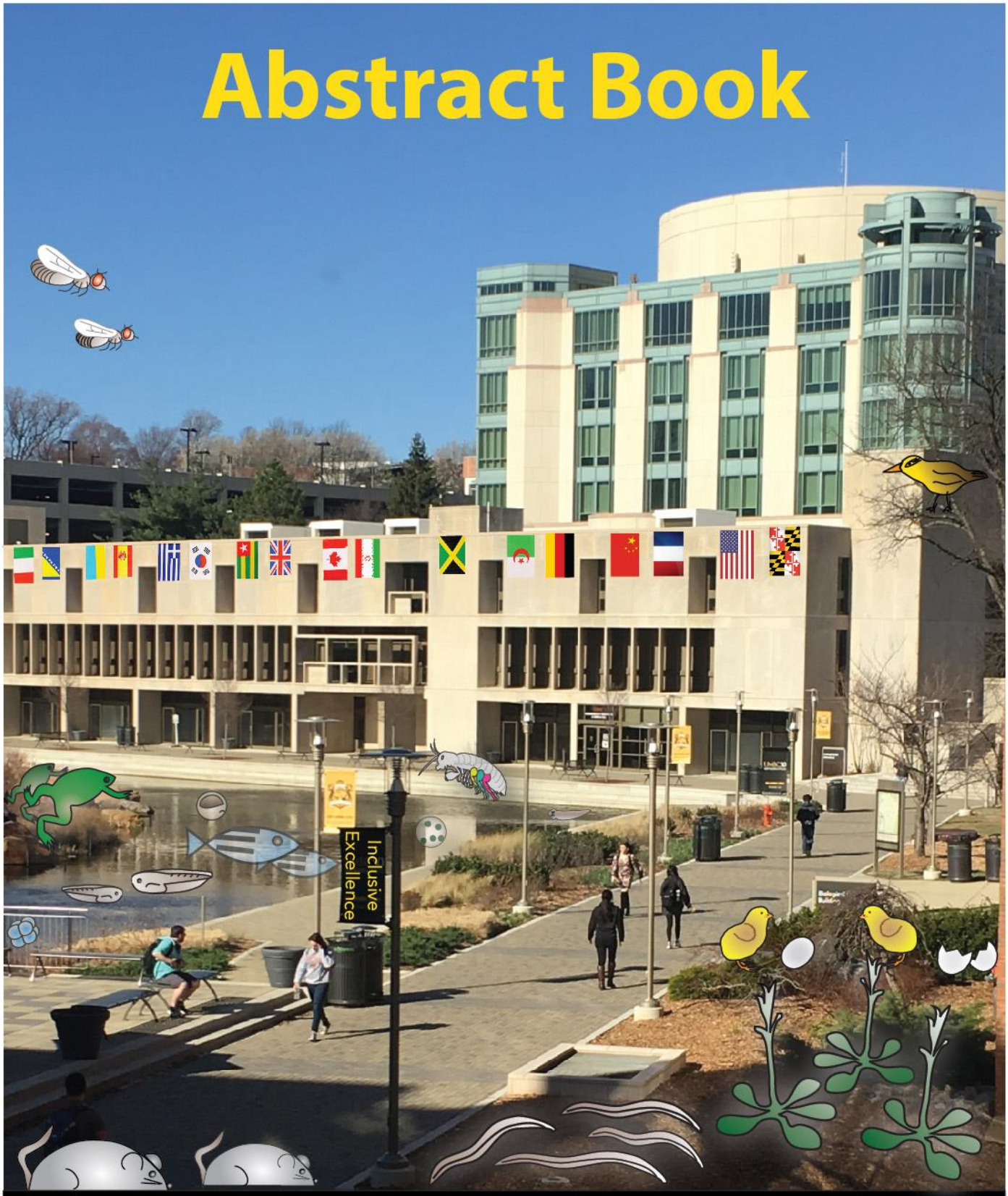
Society for Developmental Biology

Mid-Atlantic Regional Meeting 2017

Abstract Book



University of Maryland Baltimore County

May 19-20, 2017

Keynote Abstracts

WELCOME KEYNOTE: *Friday, May 19th, 1:00-2:00pm, UC Ballroom*
Rethinking the Culture of STEM Education in America: Promoting Student Success and Minority Achievement
Freeman Hrabowski, *President of UMBC*
Rapid and dramatic demographic and technological changes present our nation's schools and universities with enormous challenges for preparing students – particularly those from diverse backgrounds – for careers in science, technology, engineering, and math (STEM) fields. Over the past four decades, Hrabowski has studied minority student achievement, focusing special attention on the participation and performance of African Americans in STEM fields. Drawing on the success of UMBC's Meyerhoff Scholars Program, a national model for preparing high-achieving minority science and engineering students, and the National Academies' report, *Expanding Underrepresented Minority Participation: America's Science and Technology Talent at the Crossroads*, Dr. Hrabowski will discuss innovation in undergraduate STEM education that promotes student success, inclusive excellence, and achievement for all students in STEM.

KEYNOTE 1: *Friday, May 19th, 5:45pm-6:45pm, LH 7*
Evolution and Development: Insights from Emerging Model Systems
Nipam Patel, *University of California, Berkeley*
Studies in emerging model systems have been used to gain insight into the mechanisms of development, and how development evolves to create novel body plans. In the first part of my talk, I will describe our comprehensive analysis of Hox gene expression in the amphipod crustacean, *Parhyale hawaiensis*, and more importantly, our results from using CRISPR/Cas9 gene editing to functionally address the role of Hox genes in crustacean development. I will then describe how this experimental data leads to some new views on the evolution of the arthropod body plan. In the second part I will discuss our work on the developmental basis for structural coloration and transparency in butterflies. While both structural color and transparency have been analyzed in great detail by optical physicists, we are now making headway in uncovering the genetic and cell biological basis for these phenomena.

KEYNOTE 2: *Saturday, May 20th, 3:00-4:00pm, LH 7*
Modeling human brain development and developmental diseases using hiPSCs
Guo-li Ming, *University of Pennsylvania, USA*
Three dimensional (3D) cerebral organoid cultures from human iPSCs have been recently developed to recapitulate the cytoarchitecture of the developing brain. This system offers unique advantages in understanding molecular and cellular mechanisms governing embryonic neural development and in modeling congenital neurodevelopmental disorders, such as microcephaly. We have improved the organoid technology and developed a protocol to produce forebrain-specific organoids derived from human iPSCs using a novel miniaturized spinning bioreactor that recapitulate the human embryonic cortical development. ZIKV, a mosquito-borne flavivirus, has re-emerged as a major public health concern globally because ZIKV causes congenital defects, including microcephaly, and is also associated with Guillain-Barré syndrome in infected adults. We found that ZIKV exhibit specific tropism towards human neural progenitor cells and results in cell death and defects in neural development. I will discuss our recent work in further dissecting the molecular mechanisms underlying the ZIKV pathogenesis and microcephaly.

Invited Speaker Abstracts

1

p53 deletion rescues brain size in a novel model of microcephaly

Jessica Neville, Noelle Dwyer, *University of Virginia, USA*

The development of any tissue requires tight temporal control of cell proliferation, fates, and death. Growth of the cerebral cortex requires precise divisions of neuroepithelial stem cells (NSCs) that undergo both symmetric and asymmetric divisions to first expand and then produce post-mitotic neurons. How this is mediated is poorly understood. One consequence of impaired NSC proliferation is microcephaly, or a small brain. In a novel mouse model for microcephaly discovered by our lab, the mutation of the kinesin Kif20b results in reduced brain size at birth. Interestingly, abnormalities in cytokinesis of NSCs and increased apoptosis were noted in *Kif20b-/-* cortex. Our hypothesis is that the loss of Kif20b causes impaired cytokinesis that results in apoptosis in a subset of NSCs, decreasing the progenitor pool. To test our model for the etiology of *Kif20b-/-* microcephaly, we set out to prevent apoptosis of NSCs and observe whether microcephaly could be rescued. We crossed the Kif20b mutant mouse line to knockouts for the pro-apoptotic proteins p53 and Bax. We found that p53 deletion rescues microcephaly in *Kif20b-/-* mice, but Bax deletion does not. p53 but not Bax deletion rescued the elevated apoptosis seen in *Kif20b-/-* brains, providing further evidence for the central role of apoptosis in *Kif20b-/-* microcephaly. Although overall brain size and neurogenesis was rescued by p53 knockout, future experiments will determine if the final brain structure and lamination of double mutant mice are normal. In addition to rescuing brain size, deletion of p53 rescued neonatal lethality, decreased body size and craniofacial abnormalities in *Kif20b-/-* mice, implicating p53 activation in the phenotypes seen outside the brain in Kif20b mutants. Current work is focused on determining 1) the relationship between cytokinesis defects and p53 activation in *Kif20b-/-* mice and 2) the consequence of cytokinesis failure for undead cells when apoptosis is inhibited.

2

Cadherin-6B proteolytic fragments promote cranial neural crest cell EMT and delamination

Lisa Taneyhill, *University of Maryland, College Park*

During epithelial-to-mesenchymal transitions (EMTs), cells disassemble cadherin-based intercellular junctions to permit their segregation from the surrounding intact epithelium. Chick premigratory cranial neural crest cells reduce existing Cadherin-6B (Cad6B) levels through several mechanisms, including proteolysis, to permit their EMT and migration. Proteolytic cleavage of Cad6B generates shed N-terminal fragments (NTFs) and intracellular C-terminal fragments (CTF2s) that possess novel, adhesion-independent roles in the cranial neural crest. These findings reveal how Cad6B proteolysis orchestrates multiple pro-EMT regulatory inputs via the generation of distinct fragments, providing insight into how cadherins regulate both normal developmental and aberrant EMTs that underlie human disease.

3

Splitting signals - the impact of division on inductive signaling in the *Ciona* heart progenitor lineage

Bradley Davidson, *Swarthmore College*

Recent studies have overturned the long-held assumption that membrane trafficking is shut down during mitosis. Thus, mitotic trafficking of signaling components may play a profound, largely unrecognized role in cell fate specification. We are investigating the impact of division on

signal processing through high-resolution, in vivo analysis of Fibroblast Growth Factor (FGF) dependent induction of the heart progenitor lineage in embryos of the basal chordate, *Ciona intestinalis*. We have found that mitotic redistribution of FGF receptors (FGFRs) promotes differential heart progenitor induction. Through targeted disruption of mitotic kinases, we have begun to determine how endocytic FGFR trafficking is regulated during mitosis. We have also begun to delineate which endocytic pathways are involved in internalization and re-distribution of FGFR through co-localization analysis using pathway specific markers along with targeted disruption of pathway specific components. Our results support a two part model. During mitotic entry, it appears that CDK1 mediates Rab21 dependent internalization of FGFR into Rab11 recycling vesicles. During mitotic exit, PLK1 mediates re-distribution of FGFR along the adherent cytokinetic furrow. Our studies of mitotic receptor trafficking during *Ciona* heart progenitor induction provide fundamental insights regarding the interplay between division and signaling in both embryonic and stem cells.

4

Smooth muscle differentiation shapes domain branches in the developing mouse lung

Katharine Goodwin, Celeste M. Nelson, *Princeton University, USA*

During branching morphogenesis, a simple cluster or tube of cells proliferates and branches to generate an arborized network that facilitates the flow and exchange of gases or fluids. Branched organs are found throughout the animal kingdom, and while their function varies, their morphogenesis is driven by similar molecular programs. To achieve a specific final morphology, physical cues are required to guide branching of the epithelium into the surrounding mesenchyme. During murine lung branching morphogenesis, the airway epithelium develops concomitantly with a layer of smooth muscle, which is derived from the mesenchyme and wraps around the airways. Specific spatial patterns of smooth muscle differentiation are required for terminal bifurcation. Here, we examined the role of smooth muscle differentiation in shaping emerging domain branches (branches that bud laterally off the side of an existing branch) during early lung development. The position and morphology of domain branches are highly stereotyped: branches begin as wide buds that thin at their base as they elongate. At the same time, there is an increase in the amount of smooth muscle wrapped around the parent branch at the base of the new domain branch. Perturbing the pattern of smooth muscle differentiation results in abnormal branch positioning and morphology. Loss of smooth muscle results in ectopic branching events and slows branch thinning, and enhanced smooth muscle differentiation suppresses branch formation and elongation. Our work uncovers a role for smooth muscle differentiation in sculpting emerging domain branches, and sheds light on the physical mechanisms of branching morphogenesis of the mouse lung.

5

FGF-Notch Interactions in Somite and Vertebral Formation

Matthew Anderson, Mark Lewandoski, *National Cancer Institute, USA*

Fibroblast growth factors (FGFs) are critical components of the network of intercellular signaling events that transform the fertilized egg into the complex multi-tissue animal. One such requirement for FGFs is in extension of the vertebrate embryonic axis. The axis extends through reiterative addition of segments of tissue, called somites, to the posterior of the embryo. As development proceeds, somites transform into muscle, dermis, and vertebrae. Somites form from a progenitor tissue called the presomitic mesoderm (PSM). Somitogenesis occurs within the PSM at a position defined by opposing activities of FGF and Retinoic Acid (RA) signaling. Previously our lab has shown that FGF4 and FGF8 maintain the PSM in a progenitor state. In this study, I show that PSM-specific FGF4 has a unique role in patterning a subset of the

vertebral column. Genetic deletion of *Fgf4* within the PSM causes vertebral defects in the anterior axis, specifically in the cervical and thoracic vertebrae, that resemble human syndromes such as scoliosis and spondylocostal dysostosis. I traced these vertebral abnormalities back to defects in the formation of the corresponding somites. Utilizing multicolor fluorescent in situ hybridization of mRNA, lightsheet microscopy, and live imaging we examined signaling pathways known to be involved in somite segmentation. This analysis revealed defects in the oscillatory pattern of Notch gene expression in the PSM that are required for PSM segmentation. Furthermore, RA signaling is increased within *Fgf4* mutant PSM, and genetic reduction of RA signaling restores Notch gene oscillation to *Fgf4* mutants. I therefore propose a model, whereby *Fgf4* negatively regulates RA signaling in the PSM to allow for oscillation of Notch signals and proper somite segmentation and vertebral formation. None of the previously characterized molecular players of FGF-RA interactions are affected in this mutant, indicating a novel mechanism may be acting.

6

The IMSD Meyerhoff Graduate Fellows Program: Developing Inclusive Excellence in Doctoral STEM Training

Michael Summers, *UMBC*

The IMSD Meyerhoff Graduate Fellows Program has had a dramatic impact on PhD-level training of Underrepresented (UR) students at The University of Maryland, Baltimore County (UMBC). Since its inception in 1997, UR participation in supported departments (biology, chemistry, biochemistry, chemical/ mechanical engineering, human services psychology, and physics) has increased from an average of ~3% to ~17% today. Expansion in 2007 to the Graduate Program in Life Science (GPILS) at our sister campus, the University of Maryland Baltimore (UMB), triggered a doubling in UR GPILS enrollment at UMB. Total IMSD enrollment continues to grow, with 102 IMSD UR PhD students enrolled in the Fall of 2017. Activities that promote recruitment, retention, and high student performance outcomes will be presented.

7

Immersion Science Program: Inclusion of High School Students in Novel Laboratory

Dara Ruiz-Whalen[1], Alana O'Reilly[1,2], *[1]Fox Chase Cancer Center-Immersion Science Program, USA; [2]Fox Chase Cancer Center- Molecular Therapeutics Program, USA*

The development of all living organisms depends on a balanced diet. 75 years ago, nutritional requirements of Drosophila melanogaster were defined, resulting in development of a chemically defined culture medium that has been used to mechanistically connect genetic mutations and developmental defects without influence of dietary variation. The power of this system has not been used for the converse, however, to determine how nutrients influence developmental signal transduction. We propose that a balanced diet is defined as the sum total of dietary compounds necessary to trigger responses in multiple cell types required for organ function, with each nutrient eliciting a specific signaling response. To create a large scale and low cost method for achieving testing this idea, we developed a laboratory research training program for high school students called the Immersion Science Program (ISP). Students first learn boot-camp lab techniques to uncover specific dietary compounds that elicit phenotypic responses linked with specific developmental signal transduction pathways. Based on screening results, students develop self-designed hypotheses and experimental plans to determine the cellular and molecular consequences of nutrient treatment on flies bearing mutations in signaling effectors linked with observed phenotypes. To date, 95 students have completed our in-house program, and we recently extended the approach to high school classrooms in the

Philadelphia region, resulting in two scientific publications since 2013. Our approach is dually powerful: in the short term, we will genetically map targets of dietary supplements using a comprehensive developmental genetics approach, while providing hundreds of students each year with the skills necessary to succeed in STEM majors. Our long-term goal is to uncover new general principles governing the structural and dynamic effects of dietary compounds on protein function in vivo. This research is supported by NIH and SDB.

8

The "mixed-bag"- a year-long approach for undergraduate research

Valerie Olmo, *George Mason University*

There is no disputing the importance of undergraduate research for students receiving a natural science degree. It is known that incorporation of inquiry-based courses and independent research opportunities for undergraduates improves critical thinking skills, students' foundational knowledge of biological concepts and a deeper understanding of the scientific process. In fact, many post-baccalaureate opportunities now prefer, or even require, undergraduate research experience. As a result, many biology departments have begun incorporating varying degrees of research-oriented courses and programs to fill the demand for undergraduate research opportunities. Undergraduate research experiences are categorized as open-ended, guided-inquiry, or independent research depending on the extent to which a faculty mentor is involved in developing the research project. The "mixed-bag" approach to undergraduate research employs all three categories of undergraduate research to generate publication quality data from a year-long project conducted entirely by undergraduate researchers. Initially, students would enroll in an upper-level inquiry-based laboratory elective designed almost entirely by the instructor. Upon completion of the upper-level laboratory course, students may choose to take an advanced version of the upper-level laboratory course for credit to continue the research projects with minimal guidance from the instructor, thus making the course an open-ended research experience since the results are completely unknown. Finally, the project can be concluded as a completely independent research project. This approach provides a greater number of students the opportunity to conduct a full year of undergraduate research within the context of formalized upper-level biology laboratory electives and/or via independent research with the goal of producing publishable results

9

Project BioEYES: K-12 Student-driven science using a live developing zebrafish

Steven Farber, *Department of Embryology, Carnegie Institution*

BioEYES uses zebrafish to excite and educate K-12 students about science and teach both students and teachers how to think and act like scientists. The effort has been integrated into hundreds of under-resourced schools since 2002. During the week-long experiments, students raise zebrafish embryos to learn principles of development and genetics. Analysis of 19,463 participating students' pre- and post-tests designed to assess learning growth and attitude changes towards science found that at all grade levels, BioEYES effectively increased students' content knowledge and produced favorable shifts in students' attitudes about science. These outcomes were especially pronounced in younger students. Having served over 100,000 students, we find that our method for providing student-centered experiences and developing long-term partnerships with teachers is essential for the growth and sustainability of outreach and school collaborations.

10

The microbiota, and not its host, defines the capacity of a toxic compound to impair development and reproduction in C. elegans

Eyleen O'Rourke, *University of Virginia*

Gut microbes, also known as gut microbiota, modify how the human body responds to dietary components to influence or even determine health or disease status. A growing body of evidence shows that both the diet and the microbiota modulate drugs efficacy or toxicity. However, mechanistic understanding of drug-diet-microbiota-host interactions is lacking. Biological complexity remains a major barrier to the full elucidation of the multiple pathways through which microbiota-host co-metabolism operates. Using the genetically tractable nematode C. elegans as the host, and E. coli as the microbiota, we unraveled the mechanisms by which the diet and microbiota interact to modulate the toxicity of the chemotherapeutic agent 5'-fluoro-deoxyuridine (FUdR). I will present evidence demonstrating that toxicity is fully dependent on the microbiota's ability to covert FUdR into its more toxic fluororibonucleotide derivatives, and show that the natural range of microbe-mediated drug bioactivity (toxicity) in E. coli can vary from no effect on fertility or development to complete sterility and developmental arrest. Finally, I will show that these effects can be dramatically modified by single metabolite dietary changes. Mechanistic evidence will therefore be presented supporting a model in which the diet and the gut microbiota functionally interact to define the capacity of toxic compounds to impair reproduction and growth of the host.

11

Regulation of Hub Cell Quiescence in the Drosophila Testis Stem Cell Niche

Tiffaney Tran[1], Zelalem Demere[1], Jhanavi Sivakumar[1], Linh Pham[2], Margaret de Cuevas[3], Leah Greenspan[3], Erika Matunis[3], *[1]Department of Biology, Johns Hopkins University, USA; [2]Department of Biology, Humboldt State University, USA; [3]Department of Cell Biology, Johns Hopkins University School of Medicine, USA*

The stem cell niche is a specialized microenvironment in which stem cells reside and receive local signals that regulate their self-renewal and differentiation. In the well-characterized *Drosophila* testis stem cell niche, somatic hub cells generate many of the essential signals that maintain and regulate adjacent germline stem cells and somatic cyst stem cells (CySCs). These hub cells normally remain quiescent in the adult fly, but recent evidence from our lab has shown that under certain conditions, such as the complete genetic ablation of CySCs, hub cells can transiently exit quiescence, proliferate, and transdifferentiate into functional CySCs. Such findings suggest that upon tissue damage, changes in signaling pathways within the niche may trigger hub cells to proliferate and transdifferentiate into CySCs. To identify the pathways that maintain hub cell quiescence and identity, we conducted a reverse genetic screen in which candidate signaling molecules were knocked down or overexpressed in either the hub cells or the CySCs. Our preliminary results from the screen suggest that the Ras-MAPK and TGFß pathways may contribute to the regulation of hub cell quiescence since the misexpression of specific components from these pathways was shown to promote hub cell proliferation. Our ongoing lineage tracing analyses will determine whether these proliferating hub cells also transdifferentiate into CySCs. These experiments overall contribute to an emerging concept that *in vivo* cellular quiescence is a state that must be actively maintained over time for normal tissue homeostasis. Understanding the signals that regulate cellular quiescence and tissue homeostasis in the *Drosophila* testis may lend greater insight into mechanisms of cancer metastases and novel methods for regenerative therapies.

12

Novel functions for the RNA-binding protein ETR-1 in Caenorhabditis elegans reproduction and engulfment of germline apoptotic cell corpses

Ruby Boateng[1], Ken C.Q. Nguyen[2], David H. Hall[2], Andy Golden[3], Anna K. Allen[1]
[1]Howard University, USA; [2]Center for C. elegans Anatomy, Albert Einstein College of Medicine, USA; [3]National Institutes of Diabetes and Digestive and Kidney Diseases, USA

RNA-binding proteins are essential regulators of gene expression that act through a variety of mechanisms to ensure the proper post-transcriptional regulation of their target RNAs. ETR-1, a highly conserved ELAV-Type RNA-binding protein belonging to the CELF/Bruno protein family, is canonically known for its involvement in C. elegans muscle development. Animals depleted of ETR-1 have been previously characterized as arresting at the two-fold stage of embryogenesis. In this study, we show that ETR-1 is expressed in the hermaphrodite somatic gonad and germ line, and that reduction of ETR-1 via RNA interference (RNAi) results in reduced hermaphrodite fecundity. Detailed characterization of this fertility defect indicates that ETR-1 is required in both the somatic tissue and the germ line to ensure wild-type reproductive levels. Additionally, the ability of ETR-1 depletion to suppress the published WEE-1.3-depletion infertility phenotype is dependent on ETR-1 being reduced in the soma. Within the germline of etr-1(RNAi) hermaphrodite animals, we observe a decrease in average oocyte size and an increase in the number of germline apoptotic cell corpses as evident by an increased number of CED-1::GFP and acridine orange positive apoptotic germ cells. Transmission Electron Microscopy (TEM) studies confirm the significant increase in apoptotic cells in ETR-1-depleted animals, and reveal a delay of the somatic gonadal sheath cells to properly engulf dying germ cells in etr-1(RNAi) animals. Through investigation of an established engulfment pathway in C. elegans, we demonstrate that co-depletion of CED-1 and ETR-1 suppresses both the reduced fecundity and the increase in the number of apoptotic cell corpses observed in etr-1(RNAi) animals. Combined, this data identifies a novel role for ETR-1 in hermaphrodite gametogenesis and in the process of engulfment of germline apoptotic corpses.

13

Understanding the cis- and trans-regulation of Sex lethal in the Drosophila melanogaster germline

Raghav Goyal, Ellen Baxter, Mark Van Doren, *Johns Hopkins University, USA*

In *Drosophila*, sex determination is under the control of *Sex lethal* (*Sxl*). While in some species somatic sex is sufficient to determine germline sex via inductive signaling, in flies, germline sex is also determined cell-autonomously via intrinsic signaling dictated by its sex chromosome constitution. Interestingly, XY (male) germ cells expressing *Sxl* are able to produce eggs upon transplantation into an XX (female) somatic gonad, demonstrating that even in the germline, *Sxl* is sufficient for female identity. In both the germline and soma, the presence of two X chromosomes leads to *Sxl* expression. However, the mechanism of counting the X chromosomes in the germline differs at both *cis* and *trans* levels, and we are studying how this is done. We are performing RNA-FISH against nascent transcripts to understand *Sxl's* transcription initiation dynamics in the germline during embryogenesis. Further, the DNA elements regulating *Sxl*'s sex-specific promoter (*SxlPe*) in the germline have not been identified. To identify these, we are performing a promoter analysis assay in the developing germline. We are also identifying *trans*-acting factors that regulate germline *Sxl* expression. Based on previous studies, the X chromosome "counting genes" that activate *Sxl* in the soma are not required in the germline. However, we found that knocking down one of these genes, *sisterless A* (*sisA*), in the female germline results in an ovarian tumor phenotype and germ cell loss,

similar to masculinization of the germline due to loss of *Sxl*. Encouragingly, *Sxl* expression is lowered in *sisA* RNAi ovaries and the germline loss phenotype can be rescued by *Sxl cDNA.* In flies and humans, the sex of the germline and the soma must be properly coordinated. Since a failure to match germline and somatic sex leads to defects in gametogenesis, our work in understanding how proper sex determination leads to sex-specific gonad development is important for our understanding of reproductive biology and human health.

14

Stem cell communication regulated by hedgehog signaling in Drosophila

Eric Lee, Tiffiney Hartman, Tanu Singh, Alana O'Reilly, *Fox Chase Cancer Center, USA*

Communication between stem cell and stem cell niche has been demonstrated to be important in maintaining tissue structure and homeostasis. Drosophila provides an excellent model for studying stem cell and its niche interaction. Follicle stem cells in Drosophila ovaries and its interaction with the niche have been well studied in the past two decades. Key molecular signals provide cues for follicle stem cells to proliferate and differentiate into follicle cells. Our previous data has demonstrated that boi plays an important role in follicle stem cell proliferation by sequestrating hedgehog in starved condition. Upon feeding condition, boi releases hedgehog to stimulate proliferation of follicle stem cells. In this context, we have observed that follicle stem cells extend out projection-like structures upon feeding cycle. Our preliminary data has shown that the dynamics of projection extension is hedgehog dependent. Loss of projection is found in the absent of hedgehog (starved) or by inhibition of downstream targets of hedgehog pathway. Though hedgehog dependent, projections extend after hedgehog ligand stimulating proliferation of follicle stem cells. Proliferation stimulated by hedgehog ligand provides cues for cytoskeletal rearrangement, i.e. projection extension, thus explains the time lag. Consistent with previous literature, the extended projections are contacting cells in the stem cell niche. Our preliminary data showed that projections extend across the germarium, forming a network mesh to the germline cyst providing important signaling cues for follicle stem cells differentiation. We will further examine important communication components involved in follicle stem cells and neighboring cells interaction.

15

Symplastic signals regulate cellular patterning and cell fate decisions in the root meristem of *Arabidopsis thaliana*

Kimberly Gallagher, *University of Pennsylvania*

Plant development relies on positional information for the coordination of cellular patterning and morphogenesis. As in animals, mechanical signals, secreted peptides and hormones can all convey information between cells. However, unique to plants is the ability of cells to directly signal their neighbors via plasmodesmata. Transcription factors, small RNAs and metabolites can all move between cells in the plant. Here we exploit a novel tool that effectively blocks movement of proteins and small RNAs between cells to show that cell-to-cell signaling is important for the coordination of cell divisions and cell polarity between cell layers in the root. Tissue specific inhibition of plasmodesmata in the endodermis results in a deformation of the cortical cell layer and a failure of cells in the endodermal lineage to switch from a stem (founder) cell fate to a transit amplifying fate.

16

The Auxin Response Factor MONOPTEROS Controls Meristem Function and Organogenesis through Direct Regulation of PIN Genes

Naden Krogan[1], Danielle Marcos[2], Aaron Weiner[1], Thomas Berleth[2], *[1]American University, USA; [2]University of Toronto, Canada*

The regulatory effect auxin has on its own transport is critical in numerous self-organizing plant patterning processes. However, our understanding of the molecular mechanisms linking auxin signal transduction and auxin transport is still fragmentary. To investigate this relationship, we established an *Arabidopsis* background in which fundamental patterning processes in both shoot and root were essentially abolished and the expression of PIN FORMED (PIN) auxin efflux facilitators was dramatically reduced. In this background, we demonstrate that activating a steroid-inducible variant of the Auxin Response Factor (ARF) MONOPTEROS (MP) is sufficient to restore patterning and *PIN* expression. Further, we show that MP binds to distinct promoter elements of multiple *PIN* genes. Our work identifies a direct regulatory link between central, well-characterized genes involved in auxin signal transduction and auxin transport, and demonstrates the importance of this molecular link in multiple patterning events in both shoots and roots.

17

Characterization of New Bone Morphogenetic Protein (Bmp)-2 Regulatory Alleles

Tapan Shah[1], Youhua Zhu[1], Nadia Shaikh[1], Marie Harris[2], Stephen Harris[2], Melissa Rogers[1], *[1]Rutgers - New Jersey Medical School, USA; [2]Department of Periodontics, University of Texas Health Science Centre, San Antonio, TX, USA*

Bone morphogenetic protein 2 (BMP2, HGNC:1069, GeneID: 650) is a classical morphogen; a molecule that acts at a distance and whose concentration influences cell proliferation, differentiation, and apoptosis. Key events requiring precise Bmp2 regulation include heart specification and morphogenesis and neural development. In mesenchymal cells, the concentration of BMP2 influences myogenesis, adipogenesis, chondrogenesis, and osteogenesis. Because the amount, timing, and location of BMP2 synthesis influence pattern formation and organogenesis, the mechanisms that regulate Bmp2 are crucial. A sequence within the 3'UTR of the Bmp2 mRNA termed the "ultra-conserved sequence" (UCS) has been largely unchanged since fishes and mammals diverged. Cre-lox mediated deletion of the UCS in a reporter transgene revealed that the UCS may repress Bmp2 in proepicardium, epicardium and epicardium-derived cells (EPDC) and in tissues with known epicardial contributions (coronary vessels and valves). The UCS also repressed the transgene in the aorta, outlet septum, posterior cardiac plexus, cardiac and extra-cardiac nerves and neural ganglia. We used homologous recombination and conditional deletion to generate three new alleles in which the Bmp2 3'UTR was altered as follows: a UCS flanked by loxP sites with or without a neomycin resistance targeting vector, or a deleted UCS. Deletion of the UCS was associated with elevated *Bmp2* RNA abundance, BMP signaling, reduced fitness, and embryonic malformations.

18

5'Hoxd genes and Gli3 control dominance of primary limb axis polarity

Anna Trofka, Bau-Lin Huang, Mackem, *National Cancer Institute, USA*

In most vertebrates, the primary limb axis runs through the posterior limb with postaxial, ulna/digit4 (d4), condensations forming first. One exception is Urodele amphibians, which have anterior (pre-axial) dominance (radius/digit2 condense first), and can regenerate limbs as adults. It has been proposed that pre-axial dominance in Urodeles results from failure to expand

5'Hoxd gene expression in the late distal limb bud (LB). To test this hypothesis genetically, we examined primary axis appearance in 5'HoxdDel mice (5'Hoxd genes Hoxd11-13 deleted) and found pre-axial dominance. The 5'Hoxd homeobox transcription factors play roles in replication licensing via interactions with Geminin, and in cell adhesion, for which few specific targets are known, but include EphA3. Gli3 repressor also regulates proliferation and condensation, and antagonizes 5'Hoxd function. In compound 5'HoxdDel/Gli3 mutants, posterior axial dominance is restored. Early steps in condensation formation depend on relative proliferation rates and cell adhesion properties, and differences in anterior versus posterior LB could determine the polarity of the primary limb axis formation. We find that although proliferation rates in the posterior LB do not differ appreciably between control and 5'HoxdDel, anterior proliferation is greatly reduced in 5'HoxdDel compared to control LB at early-middle stages of axis formation. We hypothesize that the reduced proliferation state in 5'HoxdDel anterior LBs promotes an earlier cell cycle exit, accelerating onset of the condensation process and resulting in pre-axial dominance. We are analyzing differences in condensation rates between control and 5'HoxdDel LBs, by live imaging of anterior versus posterior limb cells in high-density cultures using fluorescent reporter tags. Our preliminary results suggest that mesenchymal condensation is selectively accelerated in anterior 5'HoxdDel mutant LBs.

19

Changes in a Hox gene and its downstream network drive microevolutionary changes in phenotype

Mark Rebeiz, *University of Pittsburgh*

The evolution of animal body plan organization is a macroevolutionary process that has generally been difficult to reconcile with microevolutionary processes. Here, we present the near-complete dissection of one such body plan trait, the difference in abdominal pigmentation between Drosophila yakuba and D. santomea. In D. santomea, the Hox gene Abd-B has evolved a drastic expression shift along the body axis, due to modifications of a maintenance element. In parallel, regulatory changes expanded the expression of pdm3, encoding a pigment-repressing transcription factor. In two pigment-producing enzymes, we attribute the loss of yellow primarily to trans changes, while the gain of ebony was resulted from a transposon insertion that disabled its silencer element. We have confirmed the contributions of five loci by CRISPR-Cas9 induced mutations in hybrid animals. Our results demonstrate how a Hox-regulated network has evolved at multiple tiers, providing a microevolutionary perspective of the complexities of body plan evolution.

20

Collective growth in a small cell network

Stanislav Shvartsman, *Princeton University*

Theoretical studies and work with synthetic experimental models show that many of the emergent properties associated with multicellular systems arise already in small cell networks. However, the number of established and naturally occurring systems that can be used to explore collective dynamics in well-defined cell networks is still very limited. Here we focus on collective cell behavior in the female germline cyst in Drosophila melanogaster, a stereotypically-wired network of 16 cells that grows by ~4 orders of magnitude with unequal distribution of volume among its constituents. Using 3D imaging and visualization techniques, we quantify multicellular growth with single-cell resolution and show that proximity to the oocyte is the principal factor that determines cell size; consequently, cells grow in groups. To rationalize this emergent pattern of cell sizes, we propose a tractable biophysical model that depends on intercellular transport on a cell lineage tree. In addition to correctly predicting the divergent pattern of cell sizes, our model also reveals the allometric growth of cells within the

network, an emergent property of this system, and a feature commonly associated with differential growth on a larger scale.

21

Sculpture lessons: How the zebrafish forms its vessels

Jesus Torres-Vasquez, *Skirball Institute, NYU*

How does the vertebrate vascular tree grow? Angiogenic sprouting, the formation of new vessels via the ramification of pre-existing ones, drives the bulk of the life-sustaining expansion of the early vascular tree. Sprouting angiogenesis is also pivotal for injury recovery and organ regeneration and is often deregulated in disease. In this presentation, I will speak about our zebrafish and cell culture studies revealing the cellular and molecular mechanisms by which Semaphorin-PlexinD1 (Sema-PlxnD1) signaling determines fundamental aspects of sprouting angiogenesis, such as the positioning, abundance, and shape of sprouts.

22

Microscopic fluid flows in development and disease: cilia, polarity, and scoliosis

Daniel Grimes, Rebecca Burdine , *Princeton University, USA*

Like classical signal transduction cascades, the microscopic flow of fluids within biological systems provides cues critical for development, growth, and homeostasis.Organisms are permeated with networks of fluid-filled tubes while embryos contain cavities within which flows convey patterning information. Flows are critical for kidney morphogenesis, heart and vascular development, the migration of neurons, and the establishment of left-right asymmetry. However, we know very little about how flows are generated and sensed by cells. This remains a pressing concern given the increasing number of diseases associated with flow abnormalities including structural birth defects, polycystic kidney disease, heart disease, and cancer progression. Cilia, microtubule-based organelles that project from the cell surface, are central players in flow biology. Motile cilia on specialized cells beat to generate fluid flow across epithelial sheets, while primary cilia, which are found on nearly all vertebrate cells, respond to external cues including flow forces. We use forward and reverse genetics in zebrafish to interrogate the roles of cilia and fluid flow in development and disease. I will first report data which demonstrates a molecular link between cilia motility, cilia polarization, and the planar cell polarity of multi-ciliated cells. Idiopathic scoliosis (IS) is a common disease impacting up to 4% of worlds' population in which abnormal curvatures of the spine present during adolescence. Following our work linking polarity and cilia motility, we found that motility and polarity genes are required during post-embryonic stages in order to maintain spine straightness. In mutants, the flow of cerebrospinal fluid (CSF) in the brain is lost and IS-like spinal curves develop during growth phases. Since IS is more prevalent in patients with motile cilia diseases, we suggest that aberrant cilia motility and CSF flow is an etiology of IS, a previously unexplained disease.

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Coordination of receptor tyrosine kinase signaling and interfacial tension dynamics drive radial intercalation and tube elongation

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Mammary epithelium undergoes an extensive postnatal morphogenesis program to form an interconnected ductal network. To accomplish this, a low-polarity, stratified structure, known as the terminal end bud, elongates the tissue through collective cell migration and transitions to a polarized, simple duct. Receptor tyrosine kinases (RTKs) are key regulators of collective

migration during morphogenesis. We sought to understand how RTK signaling, force generation, and single-cell behaviors coordinate to build epithelial tubes. To study this process, we utilized real-time imaging in 3D with fluorescent biosensors. We found that during tissue elongation, individual migratory epithelial cells enriched Ras activity, PIP3, and F- actin to their leading edges. Furthermore, as cells basally migrate, they enriched the same molecules to their leading edge during radial intercalation to expand the basal surface. We next sought to connect molecular activities to subcellular mechanics using force inference analysis. We found that migration through tissues requires specific anterior-posterior ratios of interfacial tension. Intercalation requires similar tension ratios and time-varying basal and posterior tension. These computational studies revealed that certain cells undergo transient intercalations, where cells intercalate but do not remain basally. We observed these transient cells experimentally and comparatively, the permanent intercalation population displayed an increased basal surface area, similar to the computational findings. Finally, we integrated our experimental and computational data to generate a finite element model of epithelial tube elongation. Our model revealed that a combination of proliferation, high basal stress, and interfacial tension dynamics is sufficient to drive cell migration and intercalation for mammary branching morphogenesis. We next seek to further understand the molecular and mechanical differences between permanent and transient intercalations.

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As the cells turn: emergent behaviors in collective cell migration through complex tissues

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Cell migration is essential in animal development, and the underlying mechanisms regulating this process are generally well-conserved. While much is known about individual cell movements, less is clear about how clusters of cells migrate together through cellular environments. To elucidate this, we focus on the migration of the border cells during Drosophila oogenesis. Here, a cluster of different cell types coalesce and traverse as a group between large cells, called nurse cells, in the center of the egg chamber. We have developed a model for this collective cell migration based on the forces of adhesion, repulsion, migration and stochastic fluctuation to generate the movement of discrete cells. Using this system, we have successfully simulated the migration of the border cell cluster through the nurse cell environment. Our simulations suggest that the forces utilized in this model are sufficient to produce behaviors of the cluster that are observed *in vivo*, such as rotation. We compare these results with cell migration in various mutant egg chambers. Our framework will be useful for not only examining aspects of *Drosophila* oogenesis, but also for modeling other two or three-dimensional systems that have multiple cell types and complex interactions between cells.

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Distinct roles for Vangl proteins in regulating neural convergent extension

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Elongation of the body axis along the anterior-posterior axis is a fundamental aspect of early vertebrate development. This process involves changes in tissue shape known as convergent extension (CE), in which the mesodermal and neural tissues undergo narrowing along along the mediolateral axis and extension along the anterior-posterior axis. CE in the neural plate is driven by polarized epithelial cell intercalation, where neighbor cells rearrange by moving between each other in a polarized way, resulting in new cell boundaries. In mice, the Loop tail mutation of the planar polarity gene Van Gogh like 2 (Vangl2), leads to embryos with a short body axis and open neural tube in the hindbrain and spinal cord regions, a condition known as craniorachischisis. We showed that the Loop tail mutation affects cell intercalation efficiency but

not its polarity in neural plate cells. As the lack of effect on polarity may be due to compensation by Vangl1, we are using a mouse line carrying a Vangl1 gene trap (gt) and Vangl2 conditional knockout (ko) to further dissect the function of Vangl proteins in neural CE. Our data show that the double mutant for Vangl1/2 (Vangl1$^{gt/gt}$; Vangl2$^{ko/ko}$) affects both the polarity and the efficiency of cell intercalation, confirming that Vangl1 can compensate functionally in regulating planar polarity of CE. In fact, in Vangl1gt/gt; Vangl2ko/+ embryos the polarity of cell intercalation is also disrupted, suggesting that Vangl1 plays a more important role in regulating planar polarity of neural CE than Vangl2. The Vangl1gt/+; Vangl2$^{ko/ko}$ genotype causes craniorachischisis but does not affect the efficiency or polarity of cell intercalation. These data show that Vangl1 and Vangl2 together cooperate to maintain cell behavior and to drive the efficiency of CE of the neural tube.

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Pairing Your Sox: Cross Species Function of Sox11 in Neural Development

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Neuronal development involves the progression of cells from progenitors to neurons and is guided by precise spatial and temporal expression of transcription factors. Sox transcription factors play critical roles in this process of neuronal development and their expression and function is conserved across species. Sox11, a member of the SoxC gene family, promotes neuronal differentiation in several species including *Xenopus laevis* (frog) and *Mus musculus* (mouse). Our functional studies reveal that frog and mouse Sox11 cannot substitute for one another in neuronal development despite the high level of Sox transcription factor conservation. To address potential causes for functional differences between frog and mouse Sox11, we compared the mouse and frog Sox11 protein domains hypothesized to be involved in protein-protein, and protein-DNA interactions and analyzed the function of cis- regulatory regions. Our results indicate that a single amino acid difference in the highly conserved protein-protein and protein-DNA domain alters the function of Sox11 in these two species and that post-transcriptional regulation via miRNAs is key to the controlling Sox11 spatial and temporal expression across species.

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Role of calcium during early neural development

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Calcium is a ubiquitous and essential messenger that regulates a wide array of physiological processes throughout life. Particularly striking is the widespread calcium activity present in vertebrate embryos during early neural development. While some progress has been made in characterizing molecular players regulating this activity, much remains unknown, including the molecular phenotype of the active cells. Studies conducted in dissected neural tubes *in vivo* have suggested that high calcium spiking increases the incidence of inhibitory (such as GABAergic) neurons and lower spiking increases the number of excitatory glutamatergic neurons. However, an assessment of whether calcium dynamics and neurotransmitter phenotype of single cells, that are identified with certainty, are cell autonomous in absence of cell-cell signaling is lacking. Therefore, we imaged calcium activity of dissociated embryonic neuronal tissue of *Xenopus laevis* and identified the neurotransmitter phenotype using labeled RNA probes against neural progenitors, differentiated neurons, inhibitory neurons and excitatory neuron marker genes. Unlike mature neurons, the signal obtained imaging these cells lacks defined spikes and periodicity. Hence to characterize these irregular calcium dynamics, we

employed spike counting as well as other data analysis methods, including average power, Hurst exponent and Markovian entropy to quantify the complexity of signal. Our data suggest that differentiated neurons exhibit more predictable and persistent calcium dynamics with a higher number of high amplitude spikes and longer spike duration compared to neural progenitor cells. Additionally, glutamatergic and GABAergic neurons show comparable spiking behavior yet differ in spectral energy content of dynamics.Since each of these methods assess different features of calcium activity, our results underscore the importance of using multiple methods to characterize calcium activity during early neural development.

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Serotonergic Signaling to the Pancreas Affects beta Cell Proliferation

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In a recent chemical screen, we found that the selective serotonin reuptake inhibitor (SSRI) paroxetine, known as Paxil, increased β-cell proliferation in both the juvenile and adult zebrafish pancreas. We wanted to characterize the mechanism by which this SSRI affects endocrine cells. Serotonin (5HT) is a neurotransmitter that is known to be secreted by both neuronal and non-neuronal cell types. SSRIs increase local concentrations of serotonin at synapses by preventing reuptake into the presynaptic cell. Because preliminary data showed that zebrafish β cells (and islet cells in general) do not express the rate-limiting enzyme for 5HT synthesis, we hypothesized that the source of serotonin must come from outside the pancreas. Currently, we are pursuing if innervation of β cells by serotonergic neurons provides serotonin to the islet. Using a *Tg(neuroD:gfp)* line, which marks all endocrine cells, we observed the development of islets in the zebrafish pancreas from 2 to 7 days post fertilization. Using immunofluorescence to detect acetylated tubulin, a marker for axons, we saw that endocrine cells develop first and then are innervated by axons coming from the gut-pancreas junction. *Sox10*-mutant (*colourless*) fish have deficient neural crest cell development and therefore show a diminished enteric nervous system and reduced innervation of the pancreas. When treated with Paxil, these mutant fish do not show the same increase in β-cell number as their wildtype siblings. This demonstrates that innervation is necessary for the proliferative effects of Paxil, and supports the hypothesis that serotonin released by neurons that innervate the islet causes proliferation of β cells. Obtaining a better understanding of how innervation affects β cells is important for the manipulation of islets in the treatment of diabetes.

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A Novel Perivascular Cell Population in the Zebrafish Brain

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The blood-brain barrier is essential for the proper homeostasis and function of the CNS, but its mechanism of function is poorly understood. Perivascular cells surrounding brain blood vessels are thought to be important for blood-brain barrier establishment, but their roles are not well defined either. We have described a novel perivascular cell population closely associated with blood vessels on the zebrafish brain. Based on similarities in their morphology, location, and scavenger behavior, these cells appear to be the zebrafish equivalent of cells variably characterized as Fluorescent Granular Perithelial cells (FGPs), perivascular macrophages, or “Mato Cells” in mammals. Despite their macrophage-like morphology and perivascular location, zebrafish FGPs appear molecularly most similar to lymphatic endothelium, and our imaging

studies suggest that these cells emerge by transdifferentiation from the endothelium of the optic choroidal vascular plexus. Our findings provide the first report of a perivascular cell population in the brain derived from vascular endothelium.

Poster Abstracts

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The Wnt beneath my wings: exploring butterfly pattern formation in the CRISPR era

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Understanding the generative mechanisms of morphological diversification requires the routine manipulation of genomes in a comparative context. Here, I present how current work using CRISPR mutagenesis has allowed to decipher developmental mechanisms that may have driven the diversification of a spectacular of morphological radiation: the color wing patterns of butterflies. Indeed, mosaic Knock-Outs induce wing both pattern and color modifications at high-efficiency in any butterfly suitable for laboratory rearing. I describe the multiple phenotypic effects of the Wnt ligand WntA in seven species, and illustrate how this signaling molecule has been essential for both pattern formation and exploration for the morphospace on the butterfly wing. A total of eighteen cis-regulatory alleles of WntA (all of adaptive relevance linked to mimicry) have been formally mapped to date in at least three radiations of butterflies. Thus, CRISPR offers an opportunity to validate genetic function in non-traditional model organisms is of tremendous importance for further understanding the genome-to-phenome relationship at different taxonomic nodes, from population levels to more macro-evolutionary scales. I will discuss this principle in the broader context of GepheBase (www.gephebase.org) , a database of known genotype-phenotype that compiles from the literature more than 1600 allele pairs across all Eukaryotes.

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Differential Growth Triggers Mechanical Feedback that Elevates Hippo Signaling

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Mechanical forces have emerged to be an important regulator of tissue growth. For example, high cytoskeletal tension enhances tissue growth while low cytoskeletal tension decreases tissue growth. On the other hand, growth has also been suggested to affect tissue mechanics: heterogeneous growth could lead to mechanical stress that feedback into cells to maintain growth homeostasis. However, whether and how such a mechanical feedback mechanism functions *in vivo* are not clear. Here we test the mechanical feedback hypothesis by inducing differential growth in *Drosophila* wing disc epithelia through distinct approaches. We show that

differential growth triggers a mechanical response that lowers cytoskeletal tension along apical cell junctions within faster-growing cells. This reduced tension modulates a biomechanical Hippo pathway, decreasing recruitment of Ajuba LIM protein and the Hippo pathway kinase Warts to junctions, thus reducing the activity of the growth-promoting transcription factor Yorkie. This provides the experimental support and a molecular mechanism for lowering growth rates within faster-growing cells by mechanical feedback. Additionally, bypassing mechanical feedback induces tissue distortions and inhomogeneous growth. Thus our research further identifies the roles of mechanical feedback in maintaining tissue shape and controlling patterned growth rates during development.

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Investigating pair-rule gene orthologs in an intermediate germ beetle, Dermestes maculatus

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A segmented body plan provides arthropods with flexibility to adapt to different habitats thus contributing to their great success. Segmentation has been well characterized in *Drosophila melanogaster* (*D. melanogaster*), in which segments are established by a genetic hierarchy including gap, pair-rule and segment polarity genes. Pair-rule genes (PRGs) are a key class of segmentation genes as they interpret upstream non-periodic input into periodic expression. While *D. melangaster* has a derived segmentation mode, with all segments specified ~simultaneously before gastrulation, most insects establish anterior segments before gastrulation but posterior segments are added sequentially. So far, systematic functional studies of PRG orthologs have only been performed in a handful sequentially segmenting species. Here, we established *Dermestes maculatus* (*D. maculatus*), a sequentially segmenting beetle, as a new model system for studying segmentation. Techniques including stable lab rearing, *in situ* hybridization, immunostaining, and both parental and embryonic RNAi have been successfully applied. We isolated all 9 *D. melanogaster* PRG orthologs. 8 of them show PR-like expression. Both *prd* and *slp* knockdown resulted in typical PR defects, suggesting they are “core” PR genes. *Dmac-eve*, *run* and *odd* have dual roles in germ band elongation and in PR segmentation as severe knockdown caused anterior-only, asegmental phenotypes while moderate knockdown resulted in PR-like defects. Elongated but asegmental germ bands resulted from *prd* and *slp* double knockdown, suggesting decoupling of germ band elongation and PR segmentation. Extensive cell death prefigured the cuticular patterns after knockdowns, seen long ago for *Drosophila* PR phenotypes, although disrupted cell mitosis was also observed after *eve* knockdown. We propose that PRGs have retained basic roles during the transition from short to long germ development and share evolutionary conserved functions in promoting cell viability.

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Cadherin-6B proteolytic N-terminal fragments promote chick cranial neural crest cell delamination

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During epithelial-to-mesenchymal transitions (EMTs), chick cranial neural crest cells simultaneously delaminate from the basement membrane and segregate from the epithelia, in part, via multiple protease-mediated mechanisms. Proteolytic processing of Cad6B in

premigratory neural crest cells by metalloproteinases not only disassembles cadherin-based junctions but also generates shed Cad6B ectodomains or N-terminal fragments (NTFs) that may possess additional roles. Here we report that Cad6B NTFs promote delamination by enhancing local extracellular proteolytic activity around neural crest cells undergoing EMT *en masse*. During EMT, Cad6B NTFs of varying lengths are observed, indicating that Cad6B may be cleaved at different sites by a disintegrin and metalloproteinases (ADAMs) 10 and 19 as well as by other matrix metalloproteinases (MMPs). To investigate Cad6B NTF function, we first generated NTF constructs that express recombinant NTFs with similar relative mobilities to those NTFs shed *in vivo*. Overexpression of either long or short Cad6B NTFs in premigratory neural crest cells reduces laminin levels within the basement membrane, which then facilitates precocious neural crest cell delamination. NTF-overexpressing cells also tend to delaminate from neighboring untreated neural crest cells. Zymography assays performed with supernatants of neural crest cell explants overexpressing Cad6B long NTFs demonstrate increased MMP2 levels and activity versus controls, suggesting that Cad6B NTFs promote delamination through a mechanism involving MMP2. Taken together, these findings reveal a novel role for Cad6B NTFs and provide insight into how cadherins regulate delamination as well as normal developmental and aberrant EMTs that underlie human disease.

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The Fgf8 subfamily is required for abdominal ventral wall closure in the mouse embryo

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Omphalocele is a severe ventral wall (VW) defect in which the abdominal contents have herniated through an enlarged umbilical ring. We generated mice in which the genes coding for the Fibroblast Growth Gactor 8 (FGF8) subfamily (*Fgf8, Fgf17* and *Fgf18*) have been conditionally inactivated in the primitive streak and emerging mesoderm using T-Cre; these compound mutants frequently display omphalocele. FGF subfamilies are grouped by sequence homology, raising the possibility of genetic redundancy between subfamily members. By removing members of the *Fgf8* subfamily in different combinations and determining the incidence of embryos with omphalocele, we identified a genetic hierarchy (*Fgf18>Fgf8>Fgf17*) in this FGF subfamily. In humans, omphalocele is associated with increased morbidity and mortality, but the causes of this condition are poorly understood. Omphalocele is thought to be the result of a failure in VW closure due to an arrest in the migration of myoblasts from the somites to the ventral midline. Histological examinations of Fgf8 subfamily mutant embryos show that muscle migration is impaired, suggesting the defect we observe is analogous to omphalocele in humans. Experiments using different Cre lines show that the *Fgf8* subfamily is required in the presomitic mesoderm (PSM), and is dispensable in the somites themselves. The PSM gives rise to the somites, which are segmented blocks of tissue that are the progenitors of the abdominal muscles, as well as other tissues. Preliminary data suggest that FGF signaling in the PSM is required to prevent ectopic cell death in the somites, which could explain the defects in the ventral muscles. We will test this genetically by suppressing cell death in *Fgf8* subfamily null mice by additionally inactivating the proapototic genes, *Bak* and *Bax*, to rescue the defect. This investigation will identify the role of FGF signaling in VW formation, which has received little attention despite its medical relevance.

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Characterizing the Role of an RNA-Binding Protein in Cell Migration

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Cell migration is an important area of scientific investigation as it is required for proper embryonic development, healing of injuries, and fighting against infection in organisms. While cell migration has these positive roles, it is also the mechanism responsible for cancer metastasis. In an attempt to obtain a full understanding of this process, we use the model organism *Drosophila melanogaster* because the genes regulating cell migration in flies are largely conserved in humans. The ovaries in the female fruit fly contain egg chambers that require a set of cells, called the border cells, to migrate for proper egg development. Previous work has shown that a gene called *shep* is expressed in the border cells. This gene encodes an RNA-binding protein that regulates gene expression post-transcriptionally. Preliminary studies suggest that loss of *shep* delays border cell migration. To test these findings, we analyzed a mutant that has an insertion of a reporter in the gene to observe where *shep* is expressed in the egg chambers, and found it is highly expressed in border cells. We also used mutants that result in knockdown of the gene's function. We found that changes in expression alter the timing of border cell migration. It is possible that the RNA-binding protein encoded by *shep* regulates miRNA function and therefore, participates with known miRNAs to govern cell migration. Future research may reveal the exact means by which *shep* regulates cell migration in Drosophila, leading researchers to explore possible implications for similar RNA-binding proteins in human cell migration.

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A Continuous Mathematical Framework for Biological Shapes Formation

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Multicellular biological organisms possess the extraordinary ability to grow and maintain intricate body shapes and forms. The processes controlling the exact dynamic shapes of a developing organism are not well understood due to the non-linearity of gene regulatory networks and the complex, systemic interactions between tissue and regulatory signals. It is now clear that to understand these dynamic processes robust mathematical approaches that are able to precisely describe the regulation of tissue growth and shape formation are necessary. To this end, we have developed a mathematical framework able to accurately demonstrate and predict the growth of biological forms and shapes. Our formulation is based on diffusion-advection-reaction-adhesion partial differential equations permitting the fast and efficient simulation of complex regulatory interactions. Our system models in a continuous fashion the proliferation of cells, their chemotactic migrations, and the adhesive forces between them, in addition to the genetic regulatory mechanisms that control these processes. We show that the feedback loops between these cell growth dynamics and their genetic regulation is sufficient to develop and maintain biological forms and shapes.

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Re-evaluating the Conservation of Primary Neurulation in Teleosts

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The tissue morphologies contributing to primary neurulation are well described in amniotes. A medial hinge point (MHP), established in the medial region of the neural plate, forms the neural groove. Paired dorsal lateral hinge points (DLHPs) localized at the base of the neural folds, where the neural plate bends, enable the neural folds to converge and fuse at the

dorsal midline. In contrast to the well-established mode of neurulation in amniotes, neural tube morphogenesis in zebrafish has remained a topic of intense debate. While the zebrafish neural plate infolds as an organized epithelial layer, a hallmark of primary neurulation, morphological landmarks identified in amniotes, including the MHP, DLHPs and neural folds, have not been reported in this organism. Here, we re-evaluate how such landmarks may manifest themselves in the multilayered zebrafish neuroectoderm in comparison to the monolayered mammalian neuroectoderm. Utilizing molecular markers implicated in hinge point formation, we describe for the first-time in the anterior zebrafish neuroectoderm structures similar to DLHPs with conserved cellular morphology, adjacent to dorsal neural folds. In addition, we identify a superficial, medial structure enriched with actomyosin that may act in a functionally similar manner to a MHP. Furthermore, we present preliminary evidence that this MHP-like structure is disrupted in embryos deficient for Vangl2, a component of the conserved Planar Cell Polarity pathway which is known to regulate MHP formation. These findings reveal a higher level of conservation in mechanisms of neurulation than previously acknowledged, paving the way towards the utilization of zebrafish as a viable model organism to dissect the molecular basis of neurulation and probe the etiology of neural tube defects.

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Regenerative angiogenesis during axolotl tail regeneration

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Therapies for enhancing angiogenesis in order to improve wound healing are an active topic of investigation in clinical research. However the timing of the dependence of tissue repair on blood vessel formation is not well understood. The extent to which vertebrate epimorphic regeneration can occur in the absence of vascularization is unclear. The objective of this project was to investigate regenerative angiogenesis during the regrowth of axolotl tails. At 7, 14, and 21 days post-amputation, the vasculature of the regenerated tail was imaged in whole mount. Tissue samples were then harvested, paraffin processed, and thick sections stained with fast green/toluidine blue. Using these methods, vasculature was undetectable to scant at 14 days post-amputation, suggesting that tissue proliferation and outgrowth may proceed in the absence of a well-developed vasculature during regeneration. Continuing work will include more sensitive methods for vascular detection, including vascular perfusion with contrast agents and immunohistochemistry.

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GTPBP10 is the Member of Obg Protein Family expressed in the Mesoderm of the Xenopus laevis Embryo

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In vertebrate organisms, cell migration and identity changes play crucial roles during early embryonic development. The capability of cells to separate and reestablish new tissues during embryogenesis is known as “cell sorting”. 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. The mesoderm further separates into axial and paraxial mesoderm, giving rise to the notochord and somites, respectively. The notochord is an essential signaling center and serves as a flexible skeletal rod that supports the body of embryonic chordate animals, while somites give rise to muscle, bone, and dermal tissues. Perturbations of cell sorting can cause several developmental defects, including cardiac deformities and generalized gastrulation irregularities. Using the model system, Xenopus laevis, we performed a screen to identify proteins involved in notochord

morphogenesis. As a result of this 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 place it in the Obg family of G-Proteins. Obg proteins are a large group of GTP-binding proteins that are conserved from bacteria to human and are vital in many organisms for cellular growth. RT-PCR shows that GTPBP10 is expressed from gastrulation through the tail bud stage. Whole mount in situ hybridization (WISH), indicates that early expression of GTPBP10 is localized to the mesoderm in a circumblastoporal pattern. Later stages show expression in the notochord, developing brain, and branchial arches. Morpholino knockdown of GTPBP10 results in an edemic phenotype. Overall, we determined that GTPBP10 is a member of the Obg family of G-proteins and is expressed at the right time and place to be involved with notochord morphogenesis.

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The Role of the Non-canonical Wnt Calcium Pathway in the Migration of Primary Mesenchyme Cells

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The highly conserved non-canonical Wnt/Calcium Signaling Pathway (ncWnt/Ca^{2+}) has been shown to regulate cell motility and play a vital role in the developmental processes of many organisms, such as ventral cell fate in Xenopus embryos, gastrulation in Zebrafish, and proper organ formation in mice. We use the sea urchin Primary Mesenchyme Cells (PMCs), which give rise to skeletogenic cells in developing sea urchin embryos, to examine directed cell migration in response to the ncWnt/Ca^{2+} pathway. The working hypothesis is that PMC migration is in part regulated by the ncWnt/Ca^{2+} signaling pathway. We have shown that activation of Protein Kinase C (PKC) downstream of the ncWnt/Ca^{2+} pathway by Phorbol 12-myristate 13-acetate (PMA) dramatically altered PMC migration patterns and decreased skeletal length in the sea urchin embryo. We observed similar phenotypes in embryos treated with the drugs Cyclosporine A, Kn-93, and NFAT inhibitor that disrupt Calcineurin, CAMKII, and NFAT transcription factor, respectively, downstream of PKC. Our lab has also produced similar phenotypes from microRNA-1 (miR-1) knockdown embryos; a microRNA with potential targets along the ncWnt/Ca^{2+} signaling cascade, including PKC and the GTPase it activates, cdc42. MicroRNAs (miRNAs) are short, non-coding RNAs that regulate gene expression by binding to the 3'UTR of target mRNA transcripts to repress translation. Since miR-1 potentially regulates PKC, we would expect the effects of the PKC-activating drug, PMA, to mimic the effects of miR-1 knockdown. We will identify the molecular mechanism of how the ncWnt/Ca^{2+} pathway impacts PMC directed migration and development.

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Protocadherin8-like (PCDH8L; PCNS) cooperates with ephrinB2 to play a role in cranial neural crest migration

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Members of the protocadherin family, the largest subgroup of the cadherin superfamily, are implicated in various neuropathies or cancers. Typically, the expression of a particular protocadherin is restricted to a specific region, which can alter during development. For example, Protocadherin in Neural Crest and Somites (PCNS) is initially expressed in the mesoderm, but later is detected in cranial neural crest (CNC) cells and somite tissue. We identified by Mass Spectrometry Analysis that PCNS is an ephrinB2 binding partner, which was confirmed by co-immunoprecipitation experiments. It was then determined that the C-terminal

PDZ binding motif of ephrinB2 and the DSR (D, S-Rich region) domain of PCNS are necessary for the interaction. Knock down (KD) of PCNS by morpholino antisense oligonucleotides induces a CNC migration defect that is rescuable by overexpression of PCNS or ephrinB2. However, a CNC migration defect caused by KD of ephrinB2 was rescuable only by ephrinB2 and not PCNS overexpression suggesting PCNS potentiates ephrinB2 signaling or plays an upstream role in CNC cell migration. Live cell imaging revealed PCNS KD CNC cells significantly lost lamella podia and filopodia. Grb4 is a known interactor of ephrinB2 in a p298 phosphorylation-dependent manner, and plays a role in cytoskeletal dynamics by modulating downstream effectors. PCNS overexpression was found to elevate the interaction of ephrinB2 and Grb4, an effect that was lost upon Y298F ephinB2 (a phosphorylation mutant) or PCNS-deltaDSR (an ephrinB2 binding mutant) co-overexpression. Upon PCNS KD, CNC cell migration was not rescued when the PCNS-deltaDSR mutant was co-overexpressed compared to wildtype PCNS, and the Y298F ephrinB2 mutant was co-overexpressed compared to wildtype ephrinB2. Taken together, these results demonstrate the role of PCNS in enhancing the interaction of ephrinB2 and Grb4 which plays a role in proper cytoskeleton dynamics to maintain the integrity of CNC cell migration.

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The Role of the Fas2 and Fas3 genes in Drosophila Border Cell Migration

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Collective cell migration is a biological mechanism that is used in immune responses, embryonic development, and cancer metastasis. We use the migrating border cell cluster in Drosophila melanogaster egg chambers to study this mechanism. Previous work has shown that manipulation of the Fas3 gene, which encodes the homophilic adhesion protein Fasciclin 3, alters cell migration. From this, we also became interested in the Fas2 gene which encodes a similar adhesion protein called Fasciclin 2. We examined protein trap expression for Fas3 and found high levels of expression in the outer cells of the border cell cluster, while previous research has found high levels of Fas2 expression between the inner cells of the cluster. From these findings we hypothesized that knockdown of both of these genes simultaneously would result in significant disruption of cell migration. To achieve this, we first knocked down each gene individually in the border cells. Knockdown of Fas3 resulted in delays in border cell migration. It is expected that knockdown of Fas2 will as well. We then performed a double knockdown of both Fas2 and Fas3, and it is expected that this will have a more severe migration delay than loss of either alone. Future research may reveal the exact means by which Fas3 and Fas2 regulate cell migration in Drosophila.

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Interdigital BMP signaling regulates digit formation and skeletogenesis during mouse limb development

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Here we show a role of the interdigital (ID) tissue as a signaling center that regulates digit development in the mouse. During limb development, mesenchymal cells in the distal limb form a pattern of tissue that will give rise to the digits (fingers and toes). These digits are initially connected by ID tissue, which is later removed by apoptosis. *Bmpr* genes encode transmembrane serine/threonine receptor kinases that are activated by extracellular BMP ligand binding. *Bmpr1b* null mice have abnormal chondrogenesis that results in short digits, known as brachydactyly. Remarkably, we find that this defect is rescued when a gene encoding a different BMP receptor, *Bmpr1a,* is inactivated in the adjacent ID tissue using tissue-specific Osr1Cre

recombination. We have previously shown the ID-specific inactivation of *Bmpr1a* does not affect digit development, but rather causes webbing due to a decrease in apoptosis. We demonstrate that this webbing is not the cause of the rescue of brachydactyly due to *Bmpr1b* loss. Thus, the ID-specific loss of BMPR1A, and not webbing, is involved in the rescue of brachydactyly. We hypothesize that in our rescued digits the absence of ID-specific BMPR1A allows unbound ligand to diffuse from the ID to the developing digit and restore normal BMP levels. In support of this, we have preliminary evidence that inactivating the gene encoding the most significant ligand in ID BMP signaling, *Bmp7,* within the ID of the rescued animals restores the *Bmpr1b* null phenotype. Surprisingly, we do not observe SMAD 1, 5, and 9 phosphorylation, a readout of canonical BMP signaling and essential component of chondrogenesis, in the rescued digits. This suggests that digit outgrowth and development is restored through a SMAD – independent mechanism. Further examining the mechanism through which ID *Bmpr1a* inactivation rescues digit outgrowth in *Bmpr1b* mutants could elucidate new therapeutic modalities for tissue engineering, fracture repair, and bone regeneration.

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Van-Gogh-Like 2, Frizzled, And Knypek Control Distinct Aspects of Polarized Cellular Migration During Neural Convergence

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The planar cell polarity (PCP) pathway plays a significant role in facilitating neural convergence (NC) – the narrowing of the neural plate cells. Evidence from literature and our laboratory suggests that NC in zebrafish requires elongation and midline-directed polarized migration of neural plate cells. Delayed NC and stages of neural tube morphogenesis can result in severe neural tube defects (NTDs) which have been observed in all vertebrates studied. Although the PCP pathway is associated with NTDs in model organisms and humans, the underlying neural cell behaviors remain elusive. To investigate the cellular effects of the PCP pathway, we used *Knypek (Kny^{fr6}), Van gogh-like* 2 *(Vangl2^{vu67}),* and *Frizzled 7a^{-}/7b^{-} (Fzd7a^{e3-}; Fzd7b^{hu3495})*, three zebrafish lines carrying null mutations. We confirmed that PCP mutations delayed NC by analyzing neural plate width through *in situ* hybridization using hindbrain probes. Next, our comparative cellular analysis revealed how cell elongation and membrane dynamics are affected in homozygotes. We show that wild type neural plate cells elongate and medially restrict membrane protrusions, thus narrowing the neural tube. Our data show that cells in all mutants failed to elongate initially and extend randomized protrusions. In addition, neural plate cells in PCP mutant embryos are unable to polarize or migrate toward the midline effectively. Live cell behaviors and NC extension movements were assessed by confocal fluorescence microscopy in embryos. Evidence from literature revealed that *Kny* is a *Fzd* co-receptor thought to present *Fzd* with various Wnt ligands in the PCP pathway. *Kny* and *Fzd* cellular phenotypes could suggest a ligand-independent aspect of PCP signaling. While current literature understands that PCP genes regulate cell polarity, the mechanisms of these genes in neural tissue and NTDs is poorly understood. By studying neural plate cell behaviors, our laboratory aims to further reveal how the PCP pathway promotes NC.

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Determining the roles of folic acid metabolism genes during morphogenesis

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Neural tube defects are common and serious birth defects in which the brain and/or spinal cord develop outside the body. Supplementation of foods with folic acid, an essential vitamin, has

been linked to a lower risk of neural tube defects. However, it is not clear how folic acid levels influence risk factors for neural tube defects. This project seeks to identify the basic cellular roles of folic acid metabolism genes in embryonic development using the roundworm *Caenorhabditis elegans (C. elegans)* and the frog *Xenopus laevis* as simple model systems. Studies in *C. elegans* are aimed at understanding the fundamental roles of folic acid genes in cell behaviors. We are studying how mutations in the folic acid metabolism gene *mel-32*/*Shmt* result in early embryonic arrest at the 100-cell stage. Our data thus far indicates that *mel-32* is required for establishment of correct cycle-cycle timing as mutations in the *mel-32* gene more than doubles the length of the cell cycle. Our studies in *Xenopus* are aimed at characterizing the RNA expression of folic acid metabolism genes during neurulation. Studies from the folic acid metabolism gene *mtfhd1* have indicated that expression of this gene is enriched in the anterior neural plate and in brain tissues. We hope these studies will allow for a better understanding of which tissues folic acid metabolism genes are required in and how folic acid genes affect cell behavior.

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Role of Microtubule-actin crosslinking factor (Macf1) functional domains in oocyte polarity

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The embryonic animal-vegetal axis of many vertebrates is first determined during oogenesis by polarity cues set up in the oocyte, preceding embryonic genome activation. This distinct asymmetry is set up by cues from a vegetally localized structure, the Balbiani body, which is conserved in oocytes from vertebrates to invertebrates. The Balbiani body (Bb) is composed of an aggregation of organelles and proteins including RNA binding proteins. However, the mechanism by which the Balbiani body establishes polarity and disassembles at the vegetal pole of the oocyte is unknown. Our lab previously identified in a zebrafish maternal-effect screen a mutant, *magellan* (*mgn*), that exhibits an egg polarity defect. The *mgn* mutant is characterized by an enlarged Balbiani body and a mislocalized nucleus. The *mgn* mutant disrupts the *microtubule actin crosslinking factor* (*macf1*) gene, known to function in other polarized cells. Macf1 is a cytoskeletal cross linker protein and is one of the largest proteins encoded in the zebrafish genome. It is a multi-domain protein that contains a plakin domain, intermediate filament binding domain and a recurring stretch of spectrin repeats, flanked between an Actin Binding Domain and Microtubule binding domain. To test the function of the cytoskeletal-interacting domains of Macf1 in regulating the Bb and positioning the nucleus, we employ CRISPR/Cas9 genome editing. Previous CRISPR deletion mutants for the Actin binding domain cause a polarity defect, whereas deletion of the intermediate filament binding domain reveals no phenotype. We also have taken advantage of a Macf1-citrine line and preliminary results reveal that Macf1 is localized to the cortex in late stage oocytes, as well as to the Balbiani body in early stage oocytes. We continue to employ CRISPR genome editing with the remaining domains. This work provides a basis for understanding the specific domain functions of Macf1 in oocyte polarity.

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beta-catenin and myosin II differentially regulate optic axon pathfinding and growth cone protrusions in the opitc tract

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The retino-tectal projection of lower vertebrates is an experimentally amenable model neuronal circuit for studying mechanisms of axon pathfinding *in situ*. To establish the retino-tectal

projection, optic axons must extend through the optic tract to their target - the optic tectum. Here, we studied how b-catenin coordinates with Myosin II to regulate optic axon pathfinding and growth cone protrusions 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 non-muscle Myosin II inhibitor Blebbistatin to, optic axons in the optic tract of intact brains. Expression of β-catNTERM increased dispersion of optic axons in the dorsal optic tract. In contrast, application of Blebbistatin inhibited growth 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 had increased numbers of filopodial protrusions. These data suggest that β-catenin and Myosin II differentially sculpt optic axonal projections and growth cone filopodial protrusions *in situ.*

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Activation of JAK/STAT signaling and cell motility require the vesicle fusion regulator a-Snap

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The well-conserved Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway is critical for normal animal development and immune function, and is implicated in cancer progression and metastasis. To unravel the regulation of this pathway, we investigated a set of follicle cells in the Drosophila ovary that switch from an epithelial to motile cell type. Two signaling cells, the polar cells, secrete the Unpaired (Upd) ligand, which activates STAT signaling in nearby cells specifying them as migratory border cells. In a mutant screen to identify novel regulators of cell migration, we uncovered a requirement for α-Soluble NSF Attachment Protein (α-Snap) in motile cell specification. α-Snap is known to function in synaptic transmission, membrane fusion, and vesicle trafficking by facilitating association of N-ethylmalemide-Sensitive Factor (NSF) and SNAP Receptors (SNAREs) during vesicle fusion. RNAi-mediated depletion of α-Snap in the follicle cells, including the polar cells, results in egg chambers that lack both polar and border cells. Over-expression of the viral anti-apoptotic gene, *p35*, in *α-Snap* depleted egg chambers rescues the polar cells and verifies that the lack of a border cell cluster in mutants is due to impaired signaling, not apoptosis. RNAi and genetic interaction studies indicate that α-Snap cooperates with the target-SNARE Syntaxin1 and NSF to regulate trafficking of a STAT signaling component. Further genetic analysis of α-Snap function provides evidence that α-Snap is specifically required for Upd secretion from the polar cells during oogenesis. These results suggest a new regulatory node – vesicle trafficking – in activating JAK/STAT signaling and induction of cell motility.

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Podosome-associated proteins Fgd1 and Tks5 are necessary for proper heart morphogenesis

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Cell migration is an essential event in numerous biological processes including the immune response, cancer metastasis, and organ morphogenesis. Congenital heart defects, which are diagnosed in roughly 1% of children and have an increasing rate of diagnosis in both adults and children, can be caused by aberrant cell migration. Two of the major events in *D. rerio* heart morphogenesis are the left-right asymmetric processes of cardiac jogging and looping, both of which are driven by cell migration. Nodal signaling drives asymmetric cell migration in the jogging heart, but the mechanisms underlying this are unknown. Our lab preliminarily identified Fgd1 as a Nodal pathway target in the zebrafish heart. Fgd1 is known to be involved in actin

reorganization and cell migration, but its contribution to cardiac cell migration has not been explored. These proteins are also implicated in the formation of podosomes; actin-rich structures that our lab observes to form during cardiac jogging. Podosomes have not been well characterized, but they are hypothesized to play a role in cell migration due to their up-regulation in metastatic cell lines. We also investigated the role of Tks5 in heart morphogenesis, which is a scaffolding protein that has been demonstrated to be necessary for podosome formation *in vitro.* We used morpholinos to knockdown Fgd1 and Tks5, which resulted in defects in heart laterality and delayed cardiac jogging, as well as abnormal heart morphology, respectively. We confirmed the morpholino results by generating mutations in Fgd1 and Tks5 with CRISPR/Cas9, which resulted in embryos with similar phenotypes to the morpholino treated embryos. These results indicate that both proteins play essential roles in heart morphogenesis, suggesting that podosomes may play an important role in the process of heart development.

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Distinct Signaling Roles for Type I Receptors Bmpr1 and Acvr1l, and the Type II Receptors Bmpr2 and Acvr2 within the BMP Receptor Complex

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The Bone Morphogenetic Protein (BMP) pathway patterns dorsal-ventral (DV) axial tissues during gastrulation. The zebrafish embryo is an excellent system to investigate the mechanism of BMP signaling during DV patterning, as many BMP pathway mutants are available and, unlike in mammals, these mutants survive to show DV patterning defects. When signaling, a dimeric BMP ligand assembles a receptor complex composed of two type I and two type II receptors. Type II receptors phosphorylate and activate type I receptors, which in turn phosphorylate Smad proteins. Phosphorylated Smad then regulates gene expression. This model, however, is overly simplistic as there are two conserved classes of type I receptor, Bmpr1 and Acvr1l, and two conserved classes of type II receptor, Bmpr2 and Acvr2, all of which are necessary for vertebrate development. Our previous findings demonstrate that Bmp2/7 heterodimers are the only ligands that signal in DV patterning. This sufficiency arises from the heterodimer's unique ability to integrate both type I receptors into the BMP receptor complex, as Bmpr1 preferentially binds the Bmp2 ligand, and Acvr1l exclusively binds Bmp7. Based on this and other data, I hypothesize that Bmpr1 and Acvr1l have distinct functional roles. I am performing a series of domain swap experiments to determine which components are required for each receptor's specific function. We do not currently know the contribution of the two BMP type II receptor classes, Bmpr2 and Acvr2, to the signaling complex acting in DV patterning. While experiments in mice suggest that both classes are necessary for early embryonic development, these embryos die before DV patterning begins. I am creating zebrafish mutants null for each type II receptor class using CRISPR technology, to determine whether both classes have independent, necessary signaling functions in DV patterning.

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Characterizing the role of RHOA in regulating blood vessel development and integrity

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The small, monomeric GTPase RHOA acts as a molecular switch, transducing stimuli from hormones, growth factors, cytokines, and transmembrane signaling proteins to downstream effectors of cellular signaling via direct phosphorylation of these downstream targets. Previous studies suggest that RHOA regulates many critical aspects of vascular endothelial cell biology,

including focal adhesion and stress fiber formation. However, most of the functional characterization of RHOA has been performed in cell culture by overexpressing dominant negative or constitutively active forms of RHOA, or by treating endothelial cells *in vitro* with exogenous factors that modulate their growth and development. The *in vivo* functions of RHOA in regulating blood vessel development and integrity are almost completely uncharacterized. We identified a mutant in a zebrafish ortholog of RHOA *(rhoaa)* with severe vascular integrity defects in a forward-genetic screen for dominant hemorrhage mutants. These *"Bloody Mary"* mutants develop extensive intracranial hemorrhage due to vascular rupture, although vascular growth and patterning appears normal. Our results suggest that vascular integrity in developing zebrafish is highly sensitive to either decreased or increased *rhoaa* gene dosage. To gain a better understanding of the molecular mechanisms and downstream effectors of RHOA activity, we are carrying out combined *in vivo/in vitro* analyses of RHOA gain- and loss-of-function in the vascular endothelium using transcriptomic and phosphoproteomic profiling, experimental manipulation, and high-resolution optical imaging of vessels in living zebrafish embryos and human endothelial cells in culture. This project will enable us to identify and characterize members of the vascular RHOA signaling network, and elucidate novel targets for stroke prevention and treatment.

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Proteolytic and transcriptional events promote cell polarity downstream of the RGMa/Neogenin signaling axis during early neural tube morphogenesis in zebrafish

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This study investigates the role of the Repulsive Guidance Molecule A (RGMa) and Neogenin (Neo) signaling as regulators of NC in the zebrafish NP. RGMa is chemorepulsive to emerging retinal ganglion cell axons and prohibitive to the regeneration of damaged nerves. However, evidence from mouse, frog, and zebrafish suggest that RGMa/Neo signaling is required for NT formation. Although this latter function is poorly understood, the Cooper lab determined that RGMa signaling directly affected NT closure, the latest stage of tube formation. Absent from the literature is an understanding of the role of RGMa/Neo signaling in the earliest, most conserved stage of anterior NT development, NC, without which adequate NT closure is improbable due increased NP tissue width. We found that insufficient ligand or receptor similarly disrupted NC. While control embryos underwent adequate NP narrowing, RGMa and Neo-deficient embryos had widened NPs at the same somitic stage. To determine the cellular behaviors contributing to this NC defect, we examined movies of hindbrain NP cells in vivo from control and loss-of-function (LOF) embryos. Our quantitative data show that control embryos extend membranous protrusions toward the midline and lateral surface of the tissue. However, both RGMa and Neo LOF randomized the protrusiveness of NP cell membranes. Cells deficient in this pathway were also rounded instead of polarized perpendicular to the midline. The lack of polarization in these embryos is reminiscent of defects observed when microtubule (MT) stability/organization is disrupted. Therefore, we investigated the requirement of RGMa and Neo for effective MT organization. In this study, control NP cells were found to have archetypical linear MT organization. However, RGMa and Neo LOF severely shortened the length of MT arrays. These observations suggest that MTs may be downstream targets of RGMa/Neo signaling to promote convergence of the NP and thus proper NT formation.

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The Role of the Neogenin Intracellular Domain (Neo-ICD) in Neurulation

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The development of the central nervous system (CNS) depends upon early, dynamic cellular behaviors that narrow and elongate the neural plate (NP) during the process of neural convergence and extension (NCE). During NCE, NP cells extend toward the midline, which consequently increases the tissue length while reducing the distance needed to close the plate into a tube. However, insufficient NCE perturbs neural tube formation and can result in neural tube defects (NTDs), the second commonest congenital disorders worldwide. By studying the proteins regulating cell polarity and migration during NC, we provide greater understanding of why mutations in the genes that encode them promote NTDs. The Neogenin receptor (Neo) transduces signals from repulsive guidance molecules (RGMs) to its host cell using poorly understood mechanisms. We show that depletion of Neo or RGM paralog A (RGMa) resulted in delayed NCE. We found that RGMa-Neo interaction promoted the sequential cleavage of Neo by alpha- and gamma-secretases to produce an intracellular domain, Neo-ICD. The Neo-ICD translocates to the nucleus of neural plate cells where it is predicted to activate the transcription of several pro-migratory genes. Future work will test these candidate genes as downstream regulators of Neo-dependent NCE.

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Noggin-like gene 7 is required for regenerative patterning in the planarian Schmidtea mediterranea

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The regeneration of lost tissue after injury requires the coordinated action of conserved developmental signaling pathways including *BMP* and *Wnt*. Recent studies in the planarian flatworm *Schmidtea mediterranea*, animals capable of whole body regeneration, have shown that muscle cells are the site of expression for many members of these pathways. In particular, BMP signaling has been shown to control dorsoventral (DV) axis specification, and its expression in muscle regulates positional identity in epidermal stem cells. Interestingly, muscle cells are also the site of expression for the canonical BMP inhibitors known as noggins. Here, we find that a noggin-like gene, *nlg7* is required for normal regenerative patterning. We find that inhibition of *nlg7* by RNAi allows ectopic tissue structures containing cells of multiple germ cell layers to form during regeneration. This suggests that BMP signaling may play a role in directing progenitor migration and/or cell-cell associations during regeneration.

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Functional role of catenin proteins in neural crest cells and placode cell-derived neurons during early cranial ganglia formation

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Tissue and organ formation often relies upon the migration, and eventual interaction, of different cell types.The assembly of the cranial ganglia, which are critical integrators of somatosensory information, typifies these processes, as these ganglia form through interactions between two migratory cell types, neural crest cells (NCCs) and placode-cell derived sensory neurons (PCs). During migration, NCCs and PCs exhibit transient cell-cell interactions, mediated in part by the formation of adherens junctions. An instrumental component of adherens junctions is a-catenin, a mechanosensory protein that provides junction stability and facilitates cell motility. The chick embryo possesses both aE (epithelial)-and aN (neural)-catenin, the latter of which is observed in later migratory NCCs and vital for proper cranial trigeminal ganglia assembly. The expression

pattern of aN-catenin during early NCC migration, and the localization of aE-catenin throughout NCC and PC formation, however, has yet to be examined. We hypothesize that aN- and aE-catenin expression is required in chick NCCs and PCs, respectively, for the migration and subsequent coalescence of these cells to assemble the cranial ganglia. To address this hypothesis, we will first define the spatiotemporal expression profile of aN- and aE-catenin via immunohistochemistry. We will next employ gene perturbation strategies to alter catenin expression and distribution in each cell population and, together with fixed and live cell confocal imaging, assess effects on migration and interactions between both cell types. Taken together, our results will not only provide valuable insight into the function of catenins in NCCs and PCs to form the cranial ganglia but also will further our overall understanding of how different cell types interact and communicate to collectively migrate and form new tissues during development.

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Migration of trunk neural crest cells in Trachemys scripta

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Turtles plastron bones develop through intramembranous ossification, suggesting they are neural crest derived, similar to some craniofacial bones. *Trachemys scripta* turtle embryos (G15-16) undergo a second migration of late-emerging skeletogenic trunk neural crest cells (NCCs) which migrate ventrally and aggregate in the region of the forming plastron. Whether this late-migrating subpopulation of NCCs results from premigratory NCCs which remain dormant in the neural tube or if a new induction event is required to produce NCCs is unknown. Through in situ hybridization and immunostaining, the expression of premigratory neural crest specifiers during and between the two waves of migration in *T. scripta* embryos is being investigated. Transcription factors Sox9, Sox10 and FoxD3 are neural crest specifiers and are expressed in premigratory and migratory NCCs. They are expressed in the dorsal neural tube of chicken embryos until the cessation of trunk NCC migration. If the expression of these premigratory markers persists in turtle embryos after the first wave of NCC migration, it would suggest that a premigratory NCC population is maintained in stage G12-14 turtle embryos. The lack of NCC migration in vivo may be due to the lack of a supportive environment, or to other factors regulating the epithelial-to-mesenchymal transition.

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Vangl1/2 function in neural tube convergent extension

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Anterior-posterior elongation of the embryo is fundamental for early vertebrate development and body plan formation. Tissue elongation is driven by convergent extension (CE), in which the tissue undergoes narrowing along one axis and extension along the perpendicular axis. One of the mechanisms involved in CE of the neural plate is epithelial cell intercalation, where neighbor cells move between each other in a polarized way, resulting in new cell boundaries. The genetic and molecular mechanisms of these polarized cell behaviors driving CE in vertebrates were identified to be components of the planar cell polarity pathway (PCP). In mice, mutations of the Van Gogh like 2 gene (Vangl2), lead to mice with a short body axes and open neural tube in the hindbrain and spinal cord regions, known as craniorachischisis (CRN). Previous data from our lab showed that the Vangl2 Loop tail mutant, which corresponds to a point mutation in the C-terminal end of Vangl2, affects cell intercalation efficiency but not polarity of neural plate cells. As Vangl1 and 2 cooperate in the regulation of polarity, our hypothesis is that Vangl1 can compensate for the loss of Vangl2 to maintain normal polarity. In this project, we are using a

Vangl1 gene trap (gt) and Vangl2 knockout (ko). Our data show that the double mutant for Vangl1/2 (Vangl1$^{gt/gt}$; Vangl2$^{ko/ko}$) affects both polarity and efficiency of cell intercalation, and reduces the cell displacement, preventing proper elongation. Vangl1gt/+; Vangl2$^{ko/ko}$ causes the same neural tube defect as Vangl2lp/lp but does not affect the efficiency or polarity of cell intercalation. In addition, in Vangl1gt/gt; Vangl2ko/+ embryos the polarity of cell intercalation is disrupted. These data show that Vangl1 and Vangl2 together cooperate to maintain cell behavior and to drive the efficiency of CE of the neural tube. Furthermore, they suggest that the polarity of CE is regulated by Vangl1 but not by Vangl2.

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The role and regulation of desmoplakin during epidermal development in the embryo

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Desmosomes are cell-cell junctions present in the epithelia and the heart and provide mechanical resistance to these tissues in adults. Thus, it is no surprise that people born with defects in desmosomal proteins can have numerous defects affecting the skin, hair and heart. However, unlike other junctional complexes, the role of the desmosome in epidermal development has largely been unexplored. Therefore, this work fills a major knowledge gap by probing the function and regulation of a critical desmosomal protein, desmoplakin (Dsp) during the development of the epidermis of Xenopus. Dsp is present in the outer epidermis at the membrane junction from 21 hpf until tadpoles stages. Morpholino-mediated decrease in Dsp results in shorter embryos with epidermal tears, cardiac edema, mild dorsal flexure, lack of an embryonic mouth, and smaller eyes. At the cellular level, keratin (type II) filaments appear to be detached from the membrane in Dsp morphants. The morphological and cellular phenotypes of Dsp morphants were recapitulated in CRISPR/ Cas9 mediated Dsp mutants in the F0 generation. Consistent with known roles of desmosomes, we demonstrate that Dsp morphants respond poorly to mechanical stress during early development. Importantly, we determined that Dsp is required for the organization and the differentiation of epidermal cells. Next, we investigated a novel role for c-jun N-terminal kinase (JNK) in Dsp regulation. Reduction of JNK activity increased desmoplakin at the membrane early in development. Further, deficient JNK also protected against EGTA-mediated dissociation of Dsp. Conversely, constitutively-active JNK led to decreased Dsp at the membrane. Finally, reduction of JNK activity improved Dsp morphant response to mechanical stress. This work shows that desmoplakin is required for proper epidermal morphogenesis and mechanical resistance in the embryo. Additionally, this study highlights a role for JNK in regulating desmosome dynamics.

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Mitotic coordination of membrane trafficking in Ciona intestinalis heart development

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During cell division, cell components must be properly segregated and inherited. Improper segregation of cell surface receptors results in abnormal signaling that can lead to cancer. Membrane trafficking can contribute to receptor segregation, but little is known about membrane trafficking during mitosis or the mechanisms that control it. Here, we demonstrate that the mitotic kinases control membrane trafficking of FGF

Receptor during cell division in embryonic *Ciona intestinalis* heart founder cells. Modulations of the mitotic kinases CDK1 and PLK1 demonstrate that each controls discrete aspects of mitotic FGF Receptor trafficking, including internalization and localization to the cytokinetic furrow, respectively. Our results suggest a role for the mitotic machinery in coordinating mitotic membrane trafficking. Uncovering the mechanisms that control mitotic membrane trafficking may define new pathways that regulate cell signaling and shed light on mechanisms controlling cell fate induction. A description of receptor segregation during mitosis will suggest ways in which improper segregation can lead to abnormal cell fate, behavior, or malignancy.

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A morphogenetic role for FGF signaling in zebrafish cardiac looping and ballooning

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The cellular events underlying proper cardiac development require the correct interpretation of signaling cues. Congenital heart defects, which affect 1 in 100 infants in the U.S. each year, arise from a failure of these events to occur. Using zebrafish as a model, we aim to better understand the links between errors in signaling, cellular aberrations, and heart malformation. To that end, we have investigated the role of Fibroblast Growth Factor (FGF) signaling, which is known to couple morphogenesis to cell migration events in zebrafish, in asymmetric heart development. We have uncovered a role for FGF signaling in the later stages of heart development during three different time windows that is independent of its earlier role in establishing left-right asymmetry. We find that FGF signaling is critical for proper chamber placement (cardiac looping) and chamber expansion (cardiac ballooning), but during different developmental windows. We hypothesize that FGF signaling influences cardiac looping by promoting the addition of a late-differentiating pool of cardiac progenitors (second heart field) to the arterial pole of the heart tube, and that the pathway influences cardiac ballooning by regulating cell shape changes in the curvatures of the looped heart.

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The newly identified ephrinB2 binding partner, TBC1d24, plays a role in neural crest cell migration.

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Although Eph-ephrin signaling contributes to the migration of cranial neural crest (CNC) cells, it is still unclear how ephrinB transduces signals affecting this event. Using ephrinB2 immunoprecipitation and mass spectrometric analysis, we identified an interaction between ephrinB2 and TBC1d24 that is mediated by Dishevelled. Both ephrinB2 and TBC1d24 morphant embryos display abnormal CNC cell migration, which is rescued by expressing their wild type counterparts. However, a TBC1d24 mutant that cannot interact with ephrinB2 fails to rescue the TBC1d24 morphant defect. TBC1d24 is known as a GAP for Rab35, and we show that it regulates contact inhibition of locomotion in CNC migration through regulating cadherin recycling. Both ephrinB2 and TBC1d24 morphants display increased E-cadherin levels that may disrupt normal CNC migration. In addition, binding of the EphB4 receptor, decreases the interaction between ephrinB2 and TBC1d24, and thus inhibits CNC cell migration. Our results indicate that

TBC1d24 is a critical player in ephrinB2 control of CNC cell migration.

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Wnt5a controls ureterovesical junction formation through apoptosis by modulating Shh

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Insertion of the ureter into the bladder in the developing embryo is dependent upon the separation of the ureter from the nephric duct during the ureter maturation process. This process is initiated by the degeneration of the common nephric duct through apoptosis. Though nephric duct insertion into the cloacal epithelium is thought to be essential for proper ureter maturation, the underlying regulatory mechanism that drives ureter-bladder connectivity is still not well understood. Here, we use conditional mouse genetic approaches to investigate the role of Wnt5a in regulating this process. Prior to E10.5, deletion of *Wnt5a* in the cloacal region subsequently led to persistent common nephric duct during ureter maturation by reducing apoptosis in the common nephric duct. Moreover, apoptosis in the nephric duct tip was also dramatically decreased in mutants at E10.5, even though the nephric duct inserted into the cloacal epithelium. Concomitant with *Wnt5a* loss, *Shh* expression in the cloacal epithelium was increased in mutants, and ablation of a single copy of *Shh* rescued common nephric duct apoptosis and suppressed the hydronephrosis/hydroureter phenotype. This study identifies a new mechanism for the regulation of apoptosis during formation of the ureterovesical junction and implicates Wnt5a in the repression of Shh from the cloacal epithelium to facilitate common nephric duct degeneration.

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Rbf intrinsically regulates niche cell quiescence, identity and niche number in the adult Drosophila testis

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Stem cells maintain homeostasis within adult tissues and require the precise regulation of signals from their surrounding microenvironment or niche to function properly. The *Drosophila* testis contains a cluster of quiescent somatic hub cells that signal to adjacent germline and somatic stem cells, providing a highly accessible system to study the regulation of a stem cell niche *in vivo*. Damaging the testis or overexpressing genes that activate the cell cycle in hub cells induces hub cell divisions, and leads to the conversion of hub cells to somatic stem cells. This change in cell fate is accompanied by the formation of new niches, which contain ectopic hubs each supporting active stem cells. Recently I found that the cell cycle inhibitor and tumor suppressor retinoblastoma homolog Rbf is a critical endogenous regulator of hub cell maintenance. Loss of Rbf in the hub is sufficient to cause hub cell proliferation, and live imaging and lineage tracing reveal that Rbf prevents hub cells from converting to somatic stem cells. Knockdown of Rbf along with the cell cycle activator E2F rescues both the over-proliferation and cell fate conversion phenotypes, suggesting that targets of E2F may drive these processes. However, these two phenotypes may be separable. Loss of Escargot (Esg) from hub cells causes conversion to somatic stem cells but not excessive hub cell divisions (Voog *et al*), and I find that overexpression of Esg upon Rbf knockdown does not suppress proliferation. In addition, extended Rbf knockdown in the hub causes ectopic niches to form, and loss of E2F or

overexpression of Esg in conjunction with Rbf knockdown suppresses this phenotype. Thus, Rbf works through the cell cycle pathway to actively regulate hub cell quiescence and identity, and likely acts upstream of Esg and additional factors during this process. Together this work reveals how precise modulation of niche cells, as opposed to the stem cells they support, can drive regeneration and disease.

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Exploring the role of cis-regulatory Evolution in Caenorhabditis Germline Sex Determination

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Self-fertile hermaphroditism arose independently multiple times in *Caenorhabditis* through the evolution of post-transcriptional gene regulation in the germline. Previous studies suggest that RNA binding proteins (RBPs) orchestrate the evolution of germline development by gaining and/or losing target transcripts. STAR domain RBPs are found across eukaryotes, and *Caenorhabditis* has a large nematode-specific family of them that includes the germline-specific GLD-1. GLD-1 has conserved, pleiotropic roles in meiotic commitment and oogenesis but has acquired a role in hermaphrodite sex determination in two androdioecious species, *C. elegans* and *C. briggsae*. Its main known function with respect to sex determination in *C. elegans* is to bind and repress *tra-2* mRNA in hermaphrodites allowing the production of sperm in an otherwise female soma. In contrast, Cbr-GLD-1 promotes oogenesis and does not detectably bind *tra-2 in vivo*. Despite GLD-1's opposing roles in the two species, Cbr-GLD-1 can rescue a *Ce-gld-1* null mutant, suggesting that mRNA targets are the changing entity. *C. briggsae* and *C. elegans* GLD-1 have both shared and species-specific GLD-1 targets, and some *C. briggsae* GLD-1 binding sites appear to lack GLD-1 binding motifs. To better understand the dynamic evolution of GLD-1 targets, we are developing PAR-CLIP to obtain the exact site of GLD-1 binding on *C. briggsae* target mRNAs. PAR-CLIP causes a sequence change that can be recognized to narrow the binding site to a 20-40 bp region. Having a clear picture of GLD-1 binding sites in two independently derived hermaphrodites will provide valuable insight into this evolutionary mechanism. In addition, to provide further evidence that GLD-1 has recently gained the *tra-2* regulator in *C. elegans*, we are performing a simpler mRNA-immunoprecipitation-qRT-PCR assays for GLD-1-*tra-2* mRNA association in *C. remanei*, *C. brenneri* and *C. japonica* (outcrossing). We expect that GLD-1 will only associate with *tra-2* in *C. elegans.*

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Potential non-canonical roles of the 26S proteasome system in C. elegans reproduction

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Cellular proteostasis is regulated by the ubiquitin proteasome system (UPS). There is evidence that the 26S proteasome of the UPS, in particular the 19S regulatory particle (RP), engages in non-proteolytic functions. We identified 8 RP subunits out of a total of 17 RP subunits that when co-depleted with WEE-1.3 suppresses the *wee-1.3(RNAi)* infertility. Seeing as 9 RP subunits did not suppress the *wee-1.3(RNAi)* infertility, we hypothesize that specific 26S proteasome components may act in a non-canonical, non-proteolytic manner during *C. elegans* reproduction to influence fertility. This could potentially be via either a direct or indirect interaction with WEE-1.3. We show that knockdown of certain proteasome components alter WEE-1.3 subcellular localization suggesting proteasome involvement in proper localization of WEE-1.3. Co-depletion of proteasome component RPN-6.1, RPN-7, RPN-8 or RPN-9 with WEE-1.3 rescues the

precocious oocyte maturation observed in *wee-1.3(RNAi)* animals. qRT-PCR was performed using RNA extracted from isolated gonads to quantify the knockdown of each transcript subjected to RNAi. Surprisingly, there is an increase, rather than decrease, in the mRNA levels of *rpn-6.1(RNAi)* animals. However, decreased levels of *rpn-6.1* mRNA levelswere observed in animals co-depleted of both RPN-6.1 and WEE-1.3, which may suggest a previously unknown interaction between the two transcripts. Future plans include endogenously tagging specific proteasome components with GFP utilizing CRISPR/Cas9 genome editing technology and *in vivo* protein degradation assays utilizing the Dendra2 reporter system. If general inhibition of proteolytic function of the proteasome in *wee-1.3(RNAi)* animals does not suppress *wee-1.3(RNAi)* infertility and is not responsible for proper WEE-1.3 subcellular localization, we will have identified a non-canonical, non-proteolytic role for the proteasome during *C. elegans* reproduction.

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Sex-specific specification of the follicle stem cells in the developing Drosophila ovary

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Sexual dimorphism is crucial for the propagation of a sexually reproducing species, thus understanding how sex-specific cells are specified is essential for our understanding of how an oocyte or sperm are produced. Our lab is interested in how the sex determination pathway controls sexual dimorphism in the gonad, including how the conserved transcription factor Doublesex (DSX) regulates sex-specific development of the somatic gonad. Follicle cells are female-specific cells that surround and nurture the developing oocyte and are conserved from flies to mammals. The germarium of the *Drosophila* ovary contains two follicle stem cells (FSCs) that give rise to the follicle cells, but how the FSCs are specified remains unknown. The best current marker for FSCs is the transcription factor Castor, which labels FSCs in addition to pre-follicle cells, and stalk cells. We have conducted a time-course immunostaining of pupal ovaries examining Castor expression to determine FSC specification. Castor is not observed at 2 hrs through 7 hrs after pupal formation APF. The earliest Castor expression can be seen at 9 hrs APF in cells intermingled with the germ cells in the middle of the developing ovarioles as well as in cells posterior to the germ cells. At 24 hrs APF, Castor expression is seen primarily in the basal stalk cells posterior to the germline. We are currently using lineage analysis to study the origins of the FSCs and if they are related to basal stalk cells. Interestingly, we also find that many aspects of follicle cell development still occur specifically in XX animals even in the absence of *dsx* function. Thus, we are investigating whether there are *dsx*-independent mechanisms controlling sex-specific follicle cell development in the soma, or whether signaling from XX germ cells influences follicle cell development.

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Frequent egg removal stimulates oviposition in the self-fertile mangrove killifish Kryptolebias marmoratus

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Genetic manipulation necessary for modern developmental biology research depends crucially on the availability of abundant embryos. Higher frequencies of oviposition facilitate both genetic crosses and the embryonic injections required for genome editing, lineage tracing, and gene knockdown experiments. The mangrove killifish *Kryptolebias marmoratus* is the only self-fertile vertebrate known, is naturally highly inbred, and is easily maintained in the laboratory. It is therefore advantageous for experimental research. However, an obstacle to development of *K.*

marmoratus research is its relatively low fecundity as compared with other fish models, such as *Danio rerio.* While investigating the timing of oviposition in *K. marmoratus*, we serendipitously found that frequent egg removal from tanks stimulated more ovipostions. This was true across several strains when compared to baseline oviposition rates for the same fish. These data suggest that olfaction or visual cues may play an integral role in stimulating oviposition, and ongoing experiments will attempt to distinguish between these alternatives.

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Germline Sexual Identity Controls Niche-Germline Stem Cell Communication

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The establishment of sexual identity in the germline is critical for the sex-specific development of germline stem cells and production of sperm vs.eggs. Germ cells depend on signals from the somatic gonad and their own sex chromosome genotype for successful sexual development and gametogenesis. When the “sex” of the germline fails to match the “sex” of the soma, germline development and gametogenesis are severely disrupted. How somatic signals and germ cell intrinsic cues act together to regulate germline sex determination is a key question about which little is known in any organism. We show that autonomous germline sexual identity is essential for driving female sex. This cell intrinsic sexual identity of the germline results in sex specific response to nearly identical niche signals. Germline chromosomal constitution, which is XX in females drives sexual identity through cell intrinsic SXL. Germline intrinsic SXL drives the female sexual identity by blocking the male sex pathway activator JAK/STAT, thereby ensuring successful oogenesis. *Sxl* regulation of JAK/STAT is important for regulating male germline identity, as either loss of SXL function or activation of JAK/STAT function are sufficient to promote spermatogenesis in XX germ cells when present in testes. We found that PHF7 is transcriptionally regulated by STAT in male germline. Since SXL suppresses JAK/STAT in the female germline, PHF7 expression is increased in female GSC’s when *Sxl* function is reduced. Loss of *Phf7* does not rescue *Sxl* mutant phenotype while loss of STAT does. Phf7 mutants have reduced fertility but are not sterile, hinting that there are multiple JAK/STAT pathway targets that regulate male germline sexual identity. We propose that JAK/STAT pathway regulates male identity in the GSCs. Intrinsic sexual identity of the GSCs, regulated by *Sxl,* is critical for how GCSs respond to niche signals; female GSCs need to block JAK/STAT signaling in order to retain their proper female identity.

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The role of DEAD-box RNA helicase Bel in Drosophila female germline development and post-transcriptional gene regulation

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RNA helicases play critical roles in post-transcriptional regulation of gene expression. Here we investigate the roles of Belle (Bel), a conserved ATP-dependent DEAD-box RNA helicase, in Drosophila female germline development and post-transcriptional gene regulation. We hypothesize that Bel ATPase activity plays crucial roles in regulating gene expression during female germline development. To investigate the roles of the ATPase activity of Bel, we expressed the following bel transgenes in a wild-type background:
(1) wild-type bel
(2) ATP-binding defective bel (K345N)
(3) ATP-hydrolysis defective bel (E460A)
(4) ADP + Pi release defective bel (E460Q)
We hypothesize that these Bel ATPase mutants remain bound to target RNAs, thus inhibiting

regulation by endogenous wild-type bel. We found that germline expression of Bel ATPase mutants in a wild-type background resulted in dominant negative phenotypes, exhibiting defects in ovary morphology, fecundity, and fertility. Moreover, we observed that Bel ATPase mutants exhibited decreased abundance of cyclin proteins in the ovary relative to controls, implicating Bel in regulating levels of cell cycle regulators. Our studies reveal that Bel ATPase mutants have dominant negative effects in female germline, suggesting that Bel ATPase activity is required for female germline development, possibly through regulating expression of cell cycle regulators.
To further investigate the molecular function of Bel in post-transcriptional gene regulation, we designed a tethered function assay where wild-type and ATPase mutant Bel were tethered to a reporter GFP mRNA. We observed that tethering wild-type Bel to the reporter resulted in increased GFP protein expression relative to control. Conversely, none of the Bel ATPase mutants exhibited changes in GFP protein level. Together, our results suggest that Bel post-transcriptionally regulates protein expression and that Bel ATPase activity is critical for this function.

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top-2 is required for proper chromosome segregation during male meiosis in C. elegans

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During sexual reproduction, haploid gametes are generated from diploid precursors through the specialized cell division of meiosis. Meiosis reduces ploidy by following one round of DNA replication with two rounds of chromosome segregation. In meiosis II sister chromatids segregate from each other similar to mitosis; but in meiosis I, it is the homologs that segregate, which requires pairing, synapsis, and recombination. Type II DNA topoisomerases are enzymes that play a crucial role in chromosome fidelity by disentangling topological problems that arise in double stranded DNA. In mitosis, Topo II enzymes solve topological problems that arise during replication, transcription, sister chromatid segregation, and recombination. However, the role Topo II plays during meiosis has not been fully elucidated. We have shown that a novel allele of Topo II, *top-2(it7ts)*, disrupts the segregation of homologous chromosomes during the meiotic divisions of spermatogenesis but not oogenesis. TOP-2 is expressed in both spermatogenic and oogenic germ lines, localizing along the lengths of chromosomes during meiotic prophase. In *top-2(it7ts)* mutants, localization of TOP-2 is disrupted in both spermatogenesis and oogenesis leading us to question why only meiotic chromosome segregation in spermatogenesis is affected. A major difference between spermatogenesis and oogenesis is chromosome morphology after pachynema. We hypothesize that TOP-2 plays a role in chromosome remodeling/architecture during spermatogenesis that facilitates homologous chromosome segregation. Preliminary evidence evaluating the role of *top-2* in chromosome architecture has found that meiotic chromosome axis components, the meiotic cohesins, COH-3 and COH-4, localize normally during spermatogenesis, but, the other meiotic cohesin, REC-8, may be prematurely removed from chromosomes. We are continuing to investigate the role of *top-2* in chromosome structure through the examination of additional architectural components.

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Drosophila ovary cell fate specification and tissue landscape

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Morphogen signaling is one of the many known biological mechanisms in cell fate determination, and therefore is critical for proper animal development. While multiple factors govern morphogen transport, we know very little about the effect of tissue architecture on signal diffusion. Morphogen signaling has been heavily investigated in the genetically-

tractable organism Drosophila. In Drosophila egg chambers the anterior polar cells secrete Unpaired (Upd), which when received by the surrounding follicle cells, activates the Signal transducer and activator of transcription (STAT) pathway and specifies follicle cells into border cells. Surprisingly we observed asymmetries in the STAT activation pattern around the polar cells, suggesting uneven Upd distribution among the follicle cells. To study this phenotype, we developed a three-dimensional computer simulation to predict the role of tissue contour in morphogen distribution. The simulation data concurred with the *in vivo* activation data, supporting the influence of tissue arrangements on morphogen spread. It is now our goal to understand the activation pattern and Upd diffusion in between the subcellular domains in a biological system. We have created a Upd-fusion to a photoswitchable protein, Dendra2, to examine Upd kinetics *in vivo*. Understanding how morphogen transport is constrained via biophysical parameters can open a whole new aspect of understanding extracellular signaling cues and their impacts on developmental disorders.

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The role of Kainate Receptor Subunit, GLR-6, in C. elegans reproduction

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Sensory perception influences the reproductive activity of *C. elegans* by driving metabolic resources towards reproduction or survival as needed. Glutamatergic signaling, mediated by the receptor subunit GLR-6 plays an important role in the processing of sensory information within the RIA neurons, second layer interneurons primarily involved in thermotaxis. Previous work has demonstrated that co-depletion of GLR-6 and the meiotic inhibitory kinase, WEE-1.3, suppresses the infertility phenotype generated by RNA interference (RNAi) depletion of WEE-1.3. Therefore, we hypothesize that GLR-6 and WEE-1.3 likely interact to influence reproduction in *C. elegans* in a manner that has yet to be reported. Preliminary results confirm that co-depletion of GLR-6 and WEE-1.3 via RNAi results in an increased brood size, indicating the suppression of the WEE-1.3 depletion infertility phenotype. In an attempt to determine whether this result is due to the affect of the overall activity of the RIA neurons or the activity of the GLR-6 specifically, we assayed several additional candidate genes. To evaluate the activity of the RIA neurons, we investigated *nlp-22*, which is uniquely expressed in the RIA neurons. Meanwhile, *glr-6* paralogs, *glr-3* and *glr-5,* as well as c-type lectin, *clec-63*, were chosen to further elucidate the role of *glr-6* in reproduction. Confocal fluorescence imaging was used to assess the effects of RNAi on the germ lines of depleted animals. Based on our preliminary data it appears that GLR-6 and WEE-1.3 may interact to effect meiotic cell cycle progression. Future work will focus on determining whether this interaction occurs directly via protein-protein interaction or indirectly by other means.

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Determining the function and regulation of polymers of nucleotide biosynthetic enzymes during Drosophila oogenesis

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CTP synthase (CTPS) and inosine monophosphate dehydrogenase (IMPDH) are two rate-limited enzymes in de novo nucleotide biosynthesis. They have been found to polymerize into filaments under conditions of nucleotide depletion or elevated demand for nucleotides in many different species and cell types. For example, these enzymes polymerize in nutrient-starved mammalian cells lines and CTPS polymerizes during normal Drosophila oogenesis, where germ cells undergo rapid cycles of endoreplication and rRNA synthesis. Our lab has demonstrated that these assemblies are present during Drosophila egg development and has pioneered the

use of this model system to understand their function and regulation. We also recently found that in vitro and in cultured cells, IMPDH filament assembly has no effect on the biosynthetic activity of IMPDH while others have found that filament assembly of CTPS can either inhibit or enhance activity of the enzyme, depending on the species. We hypothesize that assembly of these enzymes into filaments may regulate some other aspect of their biological function. In order to test how and why these filaments are assembled under normal biological conditions I am utilizing mutants of the Drosophila IMPDH gene as well as functional rescue with transgenic expression of human IMPDH2 constructs, that either inhibit or promote filament assembly without abolishing enzyme activity. This will allow me to assess the role of assembly in a biologically important in vivo context. These experiments will provide insight into the biological function of IMPDH filaments in vivo in a cell type undergoing rapid cycles of genomic replication and RNA synthesis. Thus, it may provide insights into the developmental regulation of an evolutionarily conserved pathway important for endoreplication, cell proliferation, and growth.
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Genetic dissection of TRA-1 function and its regulation within the sex-determination pathway of Caenorhabditis briggsae

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For nematodes to evolve self-fertility, the *XX* animals have to modify the sex-determination pathway within the germ line to allow for a limited window of spermatogenesis. These changes alter a conserved sex-determination pathway that centers on the transcription factor TRA-1, the worm Gli homolog. Gli proteins can function in a bipotential manner: a cleaved protein functions as a transcriptional repressor, while full-length protein functions as a transcriptional activator. It remains unclear whether TRA-1 can function as a full-length transcriptional activator in nematodes. We are characterizing a mutation in *cbr-tra-1* that may specifically prevent TRA-1 from acting as a transcriptional activator. Molecularly, *cbr-tra-1(v48)* only affects the full-length protein. Phenotypically, *cbr-tra-1(v48)* mutants produce only oocytes, which supports the hypothesis that full-length TRA-1 is required to activate spermatogenesis genes. We are also investigating how the TRA-2 receptor regulates TRA-1. The TRA-2 binding region of TRA-1 is not thought to be part of cleaved TRA-1. In *C. elegans, cel-tra-2(mx)* mutants that affect the putative TRA-1 binding region of TRA-2 block spermatogenesis. We used gene-editing to generate *cbr-tra-2(v393)* and *cbr-tra-2(v403)* mutants, which affect the similar region of TRA-2 in *C. briggsae*. Unexpectedly, these *cbr-tra-2(mx)* mutants are capable of producing sperm. Finally, we are investigating the role of FEM regulation of TRA-1. In *C. elegans*, *fem-3* mutants are feminized. But in *C. briggase*, loss of function *cbr-fem-3* mutants are self-fertile. To investigate the role of FEM-3 further, we used gene-editing to generate gain of function mutants in *cbr-fem-3* to see if upregulation of FEM-3 is sufficient to cause TRA-1 misregulation. These *cbr-fem-3* mutants are self-fertile, further supporting the hypothesis that FEM-3 is only of limited importance as a TRA-1 regulator in *C. briggsae* germ cells.

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Differential retrotransposon activity underlies fetal oocyte selection

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Naturally occurring attrition of up to 80% of fetal oocytes severely diminishes the ovarian reserve in a wide range of mammals including humans and rodents. Surprisingly little is known about the underlying cause of this phenomenon besides its coincidence with meiotic prophase I. Now, we have implicated LINE-1 (L1) retrotransposons in attrition of fetal oocytes of mice. We

discovered differential accumulation of L1ORF1p, a L1-encoded protein and critical component of L1 ribonucleoproteins, in the nuclei of fetal oocytes. Elevated nuclear L1ORF1p levels strongly correlated with DNA damage, meiotic defects and oocyte elimination. These results suggest that L1 activity underlies fetal oocyte attrition. We proposed that fetal oocyte attrition serves the purpose of selecting oocytes with minimal L1 activity, thus best suited for the next generation. We are now investigating the origin of differential accumulation of L1ORF1p in fetal oocytes. Typically repressed, L1 elements become active during epigenetic reprogramming of sexually differentiating germ cells that involve DNA methylation erasure and histone modifications. L1 expression is promptly downregulated in fetal prospermatogonia due to subsequent re-establishment of DNA methylation. However, in oocytes DNA methylation will be re-established only during adult oogenesis allowing L1 expression to persist in fetal oocytes. Therefore, we hypothesize that differential LINE-1 expression is the result of differential epigenetic repression in fetal oocytes. To start to test our hypothesis, we optimized a Fluorescent Activated Cell Sorting (FACS) strategy to isolate fetal oocytes from different developmental time points and with different L1 content. We then developed a bisulfite wide-sequencing analysis to compare levels of methylation in the different FACSed oocyte populations. We will present our experimental design and evidence that support our hypothesis.

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Live-imaging analysis of germ cell proliferation in the C. elegans adult supports a stochastic model for stem cell proliferation

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The *C. elegans* adult hermaphrodite contains a renewable pool of mitotically dividing germ cells that are contained within the progenitor zone (PZ), at the distal region of the germline. From the PZ, cells enter meiosis and differentiate, ensuring the continued production of oocytes. In this study, we investigated the proliferation strategy used to maintain the PZ pool by using a photoconvertible marker to follow the fate of selected germ cells and their descendants in live worms. We found that the most distal pool of 6-8 rows of cells in the PZ (the distal third) behave similarly, with a fold expansion corresponding to one cell division every 6 hours on average. Proximal to this region, proliferation decreases, and by the proximal third of the PZ, most cells have stopped dividing. In addition, we show that all the descendants of cells in rows 3 and above move proximally and leave the PZ over time. Combining our data with previous studies, we propose a stochastic model for the *C. elegans* PZ proliferation, where a pool of proliferating stem cells divide symmetrically within the distal most 6-8 rows of the germline and exit from this stem cell niche occurs by displacement due to competition for limited space.

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Sex-specific development of the germline stem cell niche is regulated by a novel doublesex - fruitless regulatory interaction

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In Drosophila melanogaster, sexual development of the male and female gonads is controlled by the sex-specific transcription factor Doublesex (DSX). While DSX homologs are known to control sexual development in virtually all animals, the mechanism and downstream targets remain largely unknown. Our genomic and bioinformatic approaches to identify DSX targets revealed *fruitless* (*fru*) as a candidate DSX target. It has been shown that *fru* acts in the nervous system to control sex-specific behaviors, and that male-specific expression of FRU is regulated by sex-specific alternative splicing. Surprisingly, we found that FRU is also expressed male-specifically in the developing somatic gonad. Further, sex-specific expression of FRU in the gonad does not require alternative splicing and, instead, *dsx* is necessary and sufficient to

regulate FRU expression in the male gonad as well as the nervous system. A key aspect of gonad sexual dimorphism is the formation of sex-specific germline stem cell (GSC) niches. Important components of these niches, the terminal filaments and cap cells (TFs/CCs) in females and the hub cells in males, originate from a common pool of progenitor cells but diverge to form sex-specific niches. Previously we have shown that, in the absence of dsx function, hubs are less robustly specified and stochastically switch to form TFs/CCs. Interestingly, FRU expression in the hub correlates with the time when the male niche must resist switching to form TFs/CCs. Further, ectopic FRU expression is able to block TFs/CCs fate, and reduction of *fru* function causes increased probability of hubs switching to TFs/CCs fate. In summary, this work demonstrates that *fru* expression is not only regulated by sex-specific alternative splicing but that fru expression is also regulated downstream of dsx. Further, fru not only acts to control sex-specific behaviors in the nervous system, but also functions in the somatic gonad to control sexual dimorphism of the GSC niches.

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Two mechanisms of fetal oocyte attrition select against transposon activity and DNA damage in mice

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Germ cells are faced with the responsibility of faithful transmission of genetic information to the next generation, in spite of numerous threats to the genome during development. In females, activation of transposons, intricate meiotic prophase I (MPI) events, and radical differentiation programs challenge the germ cell genome, and correlate with elimination of up to two-thirds of the maximum starting pool of oocytes by birth. Since females are born with a finite pool of oocytes, it is critical to understand the mechanisms behind this fetal oocyte attrition (FOA). Indeed, L1 retrotransposons determine the fate of fetal oocytes, with cells containing high L1 protein levels being preferentially eliminated. Due to the nature of L1 to generate DNA breaks for attempted retrotransposition, and that these breaks may interfere with meiotic events such as chromosome segregation and DNA repair, we suggest that L1-driven FOA occurs through the DNA damage checkpoint in MPI. This checkpoint serves as a quality control system, culling oocytes with persisting DNA damage in the late pachytene stage of MPI. We find that upon mutation of checkpoint kinase 2 (CHK2), a core component of the DNA damage response, FOA is prevented at late pachynema. However, CHK2 appears dispensable for FOA prior to this time. To identify the trigger of CHK2-independent FOA, we turned to another intrinsic activity of L1, reverse transcriptase (RT), required to generate a cDNA copy of a L1 RNA template for retrotransposition. Interestingly, inhibition of RT activity alone can prevent CHK2-independent oocyte loss. Moreover, by treating *chk2*$^{-/-}$mice with the RT inhibitor, we retain the entire maximum starting pool of oocytes. Now, by blocking both CHK2-dependent and independent mechanisms of FOA, we have the opportunity to test why programmed cell death is important for ovary development and reproduction in mammals, as well as investigate whether its prevention can improve poor ovarian reserve conditions.

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Increasing zebrafish egg harvests with a massive embryo production system

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Ongoing research projects in the NICHD Zebrafish Core require large numbers of wild-type zebrafish embryos every week. Specifically, one project entails a 10,000-compound screen for small molecules that can mitigate deleterious effects of hyperammonemia. To maximize throughput, we need about 1500 synchronous embryos on screening days. We are also generating mutants via microinjection of gene-targeting CRISPR/Cas9 components. To maximize CRISPR/Cas9 mutant production efficiency, we need two waves of approximately 500 freshly-fertilized embryos on microinjection days. At the onset of this project, NICHD was already in possession of two large (242-liter capacity) Massive Embryo Production Systems (MEPS) from Aquatic Habitats and two of their smaller (80-liter capacity) MEPS units. Through a combination of strategic measures, and using the two large units and one of the smaller units, we are now achieving the benchmarks of 500 embryos per each of two morning collections (9:45 and 10:45 am) 97% of the time and 1500 embryos per each of three collection days (Tuesday, Wednesday and Thursday) 98% of the time.

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Transgenesis in Rhabditophanes sp. KR3021: a model for human parasitic nematodes

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Approximately 30 to 100 million people globally are infected with the parasitic worm *Strongyloides stercoralis*, for which there are few treatment options. Due to challenges in maintaining an obligate parasite in the laboratory, *S. stercoralis* development has typically been modeled using the free-living nematode *Caenorhabditis elegans*; however, this is likely an inappropriate comparison due to the phylogenetic distance between *S. stercoralis* and *C. elegans*. Furthermore, gene regulatory sequences differ between the two species, as constructs containing *S. stercoralis* promoters expressed in *C. elegans* do not always shown comparable expression patterns with the parasite, and conversely, constructs with *C. elegans* transcription terminator sequences are not expressed in *S. stercoralis*. This suggests a high degree of functional divergence between the two species.The recent identification of *Rhabditophanes* sp. KR3021 - a close free-living relative of *S. stercoralis* – has presented an opportunity for researchers to begin to understand the developmental biology of *S. stercoralis* and related vertebrate parasites. While limited transgenesis is possible in *S. stercoralis*, passage through a mammalian host and silencing of transgenes beyond the F1 generation severely limit the study of gene function. The aim of this study has been to construct a plasmid in which green fluorescent protein (GFP) can be stably expressed by *Rhabditophanes* to create a transgenic organism. This plasmid, which consists of actin promoter and terminator sequences from *Rhabditophanes* is hypothesized to drive expression of GFP in the smooth muscle cells of transgenic *Rhabditophanes*. This proof-of-principle experiment to visualize the tissue(s) in which a specific gene is expressed will open the possibility for using transgenesis to study additional genes regulating *Rhabditiophanes* and parasitic nematode developmental biology.

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Using AngioTag fish to uncover novel vascular genes in the zebrafish

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Our lab has developed a novel transgenic line that allows for the collection of actively translating

mRNA transcripts from specific cell types through the use of epitope labeled ribosomes. The "AngioTag" line uses the kdrl promoter to drive endothelial-specific expression of an HA-tagged ribosomal subunit protein, allowing us to specifically isolate and study the endothelial translatome of the developing zebrafish. Using RNA sequencing performed on affinity-purified endothelial mRNAs from these fish, we identified four novel unannotated genes that were enriched in the endothelial translatome and showed vascular *in situ* expression. Several annotated genes with previously undocumented vascular expression were also identified in our AngioTag samples and also shown to exhibit vascular *in situ* expression patterns. We are currently using CRISPR/Cas9 technology to generate mutations in all of these genes in order to examine their potential roles in vascular development.

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Extracurricular Research in BRAIN Club

Maya Robnett, Sidwell Friends Students, *Sidwell Friends, USA*

Students at Sidwell Friends Upper School in Washington, D.C. have the opportunity to conduct original research both inside and outside of the classroom. Having completed their first year of Biology, many students find they want to explore research opportunities in an extracurricular setting. Possibilities for these students include joining BRAIN (Biological Research and Investigations in Neuroscience) Club or partnering with scientists from research institutions in the area such as the National Institutes of Health and Georgetown University Medical Center. Extracurricular research enables students to employ the critical thinking skills that they have gained through science classes in lab-like settings, like the one BRAIN Club provides, or in professional lab environments. This year, extracurricular projects conducted by students include: (i) investigating the effects of temperature on restriction enzyme potency, (ii) investigating effects of muscle-targeting chemicals on a zebrafish model of Duchenne muscular dystrophy, (iii) observing the effect of stress and other stimuli on the optomotor reflex in zebrafish, (iv) observing the effects of light exposure on zebrafish hair cell regeneration, (v) determining the effect of gluten and topical steroids on eczema. Through independent research, students have the opportunity to explore paths in STEM fields in a supportive environment. They are encouraged to continue to pursue research throughout their high school careers and beyond.

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Forays into guided group independent projects in an upper level developmental biology laboratory course

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Many research studies have shown that students who are engaged in authentic research display increased analytical and critical thinking skills, as well as an increased understanding of the scientific process. Given the large number of Biology majors at our institutions relative to the number of research faculty, many students will not have the opportunity to work in a research lab. One way to address that problem is to offer undergraduate laboratory courses that provide more investigative experiments. In our upper level developmental biology lab courses, students carry out independent research projects, in which they formulate their own hypothesis and design and execute experiments to test their hypotheses. Guiding students is an iterative process. We begin the semester with classic developmental biology experiments on model systems that have included sea urchins, zebrafish, axolotls, planarian, chick and drosophila, allowing students to hone their technical skills. In addition, we supplement the labs with reading and discussion of primary research papers to expose them to the scientific literature and to

prepare them to formulate a hypothesis. By mid-semester, students are expected to have formulated a clear testable question on a topic of interest to them. After several weeks in which they are performing their own experiments and discussing the the finding in their references with each other and with the teaching staff they turn in a research proposal. The semester culminates in a poster session where all students present their ideas and progress to each other. We will present the tools we use to guide students through the scientific process, as well as some common challenges. In addition, we will discuss the impact of our more open-ended lab in comparison to investigative project labs in which students join an ongoing research project, exemplified by the HHMI-sponsored phage hunters.

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Introductory Biology Research in a High School Setting

Maya Robnett, Sidwell Friends Students, *Sidwell Friends, USA*

At Sidwell Friends Upper School, student research is encouraged at all levels. Rising ninth graders choose between taking Biology 1 or an accelerated course, Biology 1A. In this class, the students spend much of their academic year planning, designing, and conducting their Independent Research Projects (IRPs). These IRPs provide students with the opportunity to engage in practical, investigative learning as a supplement to their traditional study. Students conduct various experiments on the development and behaviors of zebrafish (Danio rerio). By reading scientific literature, designing protocols, and partnering with scientists, students are able to refine their experimental designs and analytical procedures. This year, student IRP's include the examination of: (i) the effect of a neurotoxic pesticide on zebrafish morphology, (ii) the impact of an enzyme inhibitor on fin regeneration, (iii) the effect of ethanol on zebrafish development. Throughout these projects, students have undertaken standard protocol and also employed more novel methods as well. The IRP's have proven to be an extraordinary teaching tool: not only do these experiments help students learn more about biology, they also encourage them to pursue research beyond the classroom.

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A Role of the FUZZY ONIONS LIKE Gene in Regulating Development and Defense in Arabidopsis

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Programmed cell death is critical for development and responses to environmental stimuli in many organisms. FUZZY ONIONS (FZO) proteins in yeast, flies, and mammals are known to affect mitochondrial fusion and function. Arabidopsis FZO-LIKE (FZL) was detected in the chloroplast but not detected in the mitochondria. *FZL* did not complement the yeast strain lacking the *FZO1* gene, suggesting a functional divergence of the Arabidopsis *FZL gene* from its homologs in other organisms. A *fzl* mutant displayed severe developmental defects, showing dwarf and cell death phenotypes, and was more resistant to the bacterial pathogen *Pseudomonas syringae* and the oomycete pathogen *Hyaloperonospora arabidopsidis*. In addition to altered chloroplast morphology and cell death, *fzl* showed the activation of reactive oxygen species (ROS) and autophagy pathways. *FZL* and the defense signaling molecule salicylic acid form a negative feedback loop in defense and cell death control. Together these

data suggest that the Arabidopsis *FZL* gene is a negative regulator of development, cell death and disease resistance, possibly through regulating ROS and autophagy pathways in the chloroplast.

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Surviving low oxygen: exploring the role of AMP-activated Kinase in anoxia-tolerance in Zebrafish

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High altitude, stroke and cardiorespiratory disorders are damaging to mammalian tissues in that they cause oxygen (O2) deprivation in organs that are energy-demanding. The zebrafish model provides unique insight into mechanisms of adaptation to O2 deprivation, as zebrafish embryos can adapt to low O2 by entering a state of developmental arrest that conserves cellular energy (ATP). The zebrafish model may therefore reveal targets for therapeutic treatment of hypoxia-related injury. The Brewster lab aims to identify the signaling pathways that induce the arrested state in response to anoxia (zero O2). We hypothesize that AMPK (AMP-activated kinase) is a key mediator of developmental arrest. AMPK is a highly conserved eukaryotic energy sensor molecule that is activated under conditions of energetic stress in organisms ranging from yeast to zebrafish to plants. We therefore predict that activation of AMPK under anoxia is necessary for the zebrafish embryo to enter developmental arrest. We predict that loss of AMPK function under anoxia will prevent arrest and lead to premature death. In our ongoing study, we are testing this prediction by comparing arrest and survival rates of wild-type zebrafish embryos to that of mutant and drug-treated zebrafish embryos that are unable to activate AMPK. In order to test this prediction, we are comparing the survival of wild type, dorsomorphin-treated and LKB1 mutant zebrafish embryos under anoxia.

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Elucidating the Role of the Flowering Activator FLK in Pathogen Defense in *Arabidopsis thaliana*

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Recent studies using the model plant Arabidopsis thaliana have elucidated the crosstalk between the genetic pathways governing flowering time control and pathogen defense. Metabolically, flowering and defense control are costly processes that likely compete for the same resources during plant growth and development. Our laboratory has a unique Arabidopsis mutant, *acd6-1*, characterized by constitutive defense and diminutive size. The small size of *acd6-1* is inversely proportional to the defense level, which makes *acd6-1* an ideal readout to quickly assess defense levels in genetic analyses of defense related mutants. In a mutant screen of *acd6-1* suppressors, we identified an allele (*flk-5*) of *FLK,* a canonical flowering activator encoding a putative RNA binding protein that localizes to the nucleus. *flk* loss-of-function mutants were previously shown to exhibit delayed flowering. We confirmed suppression of *acd6-1* with another *flk* allele (*flk-1*). We additionally complemented the late flowering phenotype of *flk-1* with a wildtype *FLK* gene translationally fused with the *GFP* reporter. To further assess the defense role of *FLK*, we infected plants with a virulent *Pseudomonas syringae* strain DG3 and found that both f*lk-1* and *flk-5* mutants exhibited increased bacterial growth than wild type plants. In addition, *flk-1* and *flk-5* also showed reduced response to the treatment of flg22, a defense elicitor derived from the conserved region of *P. syringae* flagellin proteins, for reactive oxygen species (ROS) production and callose deposition

at the cell wall. Together these results affirm a role for the flowering activator gene *FLK* in pathogen defense, illustrating the crosstalk between pathogen defense and flower development. Further studies are necessary to elucidate the molecular mechanism underlying the defense role of *FLK*.

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Role of Lactate-NDRG Signaling in Low Oxygen Tolerance

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Low oxygen (O2) partial pressure results in decreased ATP levels via reduction in oxidative phosphorylation – which is most acutely experienced in organs with high metabolic demand. Hence it is not surprising that ischemic injuries cause major morbidity and mortality worldwide. Zebrafish embryos maintain function and homeostasis under low O2 by transitioning into a hypometabolic state, which is manifested by an arrest or a delay in development. Remarkably, zebrafish embryos can survive up to 50 hours in this hypometabolic state in complete absence of O2 (anoxia). Currently, the molecular mechanisms that initiate and maintain the hypometabolic state in zebrafish are unknown. Understanding these molecular mechanisms may reveal potential therapeutic targets for the prevention and treatment of ischemic injuries. We are currently using a multipronged approach to identify molecular mechanisms of hypometabolism in zebrafish embryos. A mass-spectrometry study examining metabolites whose levels change in anoxic conditions revealed a significant increase in the concentration of lactate – a molecule which was recently shown to bind to N-myc downstream-regulated gene 3 (NDRG3) to promote angiogenesis and proliferation in hypoxic cancer cells; suggesting a signaling role of lactate in cellular adaptation to low O2. In the zebrafish embryo, members of the NDRG family are expressed in a tissue-specific manner. We investigate here the function of zebrafish NDRG1 in the adaptation of the kidney and ionocytes, two metabolically demanding cell types, to anoxia.

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Evolution of the pea aphid photoperiod response

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The pea aphid, *Acyrthosiphon pisum*, exhibits a remarkable adaptive response to seasonal changes in photoperiod. In spring and summer, aphids reproduce asexually, yielding large numbers of genetically identical female offspring. The longer nights accompanying the fall induce these asexual aphids to produce sexual males and females, which mate to lay frost-resistant eggs. These eggs diapause through the cold winter months, hatch into asexually reproducing females in the spring that found new clonal populations. Pea aphid populations have been shown to exhibit latitudinal variation in this photoperiod response, presumably reflecting local adaptation to variation in the timing of the first frost. Populations from the southern United States have been reported to exhibit attenuated photoperiod responses or to have lost the ability to produce sexuals altogether. Here we describe a previously detected difference in the photoperiod response between strains from New York and Arizona. With an eye toward understanding what underlies this difference, we also describe differences in how these strains respond to juvenile hormone, which has been implicated in the induction of asexual fate.

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Understanding induction of suspended animation in Zebrafish

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Deprivation of oxygen, or ischemia, is observed in stroke, heart attack and cancer, and leads to severe depletion of intracellular energy. Depletion of adenosine triphosphate (ATP), the major cellular energy source, is believed to be irreversibly damaging to living tissues. Some organisms have adaptive mechanisms that prevent them from completely expending ATP under low oxygen conditions. These processes are not well understood, but a prevailing idea is that arrest of activity or "suspended animation" prevents complete ATP depletion. Zebrafish embryos arrest under anoxia within thirty minutes, suggesting arrest is triggered possibly involving a key change in metabolites. A favored model for oxygen sensing posits that an increase in adenosine monophosphate (AMP) may serve as the proximal signal to trigger arrest. We are utilizing metabolic profiling to identify rapidly changing metabolites after exposure to anoxia in Zebrafish. Interestingly, the levels of lactate change dramatically and may precede the previously reported changes in AMP:ATP ratio. This glycolytic byproduct was recently shown to bind to and promote the stability of N-myc downstream-regulated gene (NDRG) in hypoxic cancer cells. Ongoing studies aim to test lactate sensitivity of NDRG1 and to investigate whether members of this protein family play a protective role in anoxia-sensitive organs. This investigation was supported by a MARC Undergraduate Student Training in Academic Research (U-STAR) National Research Service Award (NRSA) Institutional Research Training Grant (2 T34 GM008663) from the National Institutes of Health, National Institute for General Medical Sciences.

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Studying the effects of human pathological mutations in Mitochondrial RNase P complex using in vivo Drosophila models

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Mitochondria supply ATP and play a critical role in cell homeostasis, apoptosis and signal transduction. Mitochondrial dysfunction is implicated in several cardiovascular and neurological disorders. Mitochondria have evolved endosymbiotically within the eukaryotic cell, retaining their own genetic material. The mitochondrial DNA (mtDNA) encodes for proteins of the electron transport chain along with ribosomal and transfer RNAs (mt tRNA). Interestingly, even though only ~9% of the mtDNA encodes for mt tRNAs, point mutations in mt tRNAs cause a disproportionally large number of mitochondrial diseases, which have been shown to manifest specific symptoms like cardiomyopathy and hypertension. Like bacteria, the mtDNA is transcribed as a polycistron. To become functional, mt tRNAs must be excised from the adjacent protein coding and ribosomal RNA region. The Mitochondrial Ribonuclease P (MRPP) complex processes the 5' end of the mt tRNAs in humans. The human MRPP is a three protein complex composed of MRPP1, MRPP2 and protein only RNase P (PRORP). PRORP is the catalytically active subunit while the exact role of MRPP1 and 2 is still unclear. Evidence suggests that mutations in the MRPP complex cause myocardial infarction, coronary heart diseases and cardiomyopathy. Our lab studies the Drosophila homologs of the MRPP complex: Roswell (MRPP1), Scully (MRPP2) and Mulder (PRORP) to understand the effect of these pathological mutations in relation to cardiovascular diseases. We demonstrated that each of these proteins co-localizes to mitochondria and are required for survival. Loss of each of these proteins is associated with mitochondrial deficits partly due to reduced mt tRNA processing. Currently, we are generating fly lines with human pathological mutations in MRPP to study their *in vivo* effect on mt tRNA processing, mitochondrial function and tissue homeostasis. Using our

in vivo fly model we strive to understand mt tRNA processing linked to human mitochondrial diseases.

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Modeling human craniofacial diseases in an organism with no bones, C. elegans

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In approximately 1 in every 2500 births, there is a premature fusion of the skull bones called craniosynostosis leading to asymmetric craniofacial development and to the inability to accommodate the rapidly growing brain in infants and children. The sole medical intervention is one or more surgeries with associated risks and complications. Therefore, a complete understanding of the underlying biology of craniosynostosis is of major public health significance.Craniosynostosis can be inherited, and one of the causes is mutations in the basic helix-loop-helix transcription factor *TWIST1*. One of TWIST1's roles is to ensure that the correct complement of proteins is expressed in bone stem cells to keep them dividing and not fusing too early. Recently, mutations in a conserved amino acid of the basic DNA binding domain in TWIST1and its paralog TWIST2 have been discovered in three distinct human craniofacial diseases. The pathological mechanisms for these diseases are not well understood. Our laboratory studies the sole *TWIST1/2* ortholog*, hlh-8,* in the model organism *Caenorhabditis elegans.* HLH-8 and its partner protein, HLH-2, and their downstream target genes are conserved with human TWIST1. Even though *C. elegans* is an invertebrate that lacks craniofacial structures, the conserved Twist pathway suggests that aspects of the transcriptional mechanisms will be conserved. We have constructed *hlh-8* mutations *in vivo* that correspond to four human craniofacial diseases associated with TWIST1 and TWIST2. The *C. elegans* mutant phenotypes and the effects on HLH-8 target genes suggest that some of the mutants retain a fair amount of function whereas others do not. Further, the phenotypes of a subset of heterozygous mutants suggest a possible dominant negative mechanism that may be consistent with distinct phenotypes in patients who all have heterozygous disease mutations.

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Zebrafish models of RASopathies: the impact of PTPN11 mutations on early embryogenesis

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Mutations that activate the RAS/ERK signaling pathway contribute to human disease; *de novo* somatic mutations in pathway components cause cancer, while mutations transmitted through the germline cause a spectrum of developmental disorders, collectively termed RASopathies. *PTPN11* missensemutations cause either Noonan Syndrome (NS) or Noonan Syndrome with Multiple Lentigines (NSML). NS patients exhibit congenital heart defects, craniofacial malformation, neurocognitive delay and an increased predisposition to cancer. NSML patients display the same symptoms, with the addition of lentigines – freckle-like lesions – and sensorineural deafness. While NS mutations increase the phosphatase activity of PTPN11 by destabilizing the inactive conformation, NSML mutations decrease phosphatase activity but cause sustained pathway activation. Modeling the comparative impact of these mutations could be informative for predicting symptom risk in patients. Using transient overexpression of variant *PTPN11*, we have compared the strength of patient mutations as measured by the effect upon early embryogenesis in zebrafish. The aspect ratio of the 11 hpf-old embryo provides a convenient readout of pathway activity, with stronger mutations presenting an increasingly oval morphology due to defects in convergence and extension. Injected embryos are also shorter at

3 dpf, display craniofacial malformations at 5 dpf and exhibit heart defects, reduced heart rate and cardiac edema, phenocopying many of the characteristic symptoms of NS and NSML. We have performed quantitative analysis and generated a rank of mutation impact for each phenotype. Intriguingly, preliminary data suggest that the rank of mutation strength generated at 11 hpf is not predictive for phenotype severity at later stages, indicating that mutations may differentially impact the development of distinct structures.

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Exploring the role of the ribonucleoprotein Clu in mitochondrial protein import

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Mitochondrial health and quality control are essential for proper cellular function. Hundreds of nuclear encoded proteins are required for proper structural and functional integrity of mitochondria. Although the majority of these proteins are post-translationally transported to the mitochondria using various molecular chaperons, there is increasing evidence that co-translational import is also used. The Drosophila protein Clueless (Clu) is required for proper mitochondrial function. *clu* mutant flies are sick, uncoordinated, sterile and have damaged nonfunctional mitochondria. These defects are a direct effect of lack of Clu because Clu also interacts with outer mitochondrial membrane proteins such as Tom20, Porin and Pink1. Recent observations from our lab and others have shows that Clu and the mammalian homolog Cluh are ribonucleoproteins that preferentially bind nucleus-encoded mitochondrial mRNAs. In addition, we have shown Clu binds ribosomal proteins on mitochondrial membrane. Our model is that Clu binds mRNAs for co-translational import into mitochondria and may act as a sensor for mitochondrial quality through Clu's interaction with the mitophagy machinery. Here we investigate Clu's role as ribonucleoprotein in further detail and identify potential candidate mRNAs to explore the function of Clu in mitochondrial protein import.

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Modeling NGLY1 deficiency in Caenorhabditis elegans

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There are ~7,000 rare diseases worldwide, 80% of which are thought to be monogenic in origin. In the United States alone, it is estimated that 1 in 13 people suffer from a monogenic rare disease. Unfortunately, the development of treatments for these patients has been difficult as disease populations are inherently small and treatment development is very expensive. As a result of this dilemma, only 5% of all rare diseases have a current treatment. To fix this, we suggest modeling rare diseases in *Caenorhabditis elegans*, a well-characterized model system. *C. elegans* are inexpensive to work with, have a fast, three-day generation time, and have a simple anatomy yielding comprehensible phenotypes. Importantly, the *C. elegans* genome has conserved orthologous genes to roughly 60% of all human genes, permitting its use to model thousands of diseases. In this project, we modeled a rare monogenic disease known as NGLY1 deficiency. Patients with this disease are homozygous or compound heterozygous for mutations in *NGLY1*, a peptide:*N*-glycanase, and suffer with developmental delay, hyperkinetic movement disorder, and hypolacrima. Studies in *C. elegans* and other organisms suggest that NGLY1 may be involved in the degradation of misfolded proteins in the ER. Using CRISPR/Cas9, we modeled one patient allele, R542X, in the *C. elegans* ortholog, *png-1*. We also made a complete gene deletion of *png-1*, serving as a null mutant. Both mutants do not yield an obvious morphological or developmental phenotype under normal growth conditions. However, when treated with the proteasome inhibitor, bortezomib, both mutants arrest at an early larval stage. Taking advantage of this phenotype, we are currently conducting a forward-based, chemical mutagenesis suppressor screen on our patient R542X model. This experiment could provide genes that, when mutated, revert the phenotype to normal growth in the presence of inhibitor.

Such genes would be great drug targets as treatment for NGLY1 deficiency.

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Limb Development is Linked to Maldaptive Behaviors in Cornelia deLange Syndrome

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Background. Cornelia deLange syndrome (CdLS) is a genetic disorder with limb deficits and intellectual disability. The association of limb deficits in CdLS such as 2,3 toe syndactyly, proximal thumb and lack of digit formation in relation to a range of autism traits was examined.
Methods: 41 children with CdLS received clinician interviews and self-report questionnaires to ascertain the diagnosis of CdLS, and assess behavioral measures and autism. Measures used were: the Childhood Autism Rating Scale (CARS) for autism features; the Aberrant Behavior Checklist–Community (ABC–C) for maladaptive behaviors; the Vineland Adaptive Behavior Scale (VABS) for adaptive function. Physical features of CdLS used a checklist developed to diagnose CdLS.
Results: 2,3 toe syndactyly ($p=0.003$) and proximal thumb placement ($p=0.042$) were significantly associated with CARS autism scores. No association was found between CARS autism score and other somatic abnormalities (cleft palate, heart defects, and others). These results remained significant after controlling for level of adaptive function.
Discussion: A strong association between developmental limb deficits and maladaptive behaviors in CdLS suggests that developmental genetic networks important for limb formation may also subserve brain structure formation. *SHH*, *BMP4*, *WNT1, 7a* are involved in both limb development and neurogenesis. *SHH* independently, and via BDNF, influences neurogenesis, while *WNT* pathways are important for neuronal apoptosis. At the same time, *SHH* and *WNT* are involved in limb patterning and interdigital formation, respectively. Any breakdown in cross-talk between these gene sets can in principle result in concurrent brain and limb defects.
Conclusion: The association of limb deficits with autistic features in children with CdLS may result from defective expression of gene sets involved in the development of both CNS and limb structures.

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Modeling Rare Diseases in *Caenorhabditis elegans*

Ben Nebenfuehr, Andrew Golden

Humans harbor approximately 7,000 rare diseases, ~80% of which are monogenic. A rare disease is defined as affecting less than one in 1,500 people. Combined, these rare diseases affect nearly 1 in 10 Americans (25 to 30 million people), and treatments only exist for around 5% of these diseases. Thanks to the advent of whole genome sequencing, the gene(s) responsible for many rare diseases are known, opening the door for more comprehensive studies. Around 60% of the more than 20,000 protein-coding genes in *Caenorhabditis elegans* are estimated to have human counterparts. We can therefore study worm phenologs of human disease, or the distinct phenotype in worms that, while different from the human phenotype, stems from mutations in a homologous gene. The CRISPR/Cas9 system also allows us to mutate conserved residues analogous to those implicit in the human diseases to mimic the patient alleles at the cellular level. Presented here are examples of three ongoing projects designed to uncover cellular interactors and potential drug targets for improving and expanding the treatments available to patients suffering from a rare disease.

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EpCAM is a transcriptional target of Sox9 in pancreatic progenitors

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All type 1 diabetics and a large proportion of type 2 diabetics suffer from a paucity of the insulin-producing beta cells. A cure for diabetes, therefore, must involve β-cell replacement. We believe that induction of beta-cell differentiation from endogenous pancreatic progenitors represents the most effective and amenable method to alleviate β-cell loss. For this reason we are interested in understanding the process of β-cell neogenesis. Unlike their human counterparts, we have shown that zebrafish readily regenerate their β cells following cell-specific ablation. We demonstrated that a specialized ductal cell, called the centroacinar cell (CAC), is the progenitor of β cells during both zebrafish development and regeneration. The mammalian pancreas contains numerous CACs but these cells are not thought to participate in regeneration. We hypothesize that knowing zebrafish-specific mechanisms behind β-cell regeneration will reveal pathways that can be pharmacologically exploited to induce human CACs to become new β cells. Recently we showed that Sox9b haploinsufficiency causes accelerated β-cell regeneration. As the downstream mediators of Sox9b are not well characterized, we employed RNAseq and SOX9 ChIP-seq on samples generated from PANC-1 cells, a human cancer cell line that acts as a model for pancreatic ductal cells. These experiments showed that SOX9 binds and regulates the promoter of *EPCAM*. Furthermore, the loss of Sox9b in zebrafish leads to the upregulation of *epcam* in pancreatic CACs. Using the CRISPR-cas9 system we have created a mutation in zebrafish *epcam*. Now we are analyzing the pancreatic phenotype in homozygous mutants to observe the effects of loss of Epcam function on CAC biology and β-cell regeneration.

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Multipotency of trunk neural crest cells in Trachemys scripta

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The bony plates that comprise the ventral part of the turtle shell (the plastron) are formed by intramembranous ossification, the same process that produces many of the bones of the skull. Several anterior skull bones and facial structures are produced by a population of migrating, multipotent cells originating from the developing central nervous system, known as neural crest cells (NCCs). Previous research has demonstrated that trunk NCCs, arising from the developing spinal cord instead of the brain, migrate in two distinct waves in turtle embryos. This experiment tested the hypothesis that the second wave of trunk NCCs in turtle embryos is capable of differentiating into bone. Turtle (*T. scripta)* NCCs were isolated and allowed to differentiate, and the resulting cell types were analyzed by antibody staining and fluorescent microscopy. The fraction that produced typical NCC-derived cells (i.e. pigment cells) was compared to the fraction that produced osteoblasts. Our results suggest that the late trunk NCCs are predisposed to differentiate into osteoblasts, and thus provide good candidates for the cells that form the plastron. Craniosynostosis is a common human developmental deformity involving premature fusion of the calvarial sutures. A better understanding of intramembranous ossification, and analysis of an enriched population of osteogenic NCCs, could result in improved treatment options.

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MicroRNA regulation of Dishevelled

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MicroRNAs (miRNAs) are highly conserved, small non-coding RNAs that regulate gene

expressions by binding to the 3' UTR of target mRNAs and silence translation. MiRNAs are key regulators of the Wnt signaling pathway that are known to affect cell proliferation, migration, polarity and other developmental processes. This study investigates miRNA regulation of different isoforms of *Dishevelled (Dsh/Dvl)*, an important signaling protein located upstream of β-catenin. The *Dvl* mRNA isoforms have similar spatial localization in early development but later show distinct ciliary staining in select isoforms. Using luciferase assays and site-directed mutagenesis, we demonstrated that the different isoforms of *Dvl* are directly regulated by miRNAs. By blocking miRNA regulation of all *Dvl* isoforms using miRNA target protector morpholino oligonucleotides (miRNA TP MASO), we observed dose-dependent defects of the length of the dorsal ventral rods and patterning of the primary mesenchyme cells. We will identify the molecular mechanism of how miRNA regulation of *Dvl* impacts early development.

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The histone methyltransferase Set1 promotes gene-specific repression of middle sporulation genes in yeast

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Sporulation is characterized by a genetic reprogramming event in which hundreds of genes are induced or repressed in a precisely timed synchrony that controls the progression of meiosis and the morphological changes required for spore formation. Sporulation occurs in three primary phases, defined as early, middle and late. During vegetative growth, middle sporulation genes are repressed by a complex comprised of the Sum1 transcriptional repressor, the histone deacetylase Hst1 and an adaptor protein Rfm1. This repressor complex serves to prevent the aberrant activation of sporulation-specific genes when cells are growing mitotically. While repression of middle genes during vegetative growth has been well-documented, we still have relatively little knowledge regarding the chromatin changes underlying the mechanism of repression for these genes. Our RNA-sequencing analysis of yeast cells lacking the histone H3 lysine 4 (H3K4) methyltransferase Set1, which is commonly linked to global transcriptional activation, has implicated Set1 in repression of middle genes. However the mechanism by which it specifically represses this gene set is unknown. Here, we analyzed the role for Set1 in regulation of middle sporulation genes. Our data reveal that Set1 and components of the COMPASS complex promote repression, likely through the deposition of H3K4me2 at middle gene loci. Additionally, cells lacking Set1 have highly correlated transcriptomes to mutants in the histone deacetylase complex that represses middle sporulation genes and display defects in sporulation. Our data show that Set1 promotes association of this complex with chromatin during vegetative growth, and that the functions for Set1 and the repressor complex diverge as cells enter meiosis. Overall, this work highlights an unexpected role for Set1 as a gene-specific repressor and provides important insights into a new mechanism associated with the control of gene expression linked to meiotic reprogramming.

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Characterizing Sox21-protein interactions and their subsequent functions in the coordination of neurogenesis in Xenopus laevis

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While transcription factors (TFs) primarily serve to alter transcription, some require a partner protein(s) to efficiently affect the transcriptional state of target genes. Since transcriptional regulation is a key factor of embryogenesis, it can be assumed these TF interactions may help

coordinate development. However, the means by which partner proteins serve to guide the function or specificity of a transcription factor is not well understood. Investigating how these interactions contribute to cell lineage coordination will contribute fundamental information not only applicable to embryogenesis, but also to future research aiming to guide pluripotent cells toward a specific fate.
The Sox family of TFs offers promising subjects to study how protein interactions contribute toward cell fate, since they are an example of TFs requiring protein interactions. Although it is known that Sox TFs require a partner protein, little is known regarding the interactomes of each Sox protein, and even less known about the functional significance of these interactions. Sox21, a SoxB2 protein, offers a particularly unique opportunity to study not only how function is influenced by partner proteins, but also how those interactions contribute to cell fate within the process of neurogenesis.
This project is focused toward two interactions, Sox21-Sox2 and Sox21-Gsx2, in effort to bridge the knowledge gap of how protein-protein interactions may serve to coordinate the specification and differentiation of neurons. Previous data suggests Sox21 is necessary to maintain neural progenitor cells in a sox2+ proliferative state, while low levels are also necessary to promote neural maturation. It is hypothesized that Sox21 is capable of having seemingly contradictory functions due to differential interactions with partner proteins across stages of development. In studying these interactions, we aim to test how these partners may differentially guide transcriptional specificity of Sox21.

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Identifying factors that interact with PAX-3, a Paired-box protein involved in hypodermal cell fate specification in C. elegans

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The development of the C. elegans hypodermis is an excellent model for understanding cell fate specification and differentiation. Early events in C. elegans embryogenesis induce hypodermal precursors to adopt dorsal, lateral or ventral fates, and there is much still to be understood about the regulatory network controlling this fate specification. While several transcription factors that function in specifying the major hypodermal cell fates have been identified, little is known about how the ventral and the lateral hypodermal cells are specified to adopt different fates and undergo distinct morphogenetic processes. Previously, the gene *pvl-4* was identified in a screen for mutants with defects in vulval development. We found by whole genome sequencing that *pvl-4* is the Paired-box gene *pax-3*, which encodes the sole PAX-3 transcription factor homolog in C. elegans. We report that *pax-3* is expressed in ventral P cells and their descendants during embryogenesis and early larval stages. *pax-3* mutants show embryonic and larval lethality as well as several body morphology defects that indicate abnormal cell fate specification in the hypodermis. Using reporter genes we observed that in *pax-3* reduction-of-function animals the ventral P cells appear to undergo a cell fate transformation and adopt a lateral seam cell fate. Furthermore, forced expression of *pax-3* in the seam cells caused them to lose expression of seam cell markers. Based on these results, we propose that *pax-3* functions in the embryonic ventral hypodermal cells to repress the lateral seam cell fate. *pax-3* promoter deletion analysis identified a cis-regulatory element that is necessary for *pax-3* expression in the embryonic P cells. Our current aim is to identify factors that regulate *pax-3* expression and that may function with *pax-3* in ventral cell fate specification.

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Dissecting the Wnt Gene Regulatory Network in Multipotent Neuro-mesodermal Progenitors (NMP)

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During development, the pluripotent ESCs that reside in the blastocyst differentiate to Epi stem cells and eventually to multi-potent Neuro-Mesodermal Progenitors (NMPs). NMPs differentiate to give rise to posterior Neural Progenitors (NP) and Paraxial Mesodermal Progenitors (PMPs). In vivo studies show that the absence of Wnt signaling results in the complete loss of NMPs and PMPs. Transcriptional profiling of *Wnt3a*$^{-/-}$ embryos revealed that the Fgf signaling pathway lies downstream of Wnt3a, however, we find that Wnt+FGFs are insufficient for long-term self-renewal of NMPs suggesting that additional signals are necessary to maintain NMPs *in vitro*. We have combined mouse genetics with ESC differentiation in vitro to address the role of Wnt signaling in NMPs. We have shown that Wnt and FGF signals enrich for NMPs through the synergistic activation of target genes such as *T, Sox2, Nkx1-2, Sp5, Sp8 and Cdx2* transcription factors; all critical markers of NMPs. To ask if any of these TFs are sufficient to maintain NMP self-renewal, we took a gain-of function approach in ESCs to examine the consequences of overexpressing T/Bra, Sox2 and Nkx1-2 on ESC differentiation and gene expression. Our data suggests that overexpression of these transcription factors alone is not sufficient to maintain NMPs, however cell biological data suggests that low level Sox2 may be maintaining stemness, while high level Sox2 over expression promotes NP differentiation. On the other hand, overexpression of T appears to promote PMP differentiation through the activation of Msgn1, a master regulator of PMP differentiation. Interestingly, overexpression of Nkx1-2 in NMPs suppressed the pluripotent Epiblast gene program, as well as the PMP and endodermal differentiation gene programs, suggesting that Nkx1-2 might promote NMP self-renewal by both suppressing pluripotency and differentiation. We conclude that Wnt3a activates a GRN that specifies the NMPs and drives NMP differentiation towards PMPs.

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Chemical exposure alters the gene expression of neurotrophins and their receptors in the main olfactory system differently between wild type and Skn-1a knockout mice

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The phospholipase C (PLC) signaling pathways mediate intracellular responses to variety of external and internal changes. We previously showed that direct activation of PLC leads to increases in intracellular Ca^{2+} levels in most olfactory sensory neurons (OSNs) and that the main olfactory epithelium (MOE) expresses multiple PLC isoform gene transcripts including γ1-2 at various levels (Szebenyi et al., 2014; Al-Matrouk et al., AChemS Abstr, 2015). PLCγ1-2 are involved in neuronal structural plasticity, differentiation and survival when activated by neurotrophins (NTs). Here, we investigated whether NTs and their receptor expression in the MOE and olfactory bulb (OB) are altered in wild type (WT) and Skn-1a knockout (Skn-1a$^{-/-}$) mice, lacking TRPM5-expressing microvillous cells (MCs), after two-week chemical exposure. Skn-1a$^{-/-}$ mice display compromised olfactory-guided food searching behavior and social preference when exposed to strong odors and chitin (Lemons et al., 2015, AChemS Abstr). Using RNA *in situ* hybridization, we found that TrkB, TrkC and p75 receptors and NT3 and NT4 gene transcripts were mostly expressed in the OSN layer. Using qPCR, we found that chemical exposure led to significantly decreased p75 in the MOE and TrkB in the OB of WT mice. In the Skn-1a$^{-/-}$ mice, expression of all of the NTs and their receptors were increased and the changes

in BDNF, p75 and NT4 were statistically significant in the MOE after exposure. Furthermore, we found that there were differences in the changes of the gene expression between the anterior and posterior MOE in both WT and Skn-1a$^{-/-}$ mice. Our data indicate TRPM5-expressing MCs in the MOE can affect the chemical exposure-induced changes in the expression of NTs and their receptors.
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Investigation of Volvox carteri cell differentiation and its evolution through functional analysis of regA and regA homologues

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Multicellularity and cell differentiation have evolved multiple times in eukaryotic organisms. The family of volvocine green algae comprise an excellent system to study the evolution of this developmental novelty, because it contains unicellular, colonial, and multicellular lineages. Species in the genus Volvox possess two different cell types, germ and soma. *Volvox carteri somatic regenerator A* (*regA*) gene is essential for maintaining differentiation of soma. *regA* encodes a nuclear protein with a ~110-amino acid region known as the Volvocine Algal *regA* like (VARL) domain, which is present in several *regA-like sequence* (*rls*) genes in *V. carteri* and in other volvocine genomes. *rlsA-D* are the closest *regA* paralogs but their functions are unknown. We are using two approaches to investigate the functions of these genes. First, we are using RNAi and genomic clones to manipulate expression of *rlsA-D*. So far, no transformants receiving *rlsA-C* RNAi transgenes express an observable vegetative phenotype, suggesting these genes do not regulate asexual development. Some *rlsD* RNAi transformants grow poorly, suggesting that *rlsD* regulates growth and reproduction. Same effect was observed when overexpressing *rlsD*. On our second approach, we are testing chimeric *regA* genes that encode a RegA protein whose VARL domain is derived from a RegA homolog—RlsA, RlsB, RlsC, RlsD, or from the RegA protein from other Volvocine, *Pandorina morum* and *Eudorina elegans* (colonial, no cell differentiation) and *Pleodorina japonica* (cell differentiation). Only the chimeric protein with *P. japonica* RegA VARL domain has rescued a *regA* mutant phenotype. In summary, our results suggest that *regA* and the *regA* VARL domain have unique and specialized functions for somatic cell differentiation, and that *rlsD* appears to be required for cell growth and reproduction. These results suggest a model for the evolution of somatic cell differentiation that involves gene duplication and diversification.

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Role of DNA methylation in cavefish eye specific gene repression

Kelly Tomins[1], Aniket Gore[1], James Iben[1], Daniel Castranova[1], Andrew Davis[1], Amy Parkhurst[1], Sara Soueidan[2], William Jeffery[2], Brant M Weinstein[1], *[1]National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892, United States; [2]University of Maryland, College Park, MD 20742, United States*
The cave morph of *Astyanax mexicanus* has a number of anatomical and physiological differences from the surface morph including increased number of facial hair cells and lack of eyes and pigmentation. Several critical eye development genes are down-regulated in the cave compared to the surface morph, however, the mechanism down-regulating these genes is largely unknown. Previous studies on eye specification genes did not identify any inactivating mutations in the coding regions. This observation suggested that other mechanisms including epigenetic and cis-regulatory changes may down-regulate gene expression in cavefish eyes. Here, we report a role for DNA methylation in cavefish eye gene repression. We performed RNA sequencing and whole genome bisulfite sequencing (WGBS) from isolated surface and cave

morph embryonic eyes. Combined analyses of the RNA and WGBS revealed that more than three hundred genes show both down-regulation and promoter hypermethylation. Further analysis revealed that out of the top thirty-five down-regulated and hypermethylated genes, twenty-two genes are associated with human eye disorders. We also found that QTLs responsible for the retinal thickness in cavefish eyes contain genes from this category. These observations suggest DNA methylation based gene repression may be an important mechanism responsible for down-regulation of multiple eye-specific gene expression, and contribute to the reduced eye phenotype. We also found upregulation of Dnmt3bb.1 transcripts in isolated eyes of both the Pachon and Sabinos cave morphs, suggesting changes in DNA methylation may be a common mechanism for modulating eye-specific gene expression in cave-adapted *Astyanax*.

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Apontic may function as an epigenetic modulator in Drosophila border cells

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The feedback inhibitor of the Janus Kinase/Signal transducer and activator of transcription (JAK/STAT) pathway, Apontic (APT), has been identified as a transcriptional regulator important for precise border cell specification in Drosophila ovaries. Border cell migration is a well-studied example of a group migratory behavior. Prior to specification and migration, border cells are stationary epithelial cells part of the follicular epithelial layer. To undergo a partial epithelial-mesenchymal transition in oogenesis, border cells must dynamically regulate transcription to express necessary pro-migratory genes. APT's predicted protein structure contains a Myb/SANT-like DNA-binding domain, a well-conserved DNA binding domain previously identified in chromatin- remodeling complexes. Thus, we hypothesize that APT functions in epigenetic changes during border cell specification. We explored APT's candidacy as an epigenetic modulator by measuring differential levels of H3K9 acetylation in border cell specification and migration. Further, we examined genetic interactions between *apt* and the catalytic Histone Acetyltransferase (HAT) domain-encoding protein *GCN5*, as well as the transcriptional HAT adapter-encoding, *Ada2* gene. Finally, we assayed positive targets of APT binding by performing chromatin immunoprecipitation with anti-APT antibodies. In all, we identify APT as a potentially influencing transcription by modulation of H3K9Ac and regulation of the epigenome in border cells.

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Deciphering the Spatial-Temporal Dynamics underlying Early Cardiopharyngeal Specification by Single-cell RNA-seq: A Blueprint of Early Cardiogenesis

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Heart and pharyngeal muscles share developmental origins from a common pool of multipotent mesoderm progenitor cells - the cardiopharyngeal progenitors (CPPs). The failure of proper CPP specification is thought to cause severe cardio-craniofacial defects, including the DiGeorge syndrome. Here, we took the advantage of a simple chordate model, the ascidian *Ciona*, to address fundamental questions regarding cardiopharyngeal specification. To obtain comprehensive transcription profiles encompassing the transition from multipotent cardiopharyngeal progenitors to distinct fate-restricted precursors, we performed single cell RNA-seq on FACS-purified cells. We obtained a total of 848 single-cell transcriptomes from 5 developmental stages covering early cardiopharyngeal specification and identified cell types based on their unique transcriptional signatures. For each lineage, we reconstructed developmental trajectories by mapping cells onto their developmental 'pseudotime' and

identified distinct regulatory states along the trajectories. We observed that a fraction of the pan-cardiac and ASM genes were both primed in the multipotent progenitors, and then they were progressively excluded from the ASM and cardiac lineages respectively due to the asymmetric activation of FGF-MAPK signaling pathway. In parallel, the *de novo* pan-cardiac genes were activated lately in both FHPs and SHPs before the FHP and SHP specific genes, indicating the preferential organ (heart) fate determination prior tocell-type determination. We also found that the FHPs differentiated into MHC2+ cardiomyocytes, whereas the SHPs differentiated into MHC2- cells, which remained as a separated domain in the conical heart. In addition, we identified a novel SHP-specific regulator *Dach* as an SHP-specific target of *Tbx1/10* and FGF-MAPK, which was required to oppose the FHP-specific program in SHPs.

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Using CRISPR mutagenesis to investigate the roles of regA paralogs rlsB and rlsC in Volvox carteri cell differentiation

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The green alga Volvox carteri possesses just two cell types—small, motile somatic cells and large, reproductive cells called gonidia—and is an excellent model for investigating fundamental mechanisms of development and their origins. Work reported here is being performed for the independent project component of the Developmental Biology Lab course at UMBC and is aimed at better understanding the functions of V. carteri genes closely related to the master cell fate regulator regA. In regA (somatic regenerator) mutants, somatic cells develop normally but then a day later dedifferentiate, enlarge, and develop as gonidia. regA encodes a somatic-cell specific protein (RegA) with a conserved DNA-binding SAND domain, which suggests that it acts as a transcriptional repressor that prevents growth and reproduction in somatic cells. regA is part of a 4-gene cluster that includes paralogs rlsA, rlsB, and rlsC (regA-like-sequence). regA, rlsA, rlsB, and rlsC transcript accumulation patterns are very similar both temporally and spatially, suggesting that rlsA, rlsB, and rlsC might also play roles in somatic cell differentiation and/or maintenance. In this study, we made sgRNA vectors that target rlsB and rlsC for Cas9-generated indel mutations. We transformed the sgRNA vectors (with hygromycin-resistance marker) into V. carteri along with a nitrate-inducible Cas9 gene, obtained hygromycin-resistant transformants, and currently are inducing Cas9 expression in them by growth in nitrate medium. We will analyze any progeny of the transformants that exhibit obvious phenotypes, to determine whether they contain indel mutations in the targeted regions of rlsB and rlsC. Successful generation of rlsB and rlsC mutants would greatly facilitate analysis of these regA paralogs and lead to a better understanding of the evolution of the regA gene family and its function.

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Chromatin accessibility underlying cardiac vs pharyngeal muscle specification in Ciona

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We harness the simplicity of the basal chordate *Ciona* to characterize the dynamics underlying the regulation of multipotency and progressive fate restriction during heart *vs* pharyngeal

muscles (ASM) specification. In *Ciona*, founder cells produce anterior tail muscle (ATM) and bipotent cardiopharyngeal (TVC) cells that are multilineage primed with regulatory cross-antagonisms between the heart and ASM programs to ensure fate segregation. To document open chromatin profiling of cardiopharyngeal precursors, we combined chromatin accessibility profiling to isolate FACS-purified cells followed by targeted molecular perturbations that altered heart *vs* ASM fate specification. From collecting ATACseq from a window encompassing developmental transitions from progenitors to heart- *vs* ASM-specific precursors, we built an atlas of accessible regions from the replicates and cells outside the cardiopharyngeal lineage to analyze differential accessibility. We revealed that chromatin accessibility in founder cells was vastly different from that in TVCs and ATMs, suggesting that chromatin remodeling cooperates with TVC/ATM induction. Moreover, the *cis*-regulatory landscape of active enhancers changed drastically between the TVCs and ATMs while moderate changes were observed at late developmental stages where the majority of accessible regions are cell-lineage-specific, with few differences in accessibility between heart and ASM precursors. Motif enrichment analyses showed pioneer factors GATA and Forkhead families motifs are enriched in TVC-specific peaks while muscle regulators and pioneer factors MyoD and Myf5 were the most enriched motifs in ATMs. These results suggest chromatin accessibility patterns are established early by pioneer transcription factors to define the competence of the cardiopharyngeal lineage by orchestrating the physical conformation of chromatin changes to form both heart and pharyngeal muscles.

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The Role of Alternative Splicing in Plant Reproductive Development

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The development of multicellular eukaryotes is driven by undifferentiated populations of stem cells that support the initiation of tissues and organs. In the model plant *Arabidopsis thaliana*, we have isolated a dominant mutation that causes stem cells to differentiate, leading to the premature cessation of reproductive growth. Multiple floral organ identity genes are strongly overexpressed in this mutant, suggesting a possible cause of the differentiation and consumption of the stem cells. We have determined that these defects are caused by a gain-of-function allele of the transcriptional regulator *SEPALLATA3* (*SEP3*). *SEP3* undergoes alternative mRNA splicing to give rise to variable protein products. Specifically, it was found that within *SEP3*, exon 6 is either retained or spliced out, hinting at some importance underlying the regulation of this exon. We are using quantitative RT-PCR to assess the relative abundance of these spliced mRNA variants in seedling and floral tissues from wildtype and *SEP3* gain-of-function lines. We want to ultimately determine when and where in plant development this exon is spliced and how this alternative splicing of *SEP*3 impacts stem cell function.

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Role of the Histone Demethylase Jarid1C in Differentiation

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Jarid1C is a histone demethylase enzyme that removes the active epigenetic mark of histone H3K4 methylation in chromatin. It is an important gene in mammalian development since many mutations of Jarid1C have been found in humans with X-Linked Intellectual Disability (XLID). Epigenetic modifiers such as Jarid1C are important for controlling gene expression since they contribute to establishing heterochromatin or euchromatin at specific chromosome loci. Stem cell characteristics are closely linked to which genes are expressed and which ones are repressed in cells at a given time, with incorrect gene expression possibly leading to severe developmental defects or even embryonic developmental arrest. Mouse embryonic carcinoma and embryonic stem cells serve as good in vitro models of stem cells and early embryonic

events. To study the role of Jarid1C in stem cells, we have developed a system in which the P19 embryonic carcinoma cell line contains a stem cell gene reporter. This reporter consists of the promoter and enhancer of the essential stem cell gene, Oct4, linked to a fusion of the enhanced green fluorescent protein (EGFP) and neomycin (Neo) resistance genes. This system allows us to easily analyze whether increased or decreased levels of factors such as Jarid1C alter stem cell properties. Using this system, we have found that changes to Jarid1C levels within these stem cells alters some stem cell properties such as differentiation. Further characterization of the role of this epigenetic gene in stem cell differentiation could impact our understanding of certain developmental disorders such as XLID, as well as provide important knowledge of the earliest stem cell specialization events.

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Role of DNA methylation in cavefish eye specific gene repression

Aniket Gore[1], Kelly Tomins[1], James Iben[1], Daniel Castranova[1], Andrew Davis[1], Amy Parkhurst[1], Sara Soueidan[2], William Jeffery[2], Brant Weinstein[1], *[1]National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892, USA; [2]University of Maryland, College Park, MD 20742, USA*

The cave morph of *Astyanax mexicanus* has a number of anatomical and physiological differences from the surface morph including increased number of facial hair cells and lack of eyes and pigmentation. Several critical eye development genes are down-regulated in the cave compared to the surface morph, however, the mechanism down-regulating these genes is largely unknown. Previous studies on eye specification genes did not identify any inactivating mutations in the coding regions. This observation suggested that other mechanisms including epigenetic and cis-regulatory changes may down-regulate gene expression in cavefish eyes. Here, we report a role for DNA methylation in cavefish eye gene repression. We performed RNA sequencing and whole genome bisulfite sequencing (WGBS) from isolated surface and cave morph embryonic eyes. Combined analyses of the RNA and WGBS revealed that more than three hundred genes show both down-regulation and promoter hypermethylation. Further analysis revealed that out of the top thirty-five down-regulated and hypermethylated genes, twenty-two genes are associated with human eye disorders. We also found that QTLs responsible for the retinal thickness in cavefish eyes contain genes from this category. These observations suggest DNA methylation based gene repression may be an important mechanism responsible for down-regulation of multiple eye-specific gene expression, and contribute to the reduced eye phenotype. We also found upregulation of Dnmt3bb.1 transcripts in isolated eyes of both the Pachon and Sabinos cave morphs, suggesting changes in DNA methylation may be a common mechanism for modulating eye-specific gene expression in cave-adapted *Astyanax*.

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Regulation of Competence to Wnt signaling during development

Melody Esmaeili, Peter Klein MD, PhD, *University of Pennsylvania Perelman School of Medicine, Cell and Molecular Biology*

As development proceeds, inductive cues must be appropriately interpreted by competent tissues for cell specification to occur. While key inductive factors and the signaling pathways within competent cells are fairly well-described, the mechanisms by which tissues lose the ability to respond to inductive signals are not understood. We have studied the loss of competence to Wnt signaling in dorsal-ventral specification in *Xenopus laevis*. Localized activation of Wnt signaling during the cleavage stage in *Xenopus laevis* leads to dorsal development. However, in cells that are competent but do not receive a Wnt signal, competence to induce dorsal genes in response to Wnts is lost by the midblastula stage, although other Wnt

responses remain intact. This loss of competence occurs at the level of Wnt target gene promoters at or downstream of Tcf3, a DNA-binding factor that mediates transcription of Wnt target genes. We hypothesize that loss of competence is mediated by changes in histone modifications at the promoter of Wnt target genes that lead to an increasingly repressive state. In support of this hypothesis, exposure to an HDAC inhibitor extends the window of competence to Wnt pathway activation through the late blastula stage and increases acetylation at the promoter of Wnt target genes. Furthermore, a preliminary study suggests the accumulation of a repressive mark at dorsal Wnt target genes promoters that correlates with the loss of competence. These data suggest that chromatin state regulates competence to an early inductive signal in a spatially and temporally regulated manner

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Regulation of Hub Cell Quiescence in the Drosophila Testis Stem Cell Niche

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The stem cell niche is a specialized microenvironment in which stem cells reside and receive local signals that regulate their self-renewal and differentiation. In the well-characterized *Drosophila* testis stem cell niche, somatic hub cells generate many of the essential signals that maintain and regulate adjacent germline stem cells and somatic cyst stem cells (CySCs). These hub cells normally remain quiescent in the adult fly, but recent evidence from our lab has shown that under certain conditions, such as the complete genetic ablation of CySCs, hub cells can transiently exit quiescence, proliferate, and transdifferentiate into functional CySCs. Such findings suggest that upon tissue damage, changes in signaling pathways within the niche may trigger hub cells to proliferate and transdifferentiate into CySCs. To identify the pathways that maintain hub cell quiescence and identity, we conducted a reverse genetic screen in which candidate signaling molecules were knocked down or overexpressed in either the hub cells or the CySCs. Our preliminary results from the screen suggest that the Ras-MAPK and TGFß pathways may contribute to the regulation of hub cell quiescence since the misexpression of specific components from these pathways was shown to promote hub cell proliferation. Our ongoing lineage tracing analyses will determine whether these proliferating hub cells also transdifferentiate into CySCs. These experiments overall contribute to an emerging concept that *in vivo* cellular quiescence is a state that must be actively maintained over time for normal tissue homeostasis. Understanding the signals that regulate cellular quiescence and tissue homeostasis in the *Drosophila* testis may lend greater insight into mechanisms of cancer metastases and novel methods for regenerative therapies.