early Infantile Epileptic Encephalopathy. *E.K. Mis*[1], A Sega[1], K Lindstrom[2], S Mercimek-Andrews[3,4,5], W Ji[1], M.T. Cho[6], J Juusola[6], M Konstantino[1], L Jeffries[1], M.K. Khokha[1,7], S.A. Lakhani[1] 1) Pediatric Genomics Discovery Program, Pediatrics, Yale University, New Haven, CT; 2) Division of Genetics and Metabolism, Phoenix Children's Hospital, Phoenix, Arizona, USA; 3) Genetics and Genome Biology Program, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Division of Clinical and Metabolic Genetics, Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada; 5) Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada; 6) GeneDx, Gaithersburg, Maryland, USA; 7) Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA.

Early infantile epileptic encephalopathies (EIEEs) are severe disorders consisting of early-onset refractory seizures often accompanied by significant developmental delay. More than 100 genes have been previously associated with EIEEs, representing a spectrum of genetic and phenotypic heterogeneity. Despite the large number of genes associated with EIEEs, the genetic burden of EIEEs has not been comprehensively explained. Utilizing whole exome sequencing (WES), we identified novel *de novo* variants in *NEUROD2* in unrelated children with drug-resistant EIEEs. Depleting *neurod2* with CRISPR/Cas9-mediated genome editing using two non-overlapping target sequences in *Xenopus tropicalis* induced spontaneous seizures in tadpoles, mimicking the patients' condition. Overexpression of wildtype NEUROD2 induces ectopic neurons in *Xenopus laevis* tadpoles; however, the patient variants were markedly less effective, suggesting that the patient variants have a loss of function and are likely pathogenic. Complete analysis of the function of Neurod2 in *Xenopus* will lead to a better understanding of the pathogenesis of EIEEs in patients.

180 AT-rich interaction domain 3A regulates the regeneration of the nephric duct through the evolutionary conserved regeneration signal response enhancer. N. Suzuki[1,2], H. Ogino[2], *H. Ochi*[1] 1) Institute for Promotion of Medical Science Research, Faculty of Medicine, Yamagata University, Yamagata, Yamagata, JP; 2) Amphibian Research Center, Hiroshima University, Higashi-hiroshima, Hiroshima, JP.

Amphibians and fish can regenerate many tissues and recover normal function after injury, whereas mammals have a limited regenerative capacity. Currently, it is considered that tissue regeneration recapitulates developmental programs because the expression of developmental genes is reactivated during regeneration in many cases. However, it is still unclear whether amphibians and fish retain the unique *cis*-regulatory elements specific to tissue regeneration. We screened regeneration signal response enhancers in *Lhx1* locus using *X. laevis* transgenic enhancer mapping system and found that the noncoding elements conserved between only highly regenerative species do not show strong enhancer activities in the regenerating amphibian nephric duct. Instead, the noncoding elements conserved from fish to human function as enhancers in the regenerating nephric duct, suggests that mammals still retain regeneration signal response enhancers in their genome. Then, we studied the transcriptional mechanisms of regeneration signal response enhancers (RSREs) that allow amphibians to reactivate the expression of developmental genes during regeneration. The putative transcription factor binding site analysis of RSREs showed that a motif of AT-rich interaction domain 3A binding site was commonly found in the RSRE. Arid3a, a member of the AT-rich interaction domain family, is a DNA binding protein and also known to as a component of H3K9me3 demethylases KDM4/JMJD2 complex. Chromatin immunoprecipitation analysis showed that Arid3a binds to RSREs, and overexpression of Arid3a in *X. laevis*resulted in reduction of H3K9me3 levels on RSREs. We found that the conditionally induced Arid3a using transgenic *X. laevis*promotes the cell cycle progression in regenerating nephric duct and this induction causes the extra regenerating nephric duct. In addition, conditional suppression of Arid3a using Photo-Morpholino inhibits the regeneration of the nephric duct. Together these results suggest that Arid3a contributes the regeneration of the nephric duct through the decreasing of H3K9me3 on RSREs.

181 Potassium channels in patients with structural heart disease: Lessons from the gastrula. *E. Sempou*[1], J. Zhu[2], KY. Kim[3], Z. Wang[3], W. Hwang[1], MJ. Caplan[2], NB. Ivanova[3], IH. Park[3], D. Zenisek[2], MK. Khokha[1] 1) Pediatrics, Yale University School of Medicine, New Haven; 2) Cellular and Molecular Physiology, Yale University School of Medicine, New Haven; 3) Genetics, Yale Stem Cell Center, Yale University School of Medicine, New Haven.

Congenital heart disease (CHD) is the most common birth defect and the leading cause of infant mortality in the US and Europe, yet its primarily genetic causes continue to be obscure. Six out of eight members of the KCNH family of voltage-gated potassium (K^+) channels have recently emerged in a large patient exome sequencing study as candidate disease genes for CHD and specifically heterotaxy. Heterotaxy is a disorder in which organs, including the heart, are mispatterned relatively to the left-right (LR) axis. It frequently results in very severe heart defects because the heart has a distinct and intricate LR architecture. Even though voltage-gated K^+ channels are well known for their connection to cardiac arrhythmias, a role in ***structural*** heart disease is undefined. We depleted candidate heterotaxy gene *kcnh6* in *Xenopus* embryos using CRISPR and morpholino and recapitulated the patient phenotype. Knockdown embryos were impaired in their LR development due to changes in plasma membrane potential at stages as early as gastrulation. Specifically, distinct meso- and ectodermal cell populations in the gastrula failed to acquire their fate, while the endoderm differentiated correctly. This conflicted with our initial hypothesis that electrical charge of cells is more likely to affect their morphogenetic behavior rather than cell fate. We also determined that the basis of these defects was aberrant activation of voltage-gated calcium channels and dramatically increased Ca^{2+} signaling, triggered by changes in cell membrane potential. Finally, we recapitulated differentiation defects caused by Kcnh6 depletion and cell membrane depolarization outside the context of embryonic structure using embryonic stem cells that we differentiated *in vitro* into endo-, meso- and ectoderm. Altogether, our findings identify potassium channels and membrane potential as novel instruments in the embryonic patterning "toolbox" as well as a potential causes of left-right patterning defects in human patients.

182 Developing *Xenopus* models of human disease. *N. Shaidani*, W. Thomas, G. Payne, A. Rodriguez-Vargas, M. Wlizla, M. Horb Bell Center for Regenerative Medicine and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.

Biallelic gene targeting in F0 embryos can be used to help elucidate gene function. However, CRISPR-Cas targeted mutagenesis often results in variable levels of penetrance and mosaicism, leaving little to be inferred. Thus, it is necessary to breed F1 heterozygous animals to create null homozygous mutant offspring in the F2 generation. This can be a constraint for researchers, as space requirements to maintain multiple

generations of a single knockout mutant line can be immense. At the NXR, we have a growing infrastructure suited for housing thousands of animals. In an effort to implement more widespread use of genome editing technologies within the *Xenopus* community, we have initiated the generation of over 100 *Xenopus* mutant frog lines in specific genes to model human disease. The availability of web-based tools for expedited sgRNA generation and germline screening has aided our efforts in developing an efficient pipeline for generating mutant *X. laevis* and *X. tropicalis* frogs. To date, we have generated over 50 mutant lines, many of which we have breed to the F1 generation and some to the F2 generation. This includes, but is not limited to, those associated with kidney disease (pkd2), heart disease (tbx5), neurological disease (fxr1), eye (prph2) and craniofacial disorders (runx2). In this poster, I will present our methodology in producing an efficient pipeline, while also circumventing embryonic lethality to develop homozygous mutant lines. In addition, I present a list of our current mutant lines with updates on our progress.

183 *Xenopus*: An alternative model system for identifying muco-active agents. *H. Sim*[1], S. Kim[2], K. Myung[1,3], T. Kwon[1], H. Lee[4], T. Park[1,3] 1) School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Korea; 2) CMRI, Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu, Korea; 3) Center for Genomic Integrity, Institute for Basic Science, Ulsan, Korea; 4) College of Natural Sciences, Kyungpook National University, Daegu, South Korea.

The airway epithelium in human plays a central role as the first line of defense against environmental contaminants. Most respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, and respiratory infections, disturb normal muco-ciliary functions by stimulating the hypersecretion of mucus. Several muco-active agents have been used to treat hypersecretion symptoms in patients. Current muco-active reagents control mucus secretion by modulating either airway inflammation, cholinergic parasympathetic nerve activities or by reducing the viscosity by cleaving crosslinking in mucin and digesting DNAs in mucus. However, none of the current medication regulates mucus secretion by directly targeting airway goblet cells. The major hurdle for screening potential muco-active agents that directly affect the goblet cells, is the unavailability of *in vivo* model systems suitable for high-throughput screening. In this study, we developed a high-throughput *in vivo* model system for identifying muco-active reagents using *Xenopus laevis* embryos. We tested mucus secretion under various conditions and developed a screening strategy to identify potential muco-regulators. Using this novel screening technique, we identified narasin as a potential muco-regulator. Narasin treatment of developing *Xenopus* embryos significantly reduced mucus secretion. Furthermore, the human lung epithelial cell line, Calu-3, responded similarly to narasin treatment, validating our technique for discovering muco-active reagents. In addition, we figured out diesel particulated matter hindered mucus secretion and α-tocopherol recovered this symptom.

184 Investigating the role of the autism-associated gene *DYRK1A* in brain development. *Y. Xu*[1,2], H.R. Willsey[1], C.R.T. Exner[1], A. Kim[2], J. Mandell[3], Q. Qi[3], A.J. Willsey[1,3], R.M. Harland[2], M.W. State[1] 1) Department of Psychiatry, Weill Institute for Neurosciences, University of California, San Francisco; 2) Department of Molecular and Cell Biology, University of California, Berkeley; 3) Institute for Neurodegenerative Diseases, Weill Institute for Neurosciences, University of California, San Francisco.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder of undetermined etiology, characterized by early onset of deficits in social communication and behavior. Advances in gene discovery have led to the identification of more than 65 ASD genes. *Dual specificity tyrosine-phosphorylation regulated kinase 1A* (*DYRK1A*) is one of the most strongly associated genes and also represents an attractive therapeutic target as small molecule modulators are well characterized. Here we leverage CRISPR/Cas9 genome editing in *Xenopus tropicalis* to understand the role of *dyrk1a* in brain development and to determine whether pharmaceutical manipulation affects its function. We observe that *dyrk1a* is expressed in neural tissues throughout neurogenesis and that CRISPR/Cas9 mutagenesis of *dyrk1a* leads to macrocephaly, particularly affecting the telencephalon. Analysis of progenitor cell number shows an expansion following loss of *dyrk1a,* and gene expression profiling suggests putatively affected pathways. Similarly, inhibition of *dyrk1a* by a specific small molecule inhibitor phenocopies these results. This suggests that activators of this gene pathway may rescue this phenotype, opening the door for future small molecule screens. This study highlights the advantages of *Xenopus tropicalis* to understand the developmental biology underlying genes associated with neurodevelopmental disorders as well as its potential for identifying therapeutics.

185 Comparative Homeolog Expression Variability and Cis-regulatory Analysis in the allotetraploid frog, *Xenopus laevis*. *R. Cutler*, J. Kim, T. Tseng, M. Saha College of William and Mary.

A convergence of two *Xenopus* diploid species in a polyploidization event 17-18 million years ago resulted in the allotetraploid *Xenopus laevis*. This recent polyploidization event has resulted in over half of the homeologous genes to be retained. However, expression patterns between homeologs have been noted to be quite variable and unstable over development. Using RNA-sequencing data over the time course of development,

we have characterized the expression variability in homeologs and find these to have greater than expected variances as compared to Non-homeologous genes in the *X. laevis* genome. In addition, we find in concordance with others that a larger proportion of expression between homeologs is L biased. However, the majority of expression variation is biased towards S homeologs. Highly similar protein-coding homeolog pairs provide a unique opportunity study contributions of cis-regulatory changes to the observed biased and variable expression patterns on a genome-wide scale. To address this, we have extracted and compared the upstream and downstream cis-regulatory regions of 10,679 homeolog pairs. From preliminary analysis, 76% of upstream regions share alignments where these are on average 2221bp long and have over 80% nucleotide identity. Homeologs with the highest alignment scores are enriched in nervous system development and patterning. Initial correlations of upstream alignment scores and homeolog expression bias were not significant, suggesting that the gene expression relationship between homeologs is complex. We also performed RNA-sequencing experiments to characterize the time course of changes in homeolog bias in response to physical and genetic developmental perturbations. We find that 67% of the differentially expressed genes are homeologs on average, which is much higher than the 23% of paired homeologs found in the genome. Moreover, an average of 80% of differentially expressed homeologs exhibit a change in expression bias due to developmental perturbation which may also be involved in the

compensatory response. These high rates of changes in bias highlight the differential role that homeologs play in the response to perturbations during development which may be either be to sub or neofunctionalization.

186 Gene structure analysis of *Xenopus* chemokines and their receptors. *A. Fukui*[1], M. Matsunami[2] 1) Faculty of Science and Engineering, Chuo University, Tokyo, JP; 2) Graduate School of Medicine, University of the Ryukyu, Okinawa, JP.

Chemokines, relatively small secreted-proteins, are involved in cell migration and function in various biological events including immunity, morphogenesis, and disease. Due to its nature, chemokines tend to be a target of hijacking of immunity by virus and therefore show particularly high mutation rate. *Xenopus laevis* is considered to be a good model to investigate the effect of whole genome duplication for gene family evolution. Because its allotetraploidization occurred just around 17-18 million years ago, ancestral subgenomes L/S were well conserved. In this study, we identified the whole chemokine and their receptor genes in *Xenopus* and estimated their evolutionary process. Based on the gene model of *Homo sapiens* and *Xenopus tropicalis*, we searched *X. laevis* genome assembly v9.1 and identified 50 chemokine genes that contain 42 homeologous genes (21 pairs) and 8 singletons. *X. tropicalis* had 28 chemokine genes and the retention rate of the gene in *X. laevis* L subgenome was 96% (27/28) and that of S was 79% (22/28). Furthermore, 26 chemokine receptors were identified in *X. laevis* genome and 18 of them (9 pairs) were homeologous pairs, and singletons were 8 genes. There were 17 genes in *X. tropicalis* and the retention rate of the gene in *X. laevis* L and S subgenome was 100% (17/17) and 53% (9/17), respectively. The average of amino acid sequence homology between homeologs was 88% for the receptor and 77% for the ligand. We conducted molecular phylogenetic analysis by using 4 vertebrate species (*H. sapiens, Gallus gallus, X. laevis, and X. tropicalis*). We found that the all receptor genes (17/17) showed clear orthology in phylogenic tree among 4 species, but only 45% (13/29) of the ligand genes retained clear orthology, suggesting that the mutation rate particularly increased in the ligand. Regarding ligand-receptor pair of *cxcl12* and *cxcr4*, the expression level of *cxcl12.S* was lower than *cxcl12.L*, while the expression levels of *cxcr4.L/S* pair are similar. Thus, both homeologs of *cxcl12* may be conserved due to gene compensation or subfunctionalization. These results revealed that the whole genome duplication in *X. laevis* promotes diversification of chemokine ligands while conserve the genes necessary for homeostasis, suggesting that selective pressure also promotes rapid evolution of the chemokines in amphibians.

187 Geneolgy and pedigrees of inbreeding strains of *Xenopus tropicalis*. *T. Igawa*[1], A. Kashiwagi[1], K. Kashiwagi[1], N. Suzuki[1], A. Watanabe[1], A. Suzuki[1], A. Noble[2], M. Guille[2], D.E. Simpson[3], M. Horb[4], T. Fujii[5], M. Sumida[1], H. Ogino[1] 1) Amphibian Research Center, Hiroshima University, Higashi-Hiroshima, Hiroshima, JP; 2) Institute of Biomedical and Biomolecular Science, University of Portsmouth, Portsmouth, UK; 3) The Wellcome Trust/Cancer Research UK Gurdon Institute, The Henry Wellcome Building of Cancer and Developmental Biology, University of Cambridge, Cambridge, UK; 4) Bell Center for Regenerative Biology and Tissue Engineering and National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA, US; 5) Department of Health Sciences, Faculty of Human Culture & Science, Prefectural University of Hiroshima, Hiroshima, JP.

The Western clawed frog, *Xenopus tropicalis*, is a highly promising model amphibian, especially in developmental and physiological research, and as a tool for understanding disease. It was originally found in the West African rainforest belt, and was introduced to the research community in the 1990s. The major strains thus far known include the *Nigerian* and *Ivory Coast* strains. However, the genetic relationship among the various strains has not yet been clarified, and establishment of inbred strains is still under the way. Since 2002 the Amphibian Research Center (ARC), Hiroshima University has maintained stocks of multiple *X. tropicalis* strains and conducted consecutive breeding as part of the National BioResource Project. We investigated the inbreeding ratio and genetic relationship of four inbred strains at ARC, as well as stocks from other institutions, using sixty highly polymorphic microsatellite markers and mitochondrial haplotypes. Our results show that the *Ivory Coast* strain clearly differs genetically from the *Nigerian* strains, and three subgroups were identified within both the *Nigerian* and *Ivory Coast* strains. It is noteworthy that the *Ivory Coast* strain has an evolutionary divergent genetic background. Over the past three years we confirmed a higher inbreeding ratio and successive reduction of heterozygosity in the genome of the four strains maintained at ARC. The offspring are still vigorous in viability, and multiple transgenic offspring for imaging analyses were effectively generated from them.

188 Neural progenitors selectively recapitulate embryonic gene regulatory programs during vertebrate regeneration. *A. Kakebeen*, A. D. Chitsazan, A. Wills Biochemistry, University of Washington, Seattle, WA.

While some vertebrates can regenerate their spinal cord (SC), we still lack a fundamental understanding of how they access a regenerative program that is not available in humans. Our lab takes advantage of the transient regenerative competency of *Xenopus tropicalis* tadpoles to reveal the molecular mechanisms enabling SC regeneration. A critical cell population for SC regeneration is the neural progenitor cell population (npc), which balance self-renewal and neurogenesis. During embryonic patterning, npc domain specification and subsequent neurogenesis are driven by opposing bmp and shh signaling and discrete transcriptional programs. I am interested in addressing whether npc regeneration recapitulates development and reuses these same processes, or applies them in a unique way to enable tissue remodeling. Addressing this question, I am taking a genomics-based approach to define the regulatory networks enabling regeneration. One limitation we previously faced in *Xenopus* was that genomic work has been restricted to mixed cell-type samples. To study specifically npc in regeneration, I optimized a method to FACs purify npc from the endogenous GFP expression of the *pax6:GFP* transgenic line and use these cells for genomic approaches. Using FACs purified npc, I examined chromatin accessibility over a regeneration time-course to define the regulatory network governing neurogenesis and self-renewal. ATAC-Seq libraries made from npc in 0, 6, 24, and 72 hours post amputation (hpa) identified accessible regions of the chromatin specific to each regeneration timepoint. Using the list of accessible regions as input for gene ontology analysis, I resolved temporally distinct chromatin accessibility landscapes associated with tube morphogenesis at 6hpa, neurogenesis prioritized at 24hpa, and stem cell maintenance and proliferation prioritized at 72hpa in npc. This temporal ordering reflects key differences from development, with differentiation prioritized earlier than proliferation. Analysis of accessible regions specific to each time-point also revealed that npc differentially utilize embryonic transcription factors at each timepoint to drive these processes during regeneration. Our data

provides critical insight into how regeneration exploits embryonic transcriptional regulatory programs during SC regeneration, and the ways regenerative programs may uniquely differ from embryogenesis.

189 RNA polyadenylation during oogenesis in *X. laevis*. *F. Yang*[1,2], W. Wang[1], M. Blower[1,2] 1) Department of molecular biology, Massachusetts General Hospital, Boston, MA; 2) Department of genetics, Harvard Medical School, Boston, MA.

It has been reported that maternal mRNAs are transcriptionally silent but translational active from the late oogenesis (Prophase I) to early development. RNA polyof adenylation is an important posttranscriptional regulation during these stages. Here we prepared and maturated the oocytes of stage VI (Prophase I) from *X.laevis*. Using H1 kinase assay, we chose oocytes at five time points from Prophase I to Metaphase II for the following genome-wide analysis of polyadenylation and translational efficiency. Using a RNA-seq method specific to 3'end of mRNA, we measured the global polyA tail length for mRNAs, and found a global polyadenylation pattern from Metaphase I which is confirmed by Hire-PAT. Our ribosomal profiling results showed that the global translational efficiency was decreased from Metaphase I. Additionally, our GO analysis found that a series of genes' expression level are increased as a result of their polyadenlyation during oogenesis. We are now studying the roles of these genes play in modulating accuracy and progress of meiosis in *X.laevis*.

190 Mechanical stimulation induces rapid phosphorylation-dependent signaling in *Xenopus laevis* embryos. *Y. Hashimoto*[1,2], N. Kinoshita[2], T.M. Greco[1], P. Jean Beltran[1], J. Federspiel[1], N, Ueno[2], I.M. Cristea[1] 1) Princeton University, Princeton, NJ; 2) National Institute for Basic Biology, Aichi, Japan.

Cells sense and respond to not only molecular factors, but also mechanical forces, which play important roles in biological events, such as tissue homeostasis and cell migration during development. Although some molecular factors, such as Wnt, have been identified as important for gastrulation, the mechanisms underlying signal transductions from physical forces remain elusive. Here, we used quantitative phosphoproteome, whole proteome, and targeted mass spectrometry analyses, as well as functional assays using kinase inhibitors, to investigate mechanosensing pathway in *Xenopus laevis* embryos. We first optimized the method of force stimulation by centrifugation of *Xenopus laevis* embryos, and confirmed that the morphology of the embryos changed from spheres to oblate spheroids following the physical force stimulus. We collected samples at different time points of mechanical force stimulation, and performed proteomic and phosphoproteomic analyses. We detected over 3,500 proteins in each whole proteome sample, and over 8,500 phosphorylated peptides in each sample following TiO_2 phosphoenrichment. Integration of these datasets and enrichment analysis based on information theory led us to identify the enrichment in cell-cell adhesion components within the upregulated phosphosites during force stimulation. We further characterized force-induced kinase profiles using kinase substrate database created from PhosphoSitePlus database. These analyses indicated that basophilic motifs are enriched rapidly upon mechanical stimulation. We confirmed the changes in phosphorylation using quantitative targeted mass spectrometry based on parallel reaction monitoring (PRM). Lastly, we interrogated the function of these alterations in phosphorylation using treatment with a kinase inhibitor. This inhibitor analysis revealed a novel crosstalk of kinases in response to mechanical force stimulation. Overall, this is the first study of proteomic and phosphoproteomic analysis in *Xenopus laevis* during mechanical sensing.

191 Effect of Passive Diffusion on Nucleocytoplasmic Partitioning. *N. Pappireddi* Princeton University, Princeton, NJ.

The distribution of proteins between the cytoplasm and the nucleus of eukaryotic cells plays a key role in determining many important cellular processes. Since proteins are synthesized in the cytoplasm, they must pass through the nuclear pore complex in order to reach the nucleus. This occurs via either passive diffusion or active transport, with the help of karyopherins and the Ran-GTP pathway. The literature contains contradicting evidence on what protein size is compatible with passive diffusion through the nuclear pore. In order to examine passive diffusion between the cytoplasm and nucleus, I worked with *Xenopus laevis* oocytes, since these cells allow manual nuclear isolation and contain large protein amounts. I used quantitative multiplexed mass spectrometry to quantify the relative amounts of hundreds of microinjected *E. coli* proteins in the cytoplasm and nucleus. Since bacterial proteins are less likely to have nuclear localization signals than eukaryotic proteins, this reduced the influence of active transport. By quantifying partitioning at various times post-injection and extrapolating the native molecular weights of the bacterial proteins in complexes, obtained from filtration experiments, I related the native molecular weight of a protein to its passive diffusion rate. These and future experiments will lead to a better understanding of how diffusion affects the cell's ability to create and maintain nucleocytoplasmic partitioning.

192 Conserved unusual wiring properties in the olfactory system of two *Xenopus* species. *T. Hassenklöver*[1], L. Weiss[1], T. Offner[1,2], K. Dittrich[1], I. Manzini[1,2] 1) Dep. of Animal Physiology and Molecular Biomedicine, Justus-Liebig University Giessen; 2) Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), University of Göttingen.

The olfactory system of *Xenopus laevis* is perfectly adapted to detect both water- and air-borne odors and displays certain morphological and functional peculiarities that are exceptional when compared to the system of rodents or fish. However, by investigating the olfactory system of *Xenopus tropicalis* we found evidence that these features might be more relevant and widespread among amphibians.
The olfactory organ of larval Pipidae consists of a main olfactory epithelium and a vomeronasal organ, with an additional epithelial surface developing during metamorphosis. Receptor cells of the tripartite epithelium project to distinct regions of the olfactory bulb. There, receptor neuron axons form synaptic connections with the dendrites of mitral tufted cells, in dense neuropil structures called glomeruli. We could show that the axons of single receptor cells of both *Xenopus* species bifurcate and mostly innervate more than one glomerulus, challenging the predominant opinion about olfactory wiring. Labelling of single mitral tufted cells by dye electroporation displayed a multiglomerular connection pattern as well. Functional assessment of the larval system was done using calcium imaging in both the main olfactory epithelium and olfactory bulb. Two segregated odor-processing streams in the receptor neuron population seem to be conserved between the two species: a cAMP-dependent medial stream and a lateral stream apparently independent of the canonical cAMP signal transduction. As one major difference we observed that cells in the olfactory epithelium as well as in the bulb of *Xenopus tropicalis* appear to be smaller than in *Xenopus laevis*, which might be due to their different ploidy level.

The vastly conserved morphology and function within the olfactory system of the Pipidae family sets the ground for future experiments in our attempt to understand the changes in the olfactory system beneficial for a successful transition from water to land.

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193 Apoptosis is Required for Embryonic Eye Regrowth in *Xenopus laevis*. *C.X. Kha*, K. Tseng School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV.

Vertebrate eye development is intricate and initiated early during embryogenesis where a coordinated, multi-step signaling process occurs during the interaction of the prospective lens ectoderm and prospective retina neuroectoderm. *Xenopus laevis* has the ability to regenerate single eye tissues such as the retina, lens, and optic nerve. However, it was unclear if young embryos would have the ability to restore lost eye tissues. We found that st. 27 tailbud embryos can regrow eyes after surgical removal of over 83% of eye tissues (Kha et al., 2018). The regrowth process is rapid as the new eyes reached comparable size and structure to age-matched controls within 5 days. The regrown eye contained the expected cell types and structures, including the retinal pigmented epithelium, retina, and lens, and was connected to the brain via an optic nerve. Tadpoles with a single regrown eye displayed visual preference in a behavioral assay, similar to tadpoles with a normal eye. In contrast, tadpoles with a single regrowth-inhibited eye failed to display visual preference. These results indicated that visual function is restored by regrowth. To identify a mechanism that drive this eye regrowth process, we assessed the role of apoptosis (programmed cell death). Apoptosis is known to be required for tissue regeneration in multiple species. Treatment with two different apoptosis inhibitors (M50054 or NS3694) blocked cell death in the eye and inhibited eye regrowth, demonstrating that this is a required mechanism. This new developmental eye repair model will serve as the basis for defining eye stem cells capable of productive repair and facilitate identification of genes that induce eye regrowth.

194 Nutrient Restriction causes Reversible G2 Arrest in Xenopus Neuronal Progenitors. *C.R. McKeown*, H.T. Cline Dept. of Neuroscience, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA.

Nutrient status affects the developing brain, yet the effect of nutrient restriction and food availability on a cellular level *in vivo* is poorly understood. In the absence of external nutrients, *Xenopus laevis* tadpoles enter a period of developmental stasis during which neural progenitor cell proliferation is drastically reduced, with proliferation synchronously resuming when food becomes available. Here we investigate the mechanisms by which neural progenitors halt cell division in response to nutrient restriction and then re-enter the cell cycle upon feeding. We demonstrate that nutrient restriction causes tectal progenitors to stop progression through the cell cycle after S phase, and that the reintroduction of nutrients triggers progenitors to synchronously re-enter the cell cycle at M-phase, suggesting cells in stasis are paused at G2. Consistent with a model for G2 arrest, we find that levels of phosphorylated cdc2 are decreased upon stasis entry and return upon the resumption of feeding. We demonstrate that progenitors along the tectal midline have increased DNA content in response to nutrient restriction, further supporting a G2 arrest model. We also show that initiation of the nutrient-restriction-induced G2 pausing is rapamycin-insensitive, but cell cycle re-entry requires mTOR signaling. This capacity of neural progenitors to pause cell cycle progression in G2 provides a mechanism to control proliferation in response to nutrient availability and yet allows cells to be poised to divide quickly when nutrients become available. This may be a general cellular mechanism that allows for developmental flexibility during times of limited resources.

195 *Xenopus* tadpoles as a model for traumatic brain injury: response to focal impact injury. Ray Torres[1], Christina Ulrich[1], Vrutant Shah[1,2], Melissa Zamora[1,3], Bryan Lavergne[1], Jonathan Teetsel[1], Kathleen Gajewski[1], *Amy Sater*[1] 1) Dept. of Biology and Biochemistry, University of Houston, Houston, TX; 2) Dept. of Epigenetics and Molecular Carcinogenesis, MD Anderson Cancer Center, Houston TX; 3) McGovern Medical School, Univ. of Texas Health Science Center, Houston TX.

We have developed a focal impact injury model to support studies of Traumatic Brain Injury (TBI) in *Xenopus laevis*tadpoles. The long-term goal of this work is to generate a model for the study of TBI that would support large-scale pharmacological screens for compounds that promote neural regeneration and repair. Metamorphic tadpoles (st. 52-56) were subjected to a focal injury to the right midbrain, consisting of a pneumatically-driven force of approximately 2.5 lb applied to an area of approximately 0.75 sq. mm. Injured tadpoles were allowed to recover for intervals and then collected for molecular, cellular, or behavioral studies. Quantitative RT-PCR assays on RNA from isolated brains demonstrate that focal injury leads to a rapid increase in expression of genes associated with mammalian reactive astrocytes (e.g., *timp1, steap4*), and elevated expression of genes characteristic of radial glia (e.g., *nestin*) and astrocytes (e.g., *fabp7, vimentin*). Focal injury also elicits increased expression of the neuroprotective gene *clusterin*, although expression of *bdnf* remained unchanged. Preliminary BrdU labeling studies showed that there is an increase in cell proliferation 24-48 hours after injury; proliferation is not localized to the injury site. Preliminary immunofluorescence studies suggest that the ependymal cilia may be disrupted by injury. Quantitative analyses of tadpole swimming behavior demonstrate that injured tadpoles show difficulty in maintaining upright orientation, shorter intervals of activity, and reduced activity overall for at least 3 days following injury. However, focal impact injury followed by a 3-day recovery does not significantly affect performance in the Visual Preference assay, suggesting that visual processing is relatively unimpaired by this treatment. Our results establish a foundation for the analysis of regenerative or inflammatory responses to traumatic brain injury, and for future pharmacological screens.

196 Ketamine Modulates Zic5 Expression via the Notch Signaling Pathway in Neural Crest Induction. *Y. Shi*[1,2,3], Jiejing Li[4], Chunjiang Chen[2,3], Yongwu Xia[1,2,3], Yanxi Li[2,3], Pan Zhang[5], Ying Xu[5], Tingyu Li[2,3], Weihui Zhou[2,3], Weihong Song[2,3,6] 1) Clinical Lab, Children's Hospital of Chongqing Medical University, Chongqing, CN; 2) Chongqing City Key Lab of Translational Medical Research in Cognitive Development and Learning and Memory Disorders, Children's Hospital of Chongqing Medical University, Chongqing, CN; 3) Ministry of Education Key Lab of Child Development and Disorders, Children's Hospital of Chongqing Medical University, Chongqing, CN; 4) Department of Clinical Laboratory,

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Ketamine is a potent dissociative anesthetic and the most commonly used illicit drug. Many addicts are women at childbearing age. Although ketamine has been extensively studied as a clinical anesthetic, its effects on embryonic development are poorly understood. Here, we applied the *Xenopus* model to study the effects of ketamine on development. We found that exposure to ketamine from pre-gastrulation (stage 7) to early neural plate (stage 13.5) resulted in disruption of neural crest (NC) derivatives. Ketamine exposure did not affect mesoderm development as indicated by the normal expression of Chordin, Xbra, Wnt8, and Fgf8. However, ketamine treatment significantly inhibited Zic5 and Slug expression at early neural plate stage. Overexpression of Zic5 rescued ketamine-induced Slug inhibition, suggesting the blockage of NC induction was mediated by Zic5. Furthermore, we found Notch signaling was altered by ketamine. Ketamine inhibited the expression of Notch targeted genes including Hes5.2a, Hes5.2b, and ESR1 and ketamine-treated embryos exhibited Notch-deficient somite phenotypes. A 15 bp core binding element upstream of Zic5 was induced by Notch signaling and caused transcriptional activation. These results demonstrated that Zic5 works as a downstream target gene of Notch signaling in *Xenopus* NC induction. Our study provides a novel teratogenic mechanism whereby ketamine disrupts NC induction via targeting a Notch-Zic5 signaling pathway.

197 Characterization of *X. muelleri* laryngeal muscle fiber type using ATPase histochemistry: behavioral and evolutionary implications. Q.R. Ferguson, D. Toglia, *E.C. Leininger* Natural Sciences, New College of Florida, Sarasota, FL.

Xenopus is an excellent model system for investigating how structure and function of peripheral effectors can shape behavior outputs in species- and sex- specific fashions. Most *Xenopus* and *Silurana* species (including *X. laevis*) exhibit sexually dimorphic vocalizations; male calls have rapid inter-pulse intervals (*Xenopus* species including *X. borealis* and *X. muelleri* exhibit reduced vocal sex differences; inter-pulse intervals are not significantly different between sexes, and are longer (>40 ms) than those of many other *Xenopus* species. We have shown previously that *X. borealis* laryngeal muscle contains fast and slow twitch fibers in both sexes, suggesting that the sexually differentiated laryngeal muscle fiber type characteristic of other species has been lost in *X. borealis*. The goal of the present study is to determine whether loss of a sexually differentiated fiber type is unique to *X. borealis* or shared with *X. muelleri*, a closely related species with similarly reduced vocal sex differences. Aspects of muscle fiber anatomy, such as cross-sectional area, are sexually dimorphic in this species. ATPase histochemistry under acidic preincubation conditions revealed a mixture of acid-sensitive and acid-stable fibers in female laryngeal muscle (indicating a mixed twitch type) but exclusively acid-sensitive fibers in male laryngeal muscle (indicating uniformly fast twitch type), suggesting a sexually differentiated laryngeal muscle fiber type. Additional histochemical and functional validations are underway to confirm this result. Our present findings suggest that evolutionary loss of a sexually differentiated fiber type is likely an isolated event in *X. borealis*, and the loss of vocal sex differences in *X. muelleri* may be more due to evolutionary changes in the vocal hindbrain circuit rather than in the physiology of the laryngeal muscle.