Program Abstract #1
Mechanical forces in epithelial morphogenesis
Celeste Nelson
Princeton University, USA
The morphogenetic patterning that generates three-dimensional (3D) tissues requires dynamic concerted rearrangements of individual cells with respect to each other. We have developed microfabrication- and lithographic tissue engineering-based approaches to investigate the mechanical forces and downstream signaling responsible for generating the airways of the lung and the milk ducts of the mammary gland. I will discuss how we combine these experimental techniques with computational models to uncover the physical forces that drive development of embryonic tissues and those engineered in the lab. I will also describe efforts to uncover and actuate the different physical mechanisms used to build the airways in lungs from birds, mammals, and reptiles.

Program Abstract #2
A customized light sheet microscopy platform for you and me
Jan Huisken
Morgridge Institute for Research, USA
Light sheet microscopy (SPIM, LSFM) has changed the field of 3D imaging dramatically by offering a versatile and simple technique to obtain optical sectioning deep inside biological specimens. By illuminating the sample with a thin sheet of light and collecting fluorescence with a fast and sensitive camera, phototoxicity is minimal and high speed acquisitions of long developmental processes have become possible. The ability to custom design an instrument around a sample has empowered many research labs to do experiments that have been impossible with commercial instruments. However, disseminating the technology is still a challenge, making it difficult for the biologist to get access to the latest technology. Typically, in order to get access to cutting-edge technology, the biologist visits an engineer’s lab or a facility that offers the technology. However, the experiments may be severely compromised by the fact that living biological samples die or otherwise degrade in transit. Experiments also need to be conducted in a short, predefined amount of time; experiments may be rushed and essential controls skipped. As an alternative, the microscope travels and is provided to the biologist for a reasonable duration, in which experiments can be performed. The biologist and the sample stay in their lab where the conditions are optimal and the biologist can take as much time as needed. The users are asked to evaluate the system and share their experiences with the developers, who can further improve the technology. Here we report a compact, reconfigurable microscopy platform realized by a confluence of optical modelling, bespoke embedded control, and rapid prototyping that can be adapted to a plurality of applications. We illustrate how different microscope geometries, sample sizes and mounting methods can be accommodated within the framework of this platform and report on the first implementation rolled out.

Program Abstract #3
Reflections on human development: From embryo to germ line
Renee A Reijo Pera
Montana State University, USA
Human development begins at fertilization, progresses through a series of cleavage divisions, compaction of the embryo and formation of the blastocyst; then just prior to or during the early stages of gastrulation, a few cells are set aside or allocated to become the germ cells of the next generation and pass the DNA of one generation to the next. Defects in germ cell differentiation are a common cause of human infertility that afflicts 10-15% of
couples and a common cause of embryo loss and/or miscarriage. Over the last decade, we have developed tools and methods to differentiate germ cells from pluripotent stem cells and tools and algorithms to assess human embryo developmental competence in the clinic. These studies will be discussed. They illustrate the precise timing of human embryo development beginning with the first cytokinesis, cleavage division from two cells to three cells and symmetry of the appearance of the third and fourth cells. Furthermore, I will review our work that has uncovered components of a transcriptional network, the OCT4-PAX5-PRDM1 circuit, whose function insures progression beyond the primordial germ cell state of development. It is our hope that knowledge of human germ cell developmental genetics will illuminate the transcriptional network governing acquisition of cell fate from pluripotency to the germ cell lineage, enable establishment of a robust genetic system that may parallel that of Drosophila and the mouse in terms of the ability to examine complex genotypes and phenotypes, and may also contribute substantially to potential novel strategies in clinical applications in diagnosis and development of novel therapeutics for infertility. Finally, I will close with reflections on career and university transitions on a personal level.

Program Abstract #4
Organization of embryonic morphogenesis via mechanical information
Dipjyoti Das¹, Dörrthe Jülich¹, Jamie Schwendinger-Schreck², Emilie Guillon¹, Andrew Lawton¹, Nicolas Dray¹, Thierry Emonet¹, Corey O’Hern¹, Mark Shattuck², Scott Holley¹
¹Yale University, USA; ²CUNY, USA
Speemann and Mangold’s seminal discovery of embryonic organizers almost a century ago motivated Alan Turing’s morphogen theory and the subsequent experimental identification of morphogen gradients. We now understand that organizers establish gradients of diffusible signaling molecules that pattern the surrounding cells in a concentration-dependent manner. Here, we elucidate an additional and fundamentally different mechanism of embryonic organizers. The vertebrate tail organizer functions within a flux of tailbud mesodermal progenitors and epidermis to direct the elongation of the developing spinal column. Using pharmacological and localized transgenic perturbations, 4D imaging of the zebrafish embryo, systematic analysis of cell motion and computer modeling, we find that the vertebrate tail organizer orchestrates morphogenesis over distances beyond the range of morphogen signaling. The organizer regulates the rate, coherence and density of cell flux in the elongating embryo using mechanical information that propagates via relay through physical interactions between neighboring cells. This mechanism is similar to a pressure wave in granular matter, but in the embryo the mechanical information emerges from self-propelled cell movement. Other embryonic organizers may similarly expand their sphere of influence beyond local cell signaling through mechanical information and long-range regulation of morphogenesis. This research was supported by award R33GM114257 from the NIH Common Fund Single Cell Analysis Program.

Program Abstract #5
Molecular basis for biological tube formation
Dicle Berfin Azizoglu, David Barry, Ke Xu, Thomas Carroll, Denise Marciano, Ondine Cleaver
UT Southwestern Medical Ctr, USA
The ability of cells to form and maintain functional systems of contiguous hollow tubes is a critical feature of organogenesis. Lumen formation, or tubulogenesis, occurs in both endothelial and epithelial cells, throughout developing embryos. Formation of vascular lumens by endothelial cells takes place prior to the establishment of blood flow and to vascular remodeling which results in a characteristic hierarchical vessel organization. While epithelial lumen formation, which has received intense attention in past decades, occurs in a variety of manners and in numerous tissues, such as lung, kidney and pancreas. Here, we examine common mechanisms involving control of junction formation and cytoskeletal rearrangements, that direct formation of lumens between cells. We find that similar mechanisms are at play, whereby cells must clear junctions at their apical membrane to open continuous lumens. We show that Rasip1 is critical in developing vascular cords, via its control of GTPase activity and recruitment, junction localization and actomyosin contractility. Similarly, loss of the scaffolding protein Afadin
disrupts de novo lumenogenesis in pancreatic epithelium, via its apicolateral placement of junctions prior to apical membrane biogenesis and control of subcellular localization of Rab GTPases. Together, these studies uncover essential roles of molecular cascades that drive lumen morphogenesis.

Program Abstract #6
Mutually Inhibitory Ras-PI(3,4)P2 Feedback Loops Mediate Cell Migration During Dictyostelium Development
Marc Edwards¹, Xiaoguang Li¹, Peter Devreotes¹
¹Johns Hopkins University, USA; ²Amherst College, USA
Signal transduction and cytoskeleton networks in a wide variety of the cells display excitability, but the mechanisms are poorly understood. Here we show that during random migration and in response to chemoattractants, cells maintain complementary spatial and temporal distributions of Ras activity and PI(3,4)P2. In addition, depletion of PI(3,4)P2 by disruption of the 5-phosphatase, Dd5P4, or by recruitment of 4-phosphatase INPP4B to the plasma membrane, leads to elevated Ras activity, cell spreading and altered migratory behavior. Furthermore, RasGAP2 and RapGAP3 bind to PI(3,4)P2 and the phenotypes of cells lacking these genes mimic those with low PI(3,4)P2 levels, providing a molecular mechanism. These findings suggest that Ras activity drives PI(3,4)P2 down causing the PI(3,4)P2-binding GAPs to dissociate from the membrane, further activating Ras, completing a positive feedback loop essential for excitability. Consistently, a computational model incorporating such a feedback loop in an excitable network model accurately simulates the dynamic distributions of active Ras and PI(3,4)P2 as well as cell migratory behavior. The mutually inhibitory Ras-PI(3,4)P2 mechanisms we uncovered here provide a novel framework for Ras regulation which may play a key role in many physiological processes.

Program Abstract #7
Frequency and asynchrony of actomyosin oscillation promote PCP-dependent convergent extension
Asako Shindo¹, Yasuhiro Inoue², Makoto Kinoshita³, John Wallingford³
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Actomyosin at cell cortices generates contractile forces that drive various cell movements during tissue morphogenesis. Convergent extension (CE) is a fundamental collective cell movement driven by actomyosin contraction at the cell-cell junction, however, how the neighboring cells interact to coordinate their actomyosin dynamics is still unclear. We perform mathematical modeling and in vivo imaging of actomyosin in the Xenopus gastrula. We show that the actomyosin oscillates in both sides of the contracting cell-cell junctions, but the pulsing in two cell cortices are not completely synchronized. Our computational simulation indicates that the asynchronous actomyosin oscillation with an optimal frequency has an advantage in moving cells efficiently during CE. Intriguingly, Prickle, a planar cell polarity (PCP) protein, is required for the proper frequency of actomyosin oscillation. Together, these results indicate that PCP signaling controls not only the orientation of actomyosin contractions, but also their frequency. Our study provides new insights into the function and control of oscillatory actomyosin contractions in convergent extension.

Program Abstract #8
Cell migration, intercalation, and growth regulate mammalian cochlear extension
Elizabeth Driver, Matthew Kelley
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Developmental remodeling of the sensory epithelium of the cochlea is required for the formation of an elongated, tonotopically organized auditory organ, but the cellular processes that mediate these events are largely unknown. We are using both morphological assessments of cellular rearrangements and time-lapse imaging to visualize cochlear remodeling in mouse. Analysis of cell redistribution within the sensory domain showed that the cochlea extends through a combination of radial intercalation and cell growth. Live imaging demonstrated that concomitant cellular intercalation results in a brief period of epithelial convergence early in cochlear
development, while subsequent changes in cell size lead to medial-lateral spreading of the sensory domain. Supporting cells, which retain contact with the basement membrane, exhibit biased protrusive activity and directed movement along the axis of extension. By contrast, hair cells lose contact with the basement membrane, but contribute to continued cochlear outgrowth through increased cell size. Treatment of cochlear explants with the myosin II inhibitor blebbistatin reveals that regulation of cellular protrusions, movement, and intercalation within the cochlea all require myosin II activity. By expressing a dominant-negative allele of myosin II in subsets of cochlear epithelial cells, we are determining how myosin II activity contributes to cell-type specific behaviors during cochlear extension. We are also analyzing the behavior of non-sensory cells located adjacent to the sensory epithelium to determine how these cell populations may influence cochlear outgrowth and coiling. These results establish the cellular processes that drive the distribution of sensory cells along the tonotopic axis of the cochlea and provide insights regarding the evolution and development of elongated auditory organs. Supported by funds from the Division of Intramural Research of the NIDCD (DC000059).

Program Abstract #9
Cell-ECM Adhesion Regulates Melanoblast Migration During Development
Amanda Haage, Caitlin Mitchell, Kelsey Wagner, Katharine Goodwin, Aaron Bogutz, Louis Lefebvre, Catherine Van Raamsdonk, Guy Tanentzapf
University of British Columbia, CA
Animal development requires precise control over the strength and duration of cell attachment to the Extracellular Matrix (ECM), a process mediated by integrin receptors. Biochemical and cell culture studies have been instrumental in uncovering strategies used to regulate integrin-mediated adhesion. However, how these strategies are used in development, particularly in vertebrates, is not well understood. A major goal of our lab is to generate tools to analyze, in developmental contexts, the roles played by regulatory mechanisms that act on integrin-based adhesion. One of the tools we have generated is a mouse containing a mutation in the gene talin (Tln1), which acts as a general activator of integrin-based adhesion. These mice have proven useful in identifying novel roles for integrin-mediated adhesion during development. Talin is a key linker between integrins and the intracellular adhesion complex that connects integrins to the cytoskeleton. The mutation we introduced blocks an important auto-inhibitory interaction, rendering talin more active. As a result of this mutation, the strength and stability of integrin-based adhesions is upregulated during embryonic development. Mice with defective talin autoinhibition exhibit pigmentation defects, which we show are due to delays in melanoblast migration. In addition, primary melanocytes display changes in cell-ECM adhesion and reduced cell migration. Melanoblasts are derived from the highly migratory neural crest cells, and undergo a long-range migration process to populate each hair follicle. We extend our analysis to early neural crest development and further show that disrupting talin autoinhibition alters cell-ECM adhesion in neural crest cells and delays migration out of the neural tube. Our results demonstrate that integrin-mediated adhesion is precisely regulated during neural crest migration and suggests that talin autoinhibition is an important mechanism for fine-tuning cell-ECM adhesion during development.

Program Abstract #10
A membrane ratchet directs progressive cell shape changes in a pulsatile system
Cayla Jewett, Timothy Vanderleest, Hui Miao, Dinah Loerke, Todd Blankenship
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In epithelial sheets, processes that drive cells to change topological relationships can be harnessed by developmental processes to effect changes in tissue architecture. An essential driver of tissue elongation is oriented cell intercalation. In the Drosophila embryonic epithelium, individual cells are able to either consolidate cell-cell contacts or direct neighbor exchange movements through the asymmetric localization of cell adhesion and polarity proteins. Interestingly, the changes in cell shape that drive cell intercalation are not continuous, but instead are pulsatile in nature. Our work has shown that a membranous ratchet centered on Rab35 is used to unidirectionally stabilize pulsed oscillations to produce contractions of interface length. Here, we examine Rab
protein distributions during *Drosophila* epithelial tissue remodeling and show that Rab35 is dynamically planar polarized. Rab35 compartments are enriched at contractile interfaces of intercalating cells and provide the first evidence of interfacial monopolarity. When Rab35 function is disrupted, apical area oscillations still occur and contractile steps are observed. However, contractions are followed by reversals and interfaces fail to shorten, demonstrating that Rab35 functions as a ratchet ensuring unidirectional movement. Although actomyosin forces have been thought to drive interface contraction, initiation of Rab35 compartments does not require Myosin II function. However, Rab35 compartments do not terminate and continue to grow into large elongated structures following actomyosin disruption. Rab35-driven ratcheting also represents a common contractile cell-shaping mechanism, as mesoderm invagination fails in Rab35 compromised embryos and Rab35 localizes to constricting surfaces. These results show that the coordination of membrane trafficking and cytoskeletal forces converge on Rab35 compartmental behaviors to direct cell shaping processes.

Program Abstract #11

**Development and evolution of a metazoan symbiosis**

*Joseph Parker*

*California Institute of Technology, USA*

Symbiotic lifestyles are pervasive in the Metazoa, but how they evolve from an ancestral free-living condition is poorly understood. Traditional model species are typically non-symbiotic, so cannot be used to probe complex interspecies relationships at a deeply mechanistic level. In this talk, I present my laboratory's work on rove beetles: Metazoa's largest family, and a group that has repeatedly evolved phenotypically elaborate symbioses with social insects. These striking and intimate relationships involve extreme changes in morphology, social behavior and chemical communication, and serve as a paradigm of intricate interspecies interactions. Using a new, genetically and behaviorally tractable model rove beetle species, I will present insights into the molecular and developmental phenomena underlying the convergent evolution of complex behavioral symbioses in this animal clade.

Program Abstract #12

**Independent evolution of limbs in cephalopod molluscs by recruitment of an ancient bilaterian appendage developmental program**

*Oscar Tarazona, Davys Lopez, Slota Leslie, Martin Cohn*

*University of Florida, USA*

Comparative studies have shown that appendage development in vertebrates (*Deuterostomia*) and arthropods (*Ecdysozoa*) is regulated by a shared genetic program. An independent origin of limbs also occurred in the third bilaterian lineage, the *Spiralia*, but because limb development in this clade is poorly understood, it is unknown whether the genetic program for appendage development evolved in the common ancestor of all bilaterians. Within spiralian, cephalopod molluscs (squid, octopus, cuttlefish, and chambered nautilus) are characterized by numerous evolutionary novelties, including highly specialized appendages - arms and tentacles - that lack obvious structural homology to any anatomical structures outside cephalopods. We studied arm and tentacle development in the cuttlefish *Sepia officinalis*, a decapod cephalopod that bears eight arms and two tentacles. Our results show that a 3-dimensional pattern of gene expression is established along three axes (proximodistal, anteroposterior, and dorsoventral) of cuttlefish limb buds by the same gene networks that pattern vertebrate and arthropod limbs. To determine whether these networks are functionally conserved, we performed *in vivo* manipulations of dorsoventral and anteroposterior signaling pathways. Repression of Bmp signaling dorsally resulted in formation of ectopic suckers, which are normally restricted to the ventral side of cephalopod limbs. Ectopic activation of Hedgehog (Hh) signaling caused mirror-image limb duplications, whereas repression of Hh caused loss of polarity, characterized by inhibition of sucker formation. These results show that the molecular mechanisms of limb development are conserved across the three major clades of *Bilateria*. Thus, independent evolution of limbs in cephalopod mollusks likely resulted from activation of the ancient developmental genetic program for appendage development that was present in the bilaterian common ancestor.
**Program Abstract #13**

*Feathered feet are just winging it: shifts in pigeon limb identity reveal conserved regulatory networks*

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¹Department of Biology, University of Utah, USA; ²Department of Molecular and Cellular Biology, University of Arizona, USA; ³Department of Genetics, University of Georgia, USA

Deciphering the genetic mechanisms of morphological variation remains a critical challenge in evolutionary and developmental biology. The domestic pigeon is an outstanding model to study the genetic and developmental programs that underlie morphological variation, as it displays striking phenotypic variation within a single species and is amenable to genetic crosses, embryonic studies, and genomic analyses. While most pigeons have scales on their feet, in some breeds scaled epidermis is replaced by skin with a range of feather morphologies. Classical genetics suggest that large feather “muffs” are caused by the synergistic effects of two loci, *grouse* (*gr*) and *Slipper* (*Sl*), which independently produce smaller foot feathers. We recently showed that *gr* and *Sl* are cis-regulatory alleles of the limb-identity genes *PITX1* and *TBX5*, respectively. Therefore, feathered feet result from a partial change in limb identity. To understand the gene regulatory network (GRN) downstream of *PITX1* and *TBX5* that causes foot feathering, we generated forelimb (FL) and hindlimb (HL) transcriptomes from scale, grouse, and muff pigeon embryos. Comparative analyses of HLs reveal a differentially expressed (DE) gene set that is enriched for transcription factors, extracellular matrix genes, and signaling pathways with known roles in limb development. A subset of the DE genes that distinguish scale, grouse, and muff HLs are also DE between pigeon FL and scale HL buds, suggesting a specific set of genes that is misregulated in the partial transformation from HL to FL identity. In addition, we compared pigeon limb bud transcriptomes to chicken, anole lizard, and mammalian datasets to identify *TBX5* and/or *PITX1*-regulated components of an evolutionarily conserved limb GRN. Our analyses reveal a set of subtle gene expression changes that are conserved across amniotes to regulate the development of morphologically distinct limbs. This work is supported by NIH 1R01GM115996 and NSF DEB-1149160.

**Program Abstract #14**

*Wnt/β-catenin regulates an ancient signaling network during zebrafish scale development*

Andrew Aman, Alexis Fulbright, David Parichy

University of Virginia, USA

Understanding how patterning influences cell behaviors to generate three dimensional morphologies is a central goal of developmental biology. Additionally, comparing these regulatory mechanisms among morphologically diverse tissues allows for rigorous testing of evolutionary hypotheses. Zebrafish skin is endowed with a coat of precisely patterned bony scales. We use in-toto live imaging during scale development and manipulations of cell signaling activity to elucidate core features of scale patterning and morphogenesis. These analyses show that scale development requires the concerted activity of Wnt/β-catenin, Ectodysplasin (Eda) and Fibroblast growth factor (Fgf) signaling. This regulatory module coordinates Hedgehog (HH) dependent collective cell migration during epidermal invagination, a cell behavior not previously implicated in skin appendage morphogenesis. Our analyses demonstrate the utility of zebrafish scale development as a tractable system in which to elucidate mechanisms of developmental patterning and morphogenesis, and suggest a single, ancient origin of skin appendage patterning mechanisms in vertebrates.

**Program Abstract #15**

*An epigenetic mechanism for cavefish eye degeneration*

Aniket Gore¹, Kelly Tomins¹, James Iben¹, Li Ma¹, Daniel Castranova¹, Andrew Davis¹, Amy Parkhurst¹, William Jeffery², Brant Weinstein¹

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Coding and non-coding mutations in DNA contribute significantly to phenotypic variability during evolution. However, less is known about the role of epigenetics in this process. Although previous studies have identified eye development genes associated with the loss of eyes phenotype in the Pachón blind cave morph of the
Mexican tetra *Astyanax mexicanus*, no inactivating mutations have been found in any of these genes. Here we show that excess DNA methylation-based epigenetic silencing promotes eye degeneration in blind cave *Astyanax mexicanus*. By performing parallel analyses in *Astyanax mexicanus* cave and surface morphs and in the zebrafish *Danio rerio*, we have discovered that DNA methylation mediates eye-specific gene repression and globally regulates early eye development. The most significantly hypermethylated and down-regulated genes in the cave morph are also linked to human eye disorders, suggesting the function of these genes is conserved across the vertebrates. Our results show that changes in DNA methylation-based gene repression can serve as an important molecular mechanism generating phenotypic diversity during development and evolution.

**Program Abstract #16**

**Progressive regionalization of neural crest gene regulatory circuits during vertebrate evolution**

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As proposed by Gans and Northcutt’s “New Head” hypothesis (1983), emergence of the vertebrate lineage was accompanied by the advent of the neural crest (NC) cell lineage and its formation of novel derivatives. Morphological novelties derived from the NC, such as jaws, enabled the origin and expansion of predation in vertebrates. In jawed vertebrates, NC derivatives are regionalized according to their rostrocaudal axial position of origin, designated as cranial, vagal, trunk, and lumbosacral. Recently, an avian “cranial-specific” NC gene regulatory subcircuit was identified that imbued the cranial population with the unique ability to form craniofacial cartilage. To determine if this axial specific circuit is an ancestral vertebrate regulatory kernel, we isolated homologues of genes in the “cranial-specific” subcircuit and examined if they were expressed in the NC of a basal, jawless vertebrate, the sea lamprey. Our results show that lamprey cranial NC lack many components of the avian “cranial-specific” gene regulatory subcircuit. To determine lamprey NC axial homology to NC of jawed vertebrates, we examined the transcriptional profiles of NC cells arising along the rostrocaudal body axis. Preliminary data suggest lamprey possess two axial populations, cranial and trunk, but these axial populations are less distinct, on a transcriptional level, than axial populations of jawed vertebrates. Our findings suggest that the emergence of vertebrates may have occurred prior to regionalization of the four distinct NC populations. Together, our data suggest that a restriction of regulatory circuits into distinct axial populations, such as the “cranial-specific” subcircuit, may be the evolutionary mechanism contributing to novel neural crest derivative formation and the elaboration of the “New Head” in jawed vertebrates. This work is supported by NIH grant R01NS08690 to MEB. MLM is supported by the Helen Hay Whitney Foundation.

**Program Abstract #17**

**Embryonic origins and cell cycling behavior of annelid stem cells**

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Most segmented worms (annelids) grow continuously throughout their lives by adding new segments from a posterior growth zone. A ring of cells within the growth zone, expressing germline/multipotency genes, have been suggested to be the stem cells that give rise to tissues of the newly-made segments. Despite their significance in the segmented worm life cycle, precise embryonic origins of the growth zone and the characteristics of the cells that make up the zone remain largely unknown. We developed methods for live-imaging the embryos and larvae of the marine annelid *Platynereis dumerilii*. Using single-cell-resolution live-imaging and cell lineage analyses, we determined the embryonic origins of the mesodermal component of the growth zone in the early larvae. We also utilized a live-cell cycle reporter and analyzed the cell cycle characteristics of this population of cells. We found that they are not quiescent but exhibit significantly slower cycling compared to other mesodermal lineages that precede the growth zone lineage. These findings provide a foundation and necessary tools for starting to understand the nature of the stem cell populations in this highly regenerative organism, and allow for evolutionary comparisons to stem cells and regenerative mechanisms in other research organisms. Studies are in
progress for testing the potential of these stem cells upon injury to identify if they can give rise to cell types such as the germline.

Program Abstract #18
Cell cycle arrest and the acquisition of cell invasive behavior – insights from fish tails and worms
Abraham Kohrman, Nicholas Palmisano, Robert Morabito, Benjamin Martin, David Matus
Stony Brook University, USA
During metazoan development the cell cycle is intrinsically coordinated with multiple aspects of embryogenesis. In particular, the decision by individual or groups of cells to pause or exit from the cell cycle appears to be critical for many morphogenetic behaviors, including gastrulation and convergent extension in many taxa. Here, we are exploring cell behaviors associated with invasion in two model systems, C. elegans anchor cell (AC) invasion into the vulval epithelium and zebrafish notochord convergent extension. Cell invasive behavior is critical for many developmental events, including cell dispersal, gastrulation, blastocyst implantation and neural crest migration, but is also dysregulated during cancer metastasis. Intriguingly, we have found that in both worms and zebrafish a single transcription factor appears to be required to maintain a G1/G0 cell cycle arrested state, and that this cell cycle arrest is necessary for the acquisition of invasive behavior. During C. elegans AC invasion, the conserved nuclear hormone receptor, nhr-67/TLX maintains the AC in a G1/G0 state required for basement membrane breach. During zebrafish posterior growth, the conserved transcription factor, ntl/Brachyury, is expressed in two small populations of notochord progenitor cells that undergo convergence and extension as they invade between each other to form the notochord. Strikingly, in both contexts manipulation of the cell cycle is sufficient to change cell behavior, revealing both functional ties between arrest and the invasive state and potentially deep evolutionary conservation of a dichotomy between cell proliferation and cell invasive behavior. These results may have important implications to how we think about limiting invasive behavior in pathogenic states such as cancer.

Program Abstract #19
A novel in vivo model of cell dissemination
Jiae Lee, Alejandra Cabrera, Young Kwon
University of Washington, USA
Cell dissemination is a multistep process leading transformed cells to leave the primary tumor site. Since metastasis is initiated by cancer-cell dissemination, targeting this initial step could be considered as an ideal strategy for the treatment of metastasis. Despite of its importance, our knowledge of cell dissemination is limited, largely due to the lack of proper in vivo models. Recently, we have established a novel model in Drosophila melanogaster, which enables us to scrutinize the cell dissemination process with an unprecedented cellular and molecular resolution. When the oncogene RasV12 is expressed in intestinal stem cells and enteroblasts, these cells proliferate initially, then however, progressively disappear from the intestine. We discovered that the RasV12-expressing cells can basally disseminate from the intestine by passing through the visceral muscle and reside outside of the intestine although apical extrusion is also frequently seen. Disseminating cells undergo striking morphological changes, which is accompanied by remodeling of actin cytoskeleton. In particular, the disseminating cells produce invadopodia – the actin-rich structure involved in invasive behavior of cancer cells – exclusively at the basal side of the cells where protrusions form across the visceral muscle. Furthermore, these cells produce large extracellular vesicles (normally bigger than 1µm), resembling those observed in the invasive cancer cells. Taken all together, this system recapitulates many features of cancer cells implicated in invasive behavior. Therefore, this model will provide us a unique opportunity to study the molecular mechanisms underlying cell dissemination in vivo with the vast genetic tools available in Drosophila, which might provide new avenues for developing intervening strategies for metastasis.
Program Abstract #20
Flamingo, a Planar Cell Polarity (PCP) component, mediates cell competition to promote tumor progression and MMP1 expression to promote invasiveness
Bomsoo Cho, Jeffrey D Axelrod
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Planar Cell Polarity (PCP) is the polarity of cells in the plane of an epithelium, reflecting the coordinated acquisition of cellular polarity orthogonal to the apical-basal axis. Components of the PCP signaling pathway are well conserved throughout evolution from flies to vertebrates, and mutations in this pathway result in a range of developmental anomalies and diseases. While PCP has been proposed to affect various stages of tumor progression, studies in mammalian model systems have not yielded a coherent picture of their functions, and therefore our understanding of PCP in tumor progression remains rudimentary. We have established in vivo epithelial tumor models in Drosophila to study roles of core PCP components during tumor progression. Tumors overexpressing Ras<sup>12</sup> and lacking scribble (scrib) were introduced either in the whole eye epithelium, or in clones to mimic the early tumor surrounded by normal cells. Eye tumor tissues were transplanted to the thorax of healthy hosts to monitor events in late stages of tumor progression, such as invasion and metastasis. We discovered that the Drosophila atypical cadherin Flamingo (Fmi), but not other core PCP components, is required for progression of clonally induced tumors but not whole eye tumors. Fmi is shown to be required for the tumor cell population to outcompete surrounding normal cells, indicating a function for Fmi in the process of cell competition required for early tumors to overgrow neighboring wildtype tissue. Fmi also appears to play roles in maintaining winner cell fates in other cell competition situations, including scrib and Myc overexpressing clones. In allografts, Fmi is required for the Matrix Metalloproteinase 1 (MMP1) expression of transplanted tumor cells, indicating that Fmi supports tumor cell migration and invasiveness. Signaling pathways in which Fmi is involved for cell competition and metastasis are being examined to understand the underlying molecular mechanisms. Funded by NIH.

Program Abstract #21
Specific Wnt-Fzd interactions are required for hematopoietic stem cell development
Stephanie Grainger<sup>1</sup>, Nicole Nguyen<sup>1</sup>, Jenna Richter<sup>1</sup>, Jordan Setayesh<sup>1</sup>, Brianna Lonquich<sup>1</sup>, Chet Huan Oon<sup>1</sup>, Jacob Wozniak<sup>1</sup>, Caramai Kamei<sup>2</sup>, Jack Houston<sup>1</sup>, Iain Drummond<sup>2</sup>, David Gonzalez<sup>1</sup>, David Traver<sup>1</sup>, Karl Willert<sup>1</sup>
<sup>1</sup>University of California San Diego, USA; <sup>2</sup>Massachusetts General Hospital, USA
Wnt genes encode a family of secreted growth factors that are upstream of a diverse set of signaling cascades required in all multicellular organisms. Wnt signaling regulates a plethora of processes during embryonic development and tissue homeostasis, including spatial organization, regulation of cellular proliferation and maintenance of stem cell niches. There are at least 19 Wnt ligands and 10 different Frizzled (Fzd) receptors in the mammalian genome. However, the mechanisms by which specificity in Wnt-Fzd interactions is required for downstream signaling remains poorly understood. Although Wnt ligands can bind to Fzd receptors with different affinities, the biological impact of these observations in vivo has yet to be determined. This is further complicated by the widely held belief that many Wnt proteins interact promiscuously with Fzd receptors. Using zebrafish and human cells, we have identified that surprisingly, the ligand Wnt9a is specifically required for the generation of hematopoietic stem and progenitor cells (HSPCs). Additionally, we demonstrate that the receptor Fzd9b signals specifically with Wnt9a, upstream of β-catenin arm of Wnt signaling. Using a combination of in vivo genetics and in vitro cell biology, we have determined that Wnt9a and Fzd9b interact to regulate HSPC development; the human homologs WNT9A and FZD9 interact to instruct the derivation of HSPCs from human embryonic stem cells, suggesting conservation. Finally, using a series of Fzd chimeric molecules, we demonstrate that signaling specificity is not mediated by the interaction of Wnt9a with the ligand binding domain of Fzd9b, but instead requires two intracellular domains in Fzd9b, suggesting that Wnt-Fzd specificity requires the input of additional transmembrane factors. Using a mass-spectrometry based approach, we have identified putative candidates for this factor, and will present a model for Wnt-Fzd specificity. This work was supported by NIH, LLS, AHA and CIRM.
Program Abstract #22
Oncogene-induced differentiation is a dominant tumor suppressive mechanism that restrains clonal expansion in skin epithelium
Zhe Ying, Madeline Sandoval, Slobodan Beronja
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Recent analyses of aged, sun-exposed yet asymptomatic human skin led to a striking discovery that oncogenic lesions can be detected in as much as 18-32% of physiologically normal epidermis. Although most of these lesions were tolerated, they exhibited a wide range of ability to drive clonal expansion, a necessary yet not sufficient step in tumorigenesis and one of the hallmarks of malignant transformation. The immediate implication of this finding was the existence of tumor suppressive mechanisms that maintain long-term homeostasis in epidermis despite possessing cancer-driving mutations. In addition, this observation suggests that oncogenic lesions can accumulate in a tissue long before any visible transformation, and that a lesion which compromises the skin’s oncogenic tolerance and drive clonal expansion can have a disproportionate effect on promoting tumorigenesis. To investigate the cellular and molecular mechanisms that maintain tissue homeostasis despite oncogenic stress, we focused on activating mutations in PI3K/AKT, the most commonly activated pathway in cancer. By employing independent assays for direct and quantitative assessment of cell fate choices in vivo, we show that oncogenic activation of PI3K/AKT promotes differentiation and cell-cycle exit of epidermal progenitors. As a result, PI3K/AKT activated epidermis exhibits a growth disadvantage even though its cells are more proliferative. To uncover the underlying mechanism behind oncogene induced differentiation, we conducted a series of genetic screens in vivo, and identified an AKT substrate SH3RF1 as a specific promoter of epidermal differentiation that has no effect on proliferation. Our study provides direct evidence that oncogene induced differentiation serves as a robust and primary block to oncogene induced clonal expansion in epidermis.

Program Abstract #23
LlamaTags: A versatile tool to image transcription factor dynamics in live embryos
Jacques Bothma, Matthew Norstad, Simon Alamos, Hernan Garcia
University of California At Berkeley, USA
Embryonic cell fates are defined by transcription factors that are rapidly deployed, yet attempts to visualize these factors in vivo often fail due to slow fluorescent protein maturation. Here we pioneer a protein tag, LlamaTag, which circumvents this maturation limit by binding mature fluorescent proteins, making it possible to visualize transcription factor concentration dynamics in live embryos. Implementing this approach in the fruit fly Drosophila melanogaster, we discovered stochastic bursts in the concentration of transcription factors that are correlated with bursts in transcription. We further used LlamaTags to show that the concentration of protein in a given nucleus depends heavily on transcription of that gene in neighboring nuclei; we show that this inter-nuclear signaling is an important mechanism for coordinating gene expression to delineate straight and sharp boundaries of gene expression. Thus, LlamaTags now make it possible to visualize the flow of information along the central dogma in live embryos. This work was supported by the NIH Director’s Pioneer Award.

Program Abstract #24
The role of the Hippo pathway in adherens junction organization
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The Hippo pathway is a highly conserved regulator of tissue growth and junctional organization during development and is responsive to changes in the mechanical environment. Actomyosin contractility promotes tissue growth through modulation of Hippo pathway activity. However, the impact of mechanical cues on growth-independent functions of the Hippo pathway is not clear. In the Drosophila embryo, Hippo signaling controls the planar polarized distribution of adherens junction proteins, which is important for cell shape and epithelial organization. We aim to understand how Hippo and Warts signal to adherens junction proteins, and whether this is directed by tension cues. In the late embryonic epidermis, we demonstrate through laser ablation studies that
mechanical forces acting on cell interfaces are anisotropic, with selected cell edges experiencing higher tension in a planar polarized fashion. In addition, we found that high-tension interfaces display an accumulation of the Hippo pathway components Ajuba and Warts. These interfaces show reduced adherens junction protein localization, suggesting that the spatial regulation of Hippo signaling by mechanical force may regulate adherens junction polarity in epithelial cells. Yet to be determined is how recruitment of Ajuba and Warts to cell interfaces affects Hippo pathway signaling, and how this regulation influences functional protein dynamics and organization. These studies of growth-independent functions of Hippo signaling may uncover novel mechanisms that regulate Hippo/Warts activity.

Program Abstract #25
Macrophages directly alter fibroblast activity during epimorphic tissue regeneration and fibrotic healing
Jennifer Simkin, Fatemeh Safaee, John Gensel, Ashley Seifert
University of Kentucky, USA
Injury culminates in tissue regeneration for some tetrapods and fibrotic healing for others. There is mounting evidence that an initial immune response is essential for both processes. Macrophages, key orchestrators of the immune response, are necessary for proper tissue regeneration across epimorphic regeneration models including mammals. Macrophages are also known to promote fibrotic healing in mammalian models of wound repair prompting the following question: how do macrophages direct tissue regeneration in one context and fibrotic healing in another? In this study, we use a model of mammalian tissue regeneration, the African spiny mouse (Acomys) to investigate macrophage activity during regeneration. Acomys are able to regenerate cartilage, nerves, and hair follicles following a 4mm ear punch injury whereas the lab mouse (Mus) repairs an identical injury with a scar. We hypothesize that unique macrophage activity in Acomys promotes regeneration over scar formation. Analyzing gene expression and cytokine production from macrophages stimulated in vitro, we find species-specific pro-inflammatory profiles. To test how local ear fibroblasts respond to these species-specific macrophage profiles we analyze changes in extracellular matrix production by Acomys and Mus fibroblasts cultured with macrophage-conditioned media. Our results suggest that pro-inflammatory macrophages from Acomys are able to reduce collagen production and increase matrix metalloproteinase production in ear fibroblasts from both species. Finally, using comparative analysis, we quantify macrophage phenotypes in vivo and observe specific differences in spatiotemporal localization of macrophage subtypes active in a regenerating injury versus a scar-forming injury. Our findings suggest the existence of a unique pro-inflammatory macrophage in Acomys that can reduce scar matrix production by fibroblasts and enhance the production of remodeling enzymes important for regeneration. Funding by R01 AR070313.

Program Abstract #26
Dynamics of developmental strategies that drive cell identity and plasticity
Camila Lopez-Anido, Laura R. Lee, Dominique C. Bergmann
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Regulation of cell fate is a crucial component of development. The transition from one cell identity to another along a developmental trajectory is a key control point, yet it is largely unclear how these transitions and cell identities are regulated. To investigate fundamental principles that direct stem cell fate and identity, we utilize the multipotent stem cell stomatal lineage in the model system Arabidopsis thaliana. Stomata are specialized organs on the surface of the leaf that are comprised of two cells. Together, these cells form a pore that opens and closes to enable the exchange of gases with the atmosphere. Stomata arise from three discrete cell lineage states (i-iii) that are sequentially driven by three conserved bHLH transcriptional regulators: SPCH, MUTE, and FAMA. Initially, a series of asymmetric cell divisions that mediate stem cell self-renewal (i) are orchestrated by SPCH, which is followed by a subsequent cell differentiation event (ii) controlled by MUTE. A final symmetric cell division and maturation event (iii) is driven by FAMA. To uncover additional layers of functionally heterogeneous and adaptable cell states, we are currently integrating single-cell systems biology with whole-tissue developmental biology and molecular genetic approaches. Our preliminary single-cell RNA-seq analysis indicates that we can
detect a range of heterogeneity within the stomatal cell lineage, especially during the self-renewing stages, which extends our understanding of evolutionarily divergent and conserved pathways in stomatal development. We hope to investigate molecular mechanisms that confer lineage adaptability and thereby render it “flexible” in response to environmental cues. Ultimately, our study should illuminate both exceptional and generalizable strategies in cell fate and re-programming. This project is supported by HHMI (to DCB) and an NIH training grant (to LL).

Program Abstract #27
Somite contribution to the HSC specification niche
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Hematopoietic Stem Cells (HSCs) are born from endothelial cells in the floor of the dorsal aorta in zebrafish, and in mammals, its cognate vessel, the primitive descending aorta. A better understanding of the cellular specification niche that regulates the birth of these cells might inform attempts to instruct their specification in vitro. We previously showed that Wnt16, a non-canonical Wnt, is required for HSC development in zebrafish. Wnt16 signals through a series of downstream relay signals, but the final signal(s) most proximal to HSC specification remain unknown. Interestingly, Wnt16 loss of function animals exhibit an earlier defect in a somite compartment, the sclerotome, which may house vascular smooth muscle cell (VSMC) progenitors. Sclerotome defects prior to HSC specification suggest that somite-derived cells might somehow be required to properly instruct definitive hematopoiesis, most obviously by contributing to the cellular regulatory environment, or “niche”, that directs HSC specification. We have perturbed sclerotome patterning by knockdown of multiple sclerotome genes, and show that in animals with defective sclerotome development, HSCs fail to be specified, indicating sclerotome is required for HSC formation. Sclerotome cells contribute directly to future arterial VSMCs, and we are investigating genetic regulation of sclerotome compartmentalization to its adult fates, such as bone and connective tissue. Our studies provide a foundation to define the complete in vivo cellular HSC specification environment and elucidate the complete set of signals involved in HSC specification, with the ultimate goal of informing clinical efforts at directed differentiation of pluripotent stem cells to an HSC fate. Funding Sources: NHLBI: 5R00HL097150 and 1F32HL129819, March of Dimes #5-FY14-42

Program Abstract #28
Conserved evolutionary mechanisms underlying collective cell migration in vertebrate neural crest cells and Drosophila border cells
Ezgi Kunttas-Tatli
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Collective cell migration requires a delicate balance between motility, cell-cell and cell substrate adhesion that is critical for embryonic development, tissue repair, and cancer metastasis. To gain insights into the conserved evolutionary mechanisms underlying collective cell behavior, we compared gene expression datasets from vertebrate neural crest cells and Drosophila border cells. Here we examine the function in vertebrate neural crest cells of genes previously implicated in collective migration of Drosophila border cells. By combining genetics, imaging and quantitative analysis, we find that, as in border cell, the JAK/STAT signaling pathway, Stat3, also is required for neural crest migration via regulation of downstream factors including cadherins. In addition, JAK/STAT signaling has a separate and early effect on neural crest cell specification by regulating expression of key genes involved in crest specification such as Ets1. Similarly, our data also suggest that Src kinase and a novel gene Ecd, both homologues of genes important for border cell migration, have an important functional role during chick and zebrafish neural crest migration. Interestingly, Ecd is associated with steroid hormone signaling, a pathway not previously implicated in the neural crest. Overall, this comparative approach enables identification of genes and signaling pathways common to collective cell migration across diverse species and cell types.
Program Abstract #29
Expanding the Human Complex Map: Integration of 9k Mass Spectrometry Experiments Improves Coverage of Ciliopathy Disease Genes

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Understanding the molecular network of developmental processes is crucial to our ability to develop therapeutics for developmental diseases. Unfortunately, we currently lack knowledge of the composition, formation and function of the human molecular network. Several recent studies using methods for high throughput discovery of protein interactions have allowed the construction of protein complex maps but the coverage of specialized proteomes important in development such as the cilium are limited. Here we take an integrated approach by reanalyzing and combining > 9k mass spectrometry experiments from published datasets and construct the most comprehensive human protein complex map to date covering over a third of the proteome and producing over 4k complexes. We expand coverage of the ciliary proteome, identifying > 230 complexes and sub-complexes involving > 150 ciliary proteins. Additionally, our integrated complex map, Hu.MAP (http://proteincomplexes.org) outperforms all other maps with a precision of >80% at a recall of 50% for predicted test interactions. We find many new complexes including ones with enrichment for cilia related developmental disorders (e.g. Joubert Syndrome, Bardet-Biedl Syndrome, Meckel-Gruber Syndrome) that have novel members suggesting candidate disease genes. We experimentally validate several of these disease candidates and show they localize to the cilia as predicted by our map. Specifically, we identify ANKRD55 as a novel member of intraflagellar transport machinery and discover CCDC138, WDR90 and KIAA1328 as members of a large centriolar complex that localizes to the basal body. The expansiveness and accuracy of this human protein complex map yields greater understanding of cellular function and provides avenues for better disease characterization. This work was funded by NIH F32 GM112495 and K99 HD092613.

Program Abstract #30
Fish scales pattern sensory axons and blood vessels during skin maturation

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The skin is a sensory organ that undergoes metamorphic-like changes during organogenesis. How sensory endings accommodate these large-scale changes to their target organ is poorly understood. We have been developing the zebrafish as a model to study sensory remodeling during skin organogenesis, which occurs during the mid-juvenile period and involves the development of bony scales along the fish trunk. The surface of each scale is covered by epidermis, which we found to be profusely innervated by nociceptive (pain-sensing) axons. Interestingly, we discovered that the pattern of innervation is stereotyped, with evenly spaced axon bundles distributed across the scale. These axon bundles share several hallmarks of peripheral nerves, including ensheathment by Schwann cells, congruence with blood vessels, and association with a laminin-rich extracellular matrix. In contrast to models of nerve-vessel alignment in mammalian skin, we found that axons and vessels are patterned independently. Axons and vessels align with radii, which are grooves in the mineralized scale surface. Imaging early stages of scale development revealed that radii arise by polarized osteoblast migration. Analysis of eda and fgfr1a mutants showed that scales are both necessary and sufficient for polarizing skin nerves, and that in the absence of scales epidermal innervation is significantly reduced. We further showed that in scale-less eda mutants cutaneous blood vessel density and nerve association are severely disrupted. Thus, the reorganization of skin innervation in zebrafish occurs in unison with other metamorphic events, involves highly orchestrated cellular migrations, and is dependent on scale development. Because scales are evolutionarily related to other dermal appendages, like feathers and hair, we speculate that birds and mammals may similarly require these appendages to pattern innervation during skin development. NIH grants to JPR (K99HD086271) and AS (R01AR064582) supported this work.
Program Abstract #31
Tissue selective effects of nucleolar stress and rDNA damage in developmental disorders
Eliezer Calo
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Craniofacial disorders are often caused by heterozygous mutations in general regulators of housekeeping cellular functions such as transcription or ribosome biogenesis. While it is understood that many of these malformations are a consequence of defects in cranial neural crest cells (cNCCs), a cell-type that gives rise to most of the facial structures during development, the mechanism underlying cell type-selectivity of these defects remains largely unknown. By exploring molecular functions of DDX21, a DEAD-box RNA helicase involved in control of both RNA polymerase (Pol) I and II dependent transcriptional arms of ribosome biogenesis, we uncovered a previously unappreciated mechanism linking nucleolar dysfunction, rDNA damage and craniofacial malformations. We demonstrate that genetic perturbations associated with Treacher Collins Syndrome (TCS), a craniofacial disorder caused by heterozygous mutations in components of the Pol I transcriptional machinery, impair cellular functions of DDX21. These effects are cell type-selective, cell-autonomous and involve activation of the tumor protein p53. This mechanism is not restricted to cNCCs, as ribosomal gene perturbations associated with Diamond Blackfan Anemia also disrupt DDX21 functions. At the molecular level, we demonstrate that impaired rRNA synthesis elicits a DNA damage response, and furthermore, that rDNA damage results in tissue-selective and dosage-dependent effects on craniofacial development. Taken together, our findings illustrate how disruption in general regulators that compromise nucleolar homeostasis can result in tissue-selective malformations.

Program Abstract #32
Evolution before the central dogma: mapping fitness landscapes of RNA
Irene Chen
University of California, Santa Barbara, USA
Evolutionary outcomes are difficult, if not impossible, to predict, largely because the effect of any possible mutation is unknown. In other words, understanding evolution requires detailed knowledge of the relationship between sequence and activity, or the fitness landscape. Inspired by the RNA World of early life, in which RNA carried information and also performed catalytic functions, we study the emergence and evolution of functional RNAs. I will describe our experimental efforts to map complete fitness landscapes for ribozymes needed to create an early genetic code and the implications for optimizing ribozyme activity and replaying the ‘tape of life’. This work was supported by the Simons Foundation (grant no. 290356), NASA (grant no. NNX16AJ32G), the Searle Scholars Program, and the Hellman Faculty Fellows Program.

Program Abstract #33
Regulation of 3'UTR-Mediated Protein-Protein Interactions
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It has been thought that genetic information is transmitted from DNA to proteins only through translation of mRNA into the amino acid sequence of proteins. However, we recently discovered that genetic information encoded in 3'UTRs can also be transmitted to proteins via 3'UTR-mediated protein-protein interactions (Berkovits & Mayr, Nature 2015). As a result, proteins with identical amino acid sequence that were generated from alternative 3'UTR isoforms have different functions. However, how the information on protein features is transferred from 3'UTRs to proteins was unclear. We found that physiological aggregation of the RNA-binding protein TIS11B is required for this process. TIS11B RNA granules form a large tubule-like meshwork that is intertwined with the endoplasmic reticulum (ER). This novel subcellular compartment has different biochemical and biophysical properties than the cytoplasm and enables the formation of 3'UTR-mediated protein-protein interactions during translation of specific mRNAs at the ER. Therefore, one function of this new subcellular compartment is the regulation of protein features not encoded in the amino acid sequence.
Program Abstract #34
Stem cell differentiation trajectories in *Hydra* resolved at single cell resolution
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Differentiation from stem cells is restricted to certain developmental stages or confined to particular adult tissues in the majority of animals. In contrast, the adult freshwater polyp *Hydra* continually renews all of its cells from either stem cells or via transdifferentiation and therefore presents a valuable system to understand cellular decision making and differentiation pathways. In the homeostatic *Hydra*, continuous cell turnover and replenishment is supported by three lineage-restricted stem cell populations. The multipotent interstitial stem cells continuously give rise to neurons, gland cells, nematocytes and, under certain conditions, to the germline. The two epithelial lineages, ectoderm and endoderm, are supported by mitotically active epithelial stem cells in the body column that are continually displaced toward the oral and aboral ends where they give rise to the head and foot epithelia. Here we elucidate and construct the complete stem cell differentiation trajectories for all three lineages using a droplet based single cell sequencing approach (Drop-seq) on whole animals. We identify molecular signatures of stem cells, terminally differentiated cells, cells in the process of differentiation from stem cells, and cells in the process of transdifferentiation. These data allow us to identify key transcription factors with likely function in specific differentiation trajectories. The data sheds new light on the molecular diversity of certain populations of cells, such as neurons, and suggest that neurons and gland cells share a common progenitor in *Hydra*. All together, these data offer an entirely new window into understanding the molecular mechanisms underlying stem cell biology and regeneration in an animal that has a long history in developmental biology.

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Program Abstract #35
The role of planar polarity and cell geometry in epidermal stem cell self-renewal
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The ability to control cell fate through oriented cell division is imperative for the proper development of many organs, such as the stratified epidermis. Basal stem cells of the epidermis can divide in two ways: 1) perpendicularly to the epithelial plane to produce one basal and one suprabasal daughter that goes on to differentiate, and 2) parallel to the epithelial plane, which generates two basal daughter cells and expands the stem cell pool. While mechanisms leading to perpendicular divisions in the murine epidermis are known, those orienting planar divisions have not been explored. Late in embryonic skin development, apical-basal polarity factors align the spindle to promote perpendicular divisions. Thus, we hypothesized that parallel cell divisions might be controlled by planar cell polarity (PCP), which relies on a set of cortical “core” transmembrane components that are asymmetrically localized along the epithelial plane. In agreement with this hypothesis, *Vangl2*⁰/−/− mutant embryos exhibit increased perpendicular, asymmetric divisions at the expense of planar, symmetric divisions. This defect was not due to increased proliferation rates, nor to mislocalization of cortical spindle anchoring proteins like LGN. Rather, we link the reduction in planar divisions to alterations in cell geometry and cell packing, but these alterations are indirectly caused by the neural tube defects characteristic of PCP mutants. We demonstrate that early in epidermal stratification, there is a close relationship between cell density, interphase cell height:width ratio, and mitotic spindle orientation. Moreover, failure of the epidermis to close over the neural tube in PCP mutants leads to cell crowding and a lower frequency of planar cell divisions. We propose a model in which basal epidermal cells utilize cell packing and shape, rather than cortical PCP cues, to inform planar division orientation.

Program Abstract #36
The airway epithelial ensemble: plasticity, regeneration, and disease
Jay Rajagopal
MGH, Center for Regenerative Medicine, USA
We use a combination of lineage tracing, genome modification, cell ablation, and computation biology to study epithelial regeneration. Our findings concerning plasticity, niches, and intraepithelial signaling all have implications for lung disease. Furthermore, we describe a new lung cell type and myriad disease relevant cell subtypes.

Program Abstract #37
The distal gonadal sheath of C. elegans regulates germ stem cell proliferation and niche exit
Kacy Gordon, Jay Zussman, David Sherwood
Duke University, USA
The C. elegans gonadal distal tip cell (DTC) is a canonical stem cell niche. By using CRISPR/Cas9 to endogenously tag gap junction constituent proteins expressed in the gonad, we found that previously undiscovered, thin, membranous cellular projections from a niche-adjacent somatic cell type, the sheath cells Sh1, intercalate deep into the stem cell zone where they interact with the DTC and underlying germ stem cells to stimulate germ cell proliferation and facilitate niche exit of stem cell progeny. The DTC and Sh1 migrate in close proximity to one another during larval gonad development, and build reciprocal elaborations as the underlying germ cells proliferate to expand the stem cell pool in the young adult animal. Live imaging of germ cell divisions reveals they are most frequent at the DTC/Sh1 interface. Contacts between the germ cells and DTC or Sh1 determine whether a cell born by asymmetric division at the DTC/Sh1 interface will remain in the niche or leave the niche to undergo transit-amplifying divisions and differentiate. Depletion of gap junction subunits expressed in the DTC causes germ cell proliferation under the DTC and at the DTC/Sh1 interface to decrease and lose polarity, but does not affect transit amplifying germ cell division under Sh1. Our findings help complete the picture of a well-studied stem cell niche and offer the first evidence of asymmetric stem cell divisions in the C. elegans gonad, as well as a mechanistic explanation for regulated stem cell niche exit. This work is supported by F32 GM121015-02 from NIGMS to KLG, and R35 GM118049 to DRS.

Program Abstract #38
Genetic analysis of the role of integrin mediated cell-ECM adhesion in hematopoiesis
Rohan Khadilkar, Bhavya Venkatesh
University of British Columbia, CA
The regulation of stem cell behavior and maintenance requires the integration of both intrinsic and extrinsic cues. One such external cue, integrin-mediated cell adhesion to the extracellular matrix (ECM), can play an important part in regulating stem cell function and maintenance. In particular, integrins help define and shape the microenvironment in which stem cells are found: the stem cell niche. In vertebrates Integrins have been implicated in maintenance of Hematopoietic stem cells (HSCs) in the bone marrow but their precise function during hematopoiesis remains elusive due to the difficulty of analyzing these defects in real time. Here we have utilized the powerful genetic tools available in Drosophila, together with long-term live imaging, to analyze the role of Integrin-mediated Cell-ECM adhesion during hematopoiesis in flies. In Drosophila larva hematopoiesis occurs in a specialized organ called the lymph gland (LG). We show that the LG contains an extensive network of ECM that surrounds the HSCs and resembles the reticular fibers found in the vertebrate bone marrow and lymphoid tissues. Long-term live imaging suggests that the ECM network in the LG helps to anchor the HSCs in place. Depletion of integrins in the HSCs disrupts the ECM network in the LG and results in increased differentiation of the HSCs. Intriguingly, following an immune challenge, such as parasitic wasp infestation, we observe a similar decrease in ECM levels in the LG and also induced differentiation of the HSCs. We have gone on to characterize in detail the composition, histology, and function of the Cell-Matrix adhesion machinery in the LG. Based on our data, we propose that integrin-mediated cell-ECM adhesion plays an important role in regulating stem cell homeostasis in the LG. Moreover, we establish the Drosophila LG as a powerful genetically tractable model to analyze the function of integrins and ECM protein during hematopoiesis.
Program Abstract #39
Modulators of tissue regeneration in zebrafish
Kenneth Poss
Duke University, USA
Adult mammals show limited heart muscle regeneration after cardiac injury. By contrast, we showed many years ago that adult zebrafish have a high innate capacity for heart regeneration. This regeneration occurs through activating proliferation of pre-existing cardiomyocytes at sites of injury, rather than de novo creation of new cardiomyocytes from stem cells. Non-muscle cells like the epicardium and endocardium help stimulate regeneration by these cardiomyocytes, by aiding neovascularization and/or releasing mitogens. Here I describe new screening and analysis tools in zebrafish that have enabled us to identify molecular signals key to heart regeneration, and to define new gene regulatory elements that activate regeneration programs.

Program Abstract #40
Teaming with Microbes: Insights from the Zebrafish Intestine
Karen Guillemin
University of Oregon, USA
Resident microbes of animals are a rich source of bioactive molecules that modulate host development. Using the gnotobiotic zebrafish model, we have identified several secreted bacterial proteins that modulate the development and inflammatory tone of the digestive tract. We are currently exploring the mechanism of action of these proteins. In addition, we wish to understand whether these secreted proteins are co-evolved bacterial signals for communicating with the host or incidental cues that the host uses to detect resident bacteria. To do this we are exploring whether the benefits to the bacteria for producing these secreted proteins are distinct from the proteins’ impact on the host.

Program Abstract #41
Biomechanical Analysis of the Embryonic Mouse Heart by Optogenetic Control
Andrew Lopez, Shang Wang
Baylor College of Medicine, USA
The role of biomechanical signaling is well accepted as a modulator of cardiac cell behavior and a requirement for cardiac morphogenesis. However, the small, fragile nature of the embryonic heart makes it difficult to determine transient mechanical homeostasis during heart development and search for causal links between biomechanical forces and cardiac cell behavior in vivo. Our work focuses on characterizing the regulatory role of biomechanical signals to direct cardiac morphogenesis and cell behavior. Towards this end, we have successfully established cardiac optogenetics to control heartbeat frequency in the embryonic mouse heart for the first time. Using a Cre-loxP system, we have generated mice that express the light-activated, cation specific transmembrane channel—Channelrhodopsin2. We have integrated a pulsed laser with our lab-built optical coherence tomography system to activate specific sites of the embryonic heart with a 30μm spatial resolution, and at the same time, generate 4D (3D+time) images of the heart and extract structural and functional information such as heart wall dynamics and blood flow velocity. We will combine this approach with second harmonic generation, an unbiased imaging approach to detect collagen deposition. Our recent preliminary data have indicated we can detect changes in collagen organization throughout the heart over development. Using optogenetic cardiac pacing and second harmonic generation imaging, we will look at how changes in heart biomechanics are consequential in the deposition and organization of cardiac collagen. This work is supported by the NIH (R01HL120140 and T32HL07676) and the Optical Imaging and Vital Microscopy Core at the Baylor College of Medicine.

Program Abstract #42
A massively parallel screen for morphogenesis genes by RNAseq of interspecific hybrid embryos
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1Georgia Institute of Technology, USA; 2University of Washington, USA; 3Friday Harbor Labs, USA; 4BEACON,
Tunicates are the invertebrate sister group to the vertebrates, and together with cephalochordates, these three groups form the Chordate phylum. Although adult tunicates and vertebrates have little resemblance to one another, their early developmental stages are very similar; both groups typically form a notochord—an elongated row of cells that provide structure for the developing embryo and larvae—the key feature that defines the phylum. However, several tunicate species have independently evolved an alternate “tail-less” body plan, losing their notochord and several other features such paraxial muscles, motor neurons, and pigmented cells associated with geotactic and light-sensing organs required for swimming and settlement. Two such species, *Molgula occulta* and *Molgula oculata* — a tailless and tailed species, respectively, are able to cross fertilize and produce hybrid offspring with a partially formed tail. These species and their hybrid provide a powerful system in which to study the mechanisms underlying body plan development and evolution. Through the use of next generation sequencing, we have assembled the genomes and transcriptomes of both species and their interspecific hybrid at key developmental stages (gastrula, neurula, and tailbud). Differential expression of parental alleles in the hybrid has enabled us to identify a set of genes whose expression has been specifically lost in tailless *M. occulta* and are thus likely to be required for morphogenesis of tailed *M. oculata* but dispensable for the alternative tailless developmental body plan. Furthermore, through the examination the genome we are able identify some of the mechanisms behind these changes such as pseudogenization and cis-regulatory module loss-of-function. **Funding** Provided by: BEACON Center at Michigan State University, and NIH/NICHD

**Program Abstract #43**

**Diverse Regenerative Strategies of the Intestinal Epithelium**

**Ophir Klein**  
**UCSF, USA**

Epithelial surfaces form critical barriers to the outside world and are continuously renewed by adult stem cells. Whereas epithelial stem cell dynamics in homeostasis are increasingly well studied, how stem cells are redirected from a tissue-maintenance program to initiate tissue repair after injury remains incompletely understood. Our initial work focused on the interrelationship between stem cell populations in the gut, and in this talk I will present more recent studies examining the response of the intestinal epithelium to damage. Using a parasitic helminth that disrupts tissue integrity during its life cycle as a model, we found that tissue damage leads to re-initiation of a developmental program, thus representing a fundamental and novel mechanism by which the intestinal crypt can remodel itself to sustain function after injury. I will also discuss our experiments using live imaging of adult intestinal organoids, which show that nascent daughter dispersal occurs when neighboring cells insert between them during cytokinesis. Our data suggest that the mechanics of progenitor cell division provide a driving force for cell mixing in elongated mammalian epithelia.

**Program Abstract #44**

**Looking for a home: the early embryo’s journey through the uterine lumen**

**Diana Flores Diaz, Ripla Arora**  
**Dept. of Ob/Gyn & Reproductive Biology, Michigan State University, USA**

How a mammalian embryo determines its site of attachment is a mystery that has puzzled researchers for decades. While in monocotous mammals it is essential to select a ‘good’ site of attachment, in polycotous species it is also essential to ensure that the embryo is spaced far enough from neighboring embryos to avoid competition for maternal resources. We use our enhanced confocal imaging and 3D image reconstruction technology to evaluate murine embryo location in the uterus, at detailed time intervals along the longitudinal oviductal-cervical axis and the mesometrial-anti mesometrial axis. Using this technology, we can discern the development stage of the embryo and the location of the inner cell mass with respect to the uterine luminal epithelium. Embryos enter the uterus around midnight of day 3 (day of plug is day 0.5) of pregnancy and move through the oviductal-cervical axis until they have traveled about half or two-thirds the length of the uterine horn. The embryos then move bidirectionally to space themselves equally through the uterus. Facilitated by 3D uterine folds that are
perpendicular to the oviductal-cervical axis, the embryos move towards the anti-mesometrial end of the lumen, allowing them to be in close proximity to the uterine glands. Upon achieving equal spacing, the embryos begin to attach to the luminal epithelium and luminal closure ensues from the anti-mesometrial to the mesometrial pole. Our data support the hypothesis that uterine implantation sites are not predetermined but instead are determined by the number of eggs ovulated and/or the number of embryos entering the uterine lumen. This allows for observed smaller distances between implantation sites when more embryos are present as compared to when fewer embryos are present in the uterine horn. We are currently investigating the mechanisms of embryo movement as determined by embryo-uterine interactions to allow for equal spacing and embryo attachment at the implantation site.

Program Abstract #45
Nematode Embryos are Susceptible to the Teratogenic Effects of a Host Pheromone
Ray Hong, Tess Renahan, Daniel Kazerskiy
California State University Northridge, USA
Kairomones are chemical cues that mediate interspecies interactions, such as the behavior between a predator and a prey. Given the intimate co-evolution between hosts and parasites however, we wondered if the host environment also controls the development of early parasite embryos. Few molecules are known to affect early nematode development due to their tough eggshells ex utero. We were therefore quite surprised to discover the host pheromone of the nematode Pristionchus pacificus is not only a volatile attractant to the larval stages, but is also a volatile teratogen against its embryos. To characterize the threshold of sensitivity to the oriental beetle pheromone ZTDO, we determined the minimum exposure duration and developmental window that would result in arrested embryos. We found that exposing embryos to volatile ZTDO for as short as 3 hours during the bean stage of embryogenesis can arrest embryonic development permanently. These embryos do not ever hatch but form incomplete lumens and can exhibit twitching- a cryptic developmental trajectory we dubbed “zombryos.” To determine if ZTDO can act on embryonic tissues directly, we utilized the membrane-specific lipophilic dye FM4-64 and found that FM4-64 specifically stained the plasma membranes of ZTDO exposed embryos, suggesting ZTDO can penetrate the eggshell and permeability barrier. The F1 progeny between a ZTDO-susceptible wildtype and a ZTDO-resistant natural isolate showed partial maternal effect, raising the possibility that the eggshell can regulate embryonic susceptibility. We are currently using single-embryo RNAseq to determine changes to the zygotic gene expression due to ZTDO exposure. Our study challenges our stereotype about the impermeable nematode eggshell and shows that early embryogenesis is susceptible to environmental cues. (NIH Award SC3GM105579)

Program Abstract #46
Bisphenol A influences expression of β-catenin in the zebrafish ovarian follicular WNT/β-catenin pathway
Heather Foote
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Bisphenol A (BPA), an estrogen mimic, has been shown to influence reproductive health. Reproduction in the female is dependent on the development of ovarian follicles through stimulation by follicle stimulating hormone (FSH) and estrogen (E2) to allow for oocyte maturation. The presence of BPA may affect hormone signaling and influence ovarian follicle development. The FSH and E2 stimulated WNT/β-catenin pathway plays an essential role in proliferation and differentiation of ovarian follicle cells, enabling ovarian follicle growth by β-catenin co-activation of cell dividing genes. In this study, the influence of BPA on the WNT/β-catenin pathway was investigated in zebrafish. Ovarian tissue was incubated in BPA (0.01, 0.1, and 1.0 µM) and analyzed for β-catenin mRNA expression by qPCR. A strong trend in decreased β-catenin mRNA expression was observed in 0.1 µM BPA (p = 0.0605) and 0.01 µM BPA (p = 0.0846; 1-way ANOVA). Western blot analysis of β-catenin protein abundance as influenced by BPA and E2 stimulation did not result in a significant difference; however, similarities in BPA and E2 β-catenin protein abundance may be informative as to how BPA influences the WNT/β-catenin pathway. Additionally, the influence of BPA on the interaction of β-catenin with a cell division gene, cyclin D1 (CCND1), using ChiP-PCR did not confirm an association; however, interactions between β-catenin and CCND1 transcription
factors, LEF/TCF, are currently being investigated. We would like to thank the University of Puget Sound and the Sherman Fairchild Foundation for funding this project.

**Program Abstract #47**

**Genetic conflicts and developmental constraints shape centromeric histone function**

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Centromeric histones (CenH3s) are fundamental to the function of chromosome segregation in most eukaryotes. Despite their essential function, they evolve rapidly in both plants and animals. We aim to understand the causes and consequences of this unexpected rapid evolution. We propose that genetic conflicts between warring chromosomes during female meiosis underlie this rapid evolution. Since only one of four ootids is retained in the oocyte in both plants and animals, we propose that chromosomes compete for inclusion by virtue of their recruitment of centromeric proteins like CenH3 (akin to a 'parasite'), whereas CenH3 proteins evolve rapidly to negate this advantage (akin to an 'immune system'). To understand the driving forces behind CenH3 evolution, we reversed the evolution of CenH3 in *Drosophila melanogaster* in situ and in vivo. Remarkably, we found a highly deleterious effect of this CenH3 replacement but exclusively in early embryonic development. Our work not only suggests a genetic incompatibility between CenH3 and divergently evolved satellite DNA, but also reveals an epigenetic basis by which even the 'wrong' CenH3 can be entrained to perform essential centromeric function.

**Program Abstract #48**

**Lineage-specific intrinsic factors govern neural stem cell proliferation decisions in response to dietary nutrient conditions**

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Stem cells switch between quiescence and proliferation as part of developmental programs, during adult homeostasis, and for repairing tissue after damage. Quiescence versus proliferation decisions require coordination of stem cell-intrinsic factors with extrinsic factors, local and systemic, that vary in response to changing animal physiology. Nutrient availability is an important extrinsic factor as nutrients provide the building blocks for macromolecular biosynthesis that drives cell growth and proliferation. We use Drosophila neuroblasts as a model to understand how neural stem cell proliferation decisions are made in response to dietary nutrient availability. We find that most neuroblasts in the developing Drosophila brain enter and exit cell cycle in a nutrient-dependent manner that is reversible and regulated by PI3-kinase. However, a small subset, the mushroom body neuroblasts, which generate neurons important for memory and learning, divide continuously, independent of dietary nutrient conditions and levels of PI3-kinase activity. We find that Eyeless (Ey), a Pax-6 orthologue, expressed in the mushroom body neuroblast subset is required for nutrient-independent proliferation. Moreover, we find that Myc, but not TOR-kinase, is also required for mushroom body neuroblast nutrient-independent proliferation. Unlike Ey, Myc is expressed in all neuroblasts, yet during dietary nutrient withdrawal Myc protein levels are maintained only in mushroom body neuroblasts. We are investigating whether Ey keeps Myc "ON" allowing for nutrient-independent proliferation in a subset of Drosophila neural stem cells. Uncoupling neural stem cell proliferation from dietary nutrient conditions could allow for preferential neurogenesis in brain subregions in nutrient poor environments.

**Program Abstract #49**

**The Hcfc1 A115V/Y mouse model of a novel, X-linked cobalamin deficiency syndrome**

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Recently, patients with cblX, an X-linked variant of the cblC cobalamin (vitamin B12) deficiency syndrome, were discovered. While cblX phenocopies the severe neurodevelopmental defects observed in the cblC syndrome, the disease is caused by mutations in the transcription cofactor *HCFC1 (Host Cell Factor C1)*. Previous research has
determined that HCFC1 directly regulates the expression of MMACHC, which encodes an enzyme essential for cobalamin metabolism and is mutated in cblC patients. Thus, the origin of the cblX phenotype is likely in part due to transcriptional reduction of MMACHC during development. In order to address this possibility and to define the exact pathophysiology of cblX, we have generated an Hcfc1 A115V mouse model, which carries the most frequent missense mutation (c.344C>T; p.Ala115Val) observed in cblX patients. We have determined that, analogous to cblX patients, these mice have a dramatic reduction of Mmachc levels in the developing brain and in fibroblasts. Unexpectedly, rather than suffering from the neurodevelopmental defects described for the cblX patients, the Hcfc1A115V/Y hemizygous mice exhibit a fully penetrant skin hypopigmentation phenotype and mild craniofacial dysmorphia. These data suggest that we may have uncovered a new transcriptional program influencing neural crest and/or melanocyte development. This also raises the intriguing possibility that disrupted cobalamin metabolism, due to reduction of Mmachc, may also play a role in neural crest development. While additional phenotyping, molecular characterization, and genetic rescue experiments are ongoing, our novel Hcfc1A115V/Y mouse model will likely lead to new mechanistic insight into cblX pathophysiology and inform strategies aimed at treating related cobalamin deficiency syndromes. Funding: NIH R01 EY024906 (Ross Poché) and NIH T32 EY007102 (Greame Mardon)

Program Abstract #50
Vitamin D signaling regulates development and diapause in the annual killifish
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Few vertebrate models offer insight about the integration of environmental cues into developmental programs and the resulting phenotypic variation. The alternative developmental pathways of the annual killifish, Austrofundulus limnaeus, present a fascinating example for investigating genetic regulation of heterochrony and heterokairy. Embryos of A. limnaeus can develop on two phenotypic trajectories associated with entrance into diapause that differ in the timing of morphological ontogeny and physiology. The two trajectories diverge many days prior to cessation of development in diapause which occurs midway through organogenesis. Embryos that "escape" dormancy in diapause develop continuously until hatching. Using incubation temperatures of 20 and 30°C, we induced embryos to develop along the diapause and escape trajectories and profiled phenotype-specific gene expression using RNA-seq. Applying a weighted gene co-expression analysis (WGCNA), we found that vitamin D receptor (VDR) signaling induces transcriptional networks that regulate developmental trajectory in A. limnaeus. Furthermore, exposure of embryos to vitamin D3 analogues directs continuous development even under diapause-inducing conditions. This is the first description of a molecular pathway that regulates developmental trajectory and metabolic dormancy in a vertebrate and demonstrates a striking homology to the regulation of dormancy in C. elegans and Drosophila. We suggest that hormones derived from 7-dehydrocholesterol and their associated nuclear receptors represent a conserved pathway for the integration of environmental information into developmental programs associated with life history transitions in animals.

Program Abstract #51
It takes guts to live in a cave: Morphogenesis, motility, and homeostasis of the blind Mexican cavefish gastrointestinal tract
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We are using the Mexican tetra, Astyanax mexicanus, to study the genetic changes that alter development and drive evolution of the gastrointestinal (GI) tract. This species has both a river-dwelling population (surface fish) living in an environment with abundant food, and multiple eyeless cave-dwelling populations (cavefish) that evolved in perpetual darkness with limited food. Cavefish have increased appetite, fat storage, insulin resistance and can survive long periods of starvation. All of the populations are interfertile, easily bred in the lab and the A. mexicanus genome is sequenced and annotated. We found that post-larval cavefish exhibit bi-directional churning motility in the stomach that is largely absent in surface fish. Churning is associated with slower transit of food
from the stomach to the midgut and coincides with an overall greater number of enteric neurons that coordinate intestinal contractions. We are currently investigating enteric neuron development and connectivity and the impact of altered intestinal motility on nutrient absorption. In adult cavefish, the surface area for nutrient absorption is maximized by a more densely folded intestinal epithelium. Using a genetic mapping approach, we identified KLF5, a gene associated with cell proliferation and colon cancer, as a potential contributor to cavefish hindgut length. In line with a role for KLF5, we found that under a fed state the intestinal epithelium in cavefish is more proliferative than in surface fish. Interestingly, in response to starvation, the cavefish gut decreases proliferation and remains intact, while the surface fish gut maintains proliferation and atrophies. Our results reveal developmental and genetic changes that may be important for adaptation of the post-larval and adult GI tract to the cave environment. Funding sources: NIH grant (HD089934) and National Research Service Award (DK108495).

Program Abstract #52
Non-Academic Careers: Tips for Trainees and PI Mentors
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Are you a grad student or post-doc considering a career outside of academia? Are you a PI wondering how to support your trainees when they express the wish to explore alternative careers? In this session, experts from industry, publishing, law, outreach, and science communication will share their career paths. We will learn how their training in science has translated to skills needed in their current careers, and learn how to better support our members as they explore alternative fulfilling careers. This session will be an open dialog and exchange.

Program Abstract #53
Global Views of Development
Jay Shendure
University of Washington, USA
I will describe our ongoing efforts to develop technologies supporting organism-scale single cell atlases of transcription, chromatin accessibility and lineage, and to apply these to organisms including the worm, fly, fish, mouse and human.

Program Abstract #54
Precision and Plasticity in Animal Transcription
Angela DePace
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The regulatory DNA that controls transcription harbors natural sequence variants at multiple DNA length scales, from single nucleotide polymorphisms to large scale structural variants. Given that changes in gene regulation are critical in development, disease and evolution, a central challenge is to understand how sequence variants at all of these scales impact proximal molecular phenotypes and downstream organismal phenotypes. I will present our work probing the molecular mechanisms that govern transcription in the developing Drosophila embryo, with a view to understanding how regulatory DNA can drive precise gene expression patterns while the underlying sequence is quite plastic. I will discuss examples at different levels of complexity, from single enhancers to entire developmental loci, and emphasize how coupling quantitative measurements to computational models can decipher underlying principles of transcription.

Program Abstract #55
Histone acetylation provides a timer to zygotic genome activation
Shun Hang Chan, Antonio Giraldez
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During the maternal to zygotic transition, the genome is initially silent and maternal factors control initial competency for transcription. We have recently identified that transcription factors Nanog, Oct4 and SoxB1 are important to mediate the proper activation of the zygotic program of development, yet the mechanisms that control the timing of these events remains poorly understood. To identify the temporal dynamics of genome activation and the factors that mediate this process, we combined CRISPR-Cas9 mediated live imaging during embryogenesis with chromatin and transcription analysis. We observed that genome activation is gradual and stochastic, and the active state is inherited in the daughter cells. We used the first gene transcribed during the transition, miR-430, as a model to show that H3K4me3 and H3K27Ac are key marks labeling active loci. Chemical inhibition of K27Ac readers blocks activation, while induction of H3K27Ac triggers premature transcription of the genome. We have identified a set of factors that control the timing of genome activation and our progress will be presented at this meeting. Taken together, our findings challenge current models of genome activation and reveal key factors that initiate zygotic development, a process relevant to developmental reprogramming in vivo and in vitro.

Program Abstract #56
A single-cell biochemistry approach to cell polarity during development
Daniel Dickinson
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Dynamically regulated protein-protein interactions are ubiquitous in biology and play critical roles in cell signaling, differentiation and development. To elucidate the dynamics and regulation of protein complexes in cells, it is crucial to employ techniques that yield time-resolved information about protein-protein interactions. Regulated interactions often occur in the context of large, interconnected signaling networks that require detection of multiple binding partners simultaneously. Moreover, because cell signaling is strongly context dependent, protein interaction networks that are involved in signaling must be studied in vivo in the context of a living animal where at all possible. With these goals in mind, we developed a rapid, nanoscale biochemical method that can detect protein complexes at the single molecule level in samples as small as a single cell. We demonstrated the utility of this approach by studying PAR polarity proteins, which mediate polarization of a wide variety of animal cell types including epithelia, neurons and stem cells. Although PAR complexes are generally thought of as static, we uncovered striking regulation of PAR complex composition and stoichiometry over the course of the first cell cycle in the C. elegans embryos, which lasts only 30 minutes. These results established a novel approach to the study of dynamic biochemical events in vivo. Moving forward, we are applying this approach to study how cell polarity responds to environmental signals in a variety of developmental contexts. NIH R00 GM115964 (to D.J.D.)
Cancer Prevention and Research Institute of Texas (CPRIT) RR170054 (to D.J.D.)

Program Abstract #57
Smooth muscle differentiation sculpts domain branches in the developing mouse lung
Katharine Goodwin, Andrej Kosmrlj, Celeste M. Nelson
Princeton University, USA

During branching morphogenesis, a simple tube of cells grows and bifurcates to generate an arborized network. To achieve a specific final morphology, physical cues are required to guide branching of the epithelium into the surrounding mesenchyme. In the mouse lung, the overall architecture is established by domain branching, in which new branches form laterally off the side of an existing branch, while bifurcations build a space-filling network. The airway epithelium develops concomitantly with a layer of smooth muscle that is derived from the embryonic mesenchyme and wraps circumferentially around the airways. Stereotyped patterns of smooth muscle differentiation are required for terminal bifurcation. Here, we examined the role of smooth muscle in shaping emerging domain branches during early murine lung development. We found that the position and morphology of domain branches are highly stereotyped: branches begin as wide buds that thin at their base as they extend. At the same time, there is an increase in the amount of smooth muscle wrapped around the parent bronchus at the base of each nascent domain branch. Perturbing smooth muscle differentiation results in abnormal branch
positioning and morphology. Loss of smooth muscle leads to ectopic branching events and slows branch thinning. Enhanced smooth muscle differentiation suppresses branching. Using experiments and modeling, we investigated the roles of epithelial proliferation and smooth muscle-mediated constraints during domain branching. Smooth muscle wrapping restricts proliferation in the parent branch adjacent to emerging domain branches and controls parent branch morphology during domain branching. Our work uncovers a role for smooth muscle differentiation in physically sculpting domain branches, and sheds additional light on the physical mechanisms of branching morphogenesis of the mouse lung. Funding Sources: HHMI Faculty Scholar’s Award; NIH/NHLBI R01 HL120142; NSF CMMI-1435853.

Program Abstract #58
A mesoderm-independent role for Nodal signaling in convergence & extension
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All early vertebrate embryos, initially shaped as a sphere or disc, become elongated along the head-to-tail axis during gastrulation by convergence and extension (C&E). This highly conserved morphogenetic process is accomplished in part by biased intercalation of cells that are planar polarized orthogonal to the anteroposterior (AP) axis. Patterning of the AP embryonic axis is required for its extension, implying that patterning information is communicated to the cellular machinery that drives C&E via as yet unknown mechanisms. The morphogen Nodal has well-described roles in AP patterning and C&E. Zebrafish embryos lacking Nodal signaling exhibit severe mesoderm deficiencies and axis extension defects, but it is unclear whether failure of extension results entirely from the absence of mesoderm, or also from loss of Nodal per se. Here, we present evidence that Nodal signaling contributes directly to cell behaviors underlying C&E in zebrafish. We found that planar cell polarity is disrupted in the neuroectoderm of Nodal-deficient mutants, and cannot be fully restored by the presence of mesoderm when transplanted into wild-type hosts. Nodal signaling is also necessary and sufficient to promote C&E ex vivo in zebrafish blastoderm explants, a simplified model of axis extension. These explants exhibit organizer activity, but do not require ß-catenin pre-patterning for extension to occur. Asymmetry of Nodal activity is apparent prior to C&E onset, but inhibitor treatments reveal that Nodal signaling is required throughout blastula and gastrula stages for explant extension. Strikingly, Nodal inhibition after C&E onset prevents full explant extension, consistent with a role for Nodal signaling during this morphogenetic process. Together, these data confirm the importance of Nodal-specified mesoderm in axial extension and reveal an additional direct role for Nodal signaling in polarized C&E cell behaviors. Supported by an NIGMS F32 NRSA to MW and NIGMS R35 MIRA award to LSK

Program Abstract #59
Making a Pore: Regulatory circuit orchestrating a single cell division event to create functional stomata
Keiko U. Torii
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Precise cell division control is critical for developmental patterning. The presence of rigid cell walls imposes a special challenge for plant development, as cell movement is limited and patterns must emerge through controlled cell proliferation and differentiation. For the differentiation of a functional stoma, a cellular valve for efficient gas exchange and water control, the single symmetric division of an immediate precursor is absolutely essential. Yet, the mechanism governing this event remains unclear. To address this question, we have profiled comprehensive inventories of gene expression by the Arabidopsis bHLH protein MUTE, a potent inducer of stomatal differentiation. Through the analysis of its target genes, we reveal that MUTE switches the cell-cell signaling program that generates stomatal initial cells via Turing-pattern like mechanism to differentiation. Furthermore, MUTE directly induces a suite of cell-cycle genes whose introduced expression triggers the symmetric divisions of arrested precursor cells in mute, and their transcriptional repressors, FAMA and FOUR LIPS. The regulatory network initiated by MUTE represents an Incoherent Type 1 Feed-Forward Loop. Our mathematical modeling and experimental perturbations support a notion that MUTE orchestrates a
transcriptional cascade leading to a tightly-restricted pulse of cell-cycle gene expression, thereby ensuring the single cell division to create functional stomata.

Program Abstract #60
An emerging role for neuronal guidance cues in coordinating individual cell movements for collective migration
Sally Horne-Badovinac
University of Chicago, USA
The collective migration of cells within an epithelial sheet underlies tissue remodeling events associated with morphogenesis, wound repair, and the spread of many cancers. Yet little is known about how each epithelial cell coordinates its movements with those of its neighbors. Studying the rotational migration of the follicular epithelium in *Drosophila*, we have identified two signaling pathways that these cells use to coordinate their movements for collective motility. The cadherin Fat2 and the receptor tyrosine phosphatase Lar had long been known to have a planar polarized distribution at the basal surface of this tissue. In the first half of the talk, I will show that Fat2 and Lar send short-range signals within the tissue plane to coordinate leading edge and trailing edge dynamics between neighboring cells. In the second half of the talk, I will then introduce Sema-5c and Plexin A as two new regulators of epithelial migration. These proteins show a similar planar polarized distribution to that of Fat2 and Lar. Moreover, Sema-5c appears to antagonize Lar activity in a manner that is independent of. All four of these proteins play key roles in wiring the nervous system. Thus, our data suggest a model in which the same cues that guide growing axons though the body can be deployed within individual epithelial cells to allow them to influence the migratory behavior of their neighbors, and to ultimately drive collective cell movement.

Program Abstract #61
Tissue-specific degradation of essential centrosome components reveals distinct microtubule populations at microtubule organizing centers
Maria Sallee, Jennifer Zonka, Taylor Skokan, Brian Rafetry, Jessica Feldman
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The centrosome is the best characterized microtubule organizing center (MTOC), and is critical for microtubule organization in dividing animal cells. However, most differentiated cells establish MTOCs at non-centrosomal sites (ncMTOCs), and how these ncMTOCs regulate microtubule organization and dynamics is largely unknown. The essential centrosomal microtubule nucleators γ-tubulin ring complex (γ-TuRC) and AIR-1/Aurora A localize to the apical ncMTOC of intestinal epithelial cells in *C. elegans* embryos (Feldman and Priess 2012), so we tested whether γ-TuRC and AIR-1 are also required for microtubule nucleation and organization at this apical ncMTOC. We first optimized the ZIF-1/ZF degradation system (Armenti et al. 2014) to allow intestine-specific depletion of AIR-1 and γ-TuRC. Using this method, we find that depleting the core γ-TuRC component GIP-1/GCP3 disrupts the apical localization of other γ-TuRC members including MZT-1/Mozart1, which we characterize for the first time in animal development. This result suggests that γ-TuRC localizes as a complex at the apical ncMTOC, as it does at the centrosome. We observe that AIR-1 and MZT-1 are required to recruit γ-TuRC to the centrosome in dividing intestinal cells as expected. Surprisingly, AIR-1 and MZT-1 are not required to recruit γ-TuRC to centrioles or the apical ncMTOC, indicating that different regulators control apical MTOC function and activity. Further, we find that the general microtubule organization at the apical ncMTOC is not affected by co-depletion of γ-TuRC and AIR-1, nor by depletion of other microtubule regulators including TPXL-1/TPX2, PTRN-1/CAMSAP, or NOCA-1/Ninein. However, loss of GIP-1 and AIR-1 does remove a subset of dynamic EBP-2/EB1-marked microtubules. Together, these results suggest that different MTOCs use discrete proteins for their function, and that the apical ncMTOC is composed of distinct populations of microtubules that compete for a limited pool of resources. (NIH F32, DP2)

Program Abstract #62
Differential expression of Cadherin-2 patterns RhoA and Myosin activity to drive zippering and neural tube closure in a simple chordate
Hidehiko Hashimoto
Forces generated at tissue boundaries control many forms of morphogenesis, but the mechanisms remain poorly understood. We are addressing this question in the context of zippering and neural tube closure in the basal chordate, Ciona robusta. We previously showed that zippering and neural tube closure are driven by local RhoA-dependent activation of Myosin II along the neural/epidermal (Ne/Epi) boundary ahead of the advancing zipper. Here we show that local activation of RhoA and Myosin II is governed by neural-specific expression and homotypic localization of a classical cadherin, Cadherin-2. During zippering, Cadherin-2 accumulates along homotypic Ne/Ne junctions, but is absent from heterotypic Ne/Epi junctions, where RhoA and Myosin II are locally activated during zipper progression. Equalizing Cadherin-2 expression across the Ne/Epi boundary inhibits RhoA/Myosin II activation and zipper progression, while creating ectopic Cadherin-2 expression boundaries in neural or epidermal domains is sufficient to direct RhoA/Myosin II activity to those boundaries. Cadherin-2 directs RhoA activity to heterotypic junctions by recruiting the Rho GTPase activating protein, GAP-21/23, to homotypic junctions and away from heterotypic junctions. By activating Myosin II along Ne/Epi junctions ahead of zipper and inhibiting Myosin II at new Ne/Ne junctions behind zipper, Cadherin-2 promotes tissue level contractile asymmetry to drive zipper progression. This work was funded by NICHD grant 1R01HD088831.

Program Abstract #63
Signals, forces, and cells: Decoding tissue morphogenesis
Jennifer Zallen
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A major challenge in developmental biology is to understand how changes in tissue structure are generated by processes that occur on a cellular and molecular scale. In Drosophila, the characteristic elongated shape of the body axis is achieved through the coordinated movements of hundreds of cells. We discovered that these movements are generated by subcellular asymmetries in the localization and activity of proteins that generate contractile and adhesive forces within cells. In addition, we identified a positional code that systematically orients cell movements throughout the embryo and demonstrate that this spatial information is provided by an ancient family of Toll-related receptors that are widely used for pathogen recognition by the innate immune system. Using quantitative live imaging and automated cell tracking, we showed that these molecular asymmetries result in dynamic, collective cell behaviors in which multicellular rosette structures form and resolve directionally, driving tissue elongation. Planar polarized actomyosin contractility and rosette behaviors have now been shown to promote tissue elongation in chicks, frogs, and mice, and represent a general mechanism linking cellular asymmetry to global tissue reorganization. We are currently using genetic, cell biological, biophysical, and live imaging approaches to understand how genes encode the forces that shape tissues, and to elucidate the mechanotransduction mechanisms that allow cells to modulate their behavior in response to changes in their mechanical environment.

Program Abstract #64
Neuroinflammatory signals drive spinal curve formation in zebrafish models of idiopathic scoliosis
Brian Ciruna1,2
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Idiopathic scoliosis (IS) is a complex genetic disorder characterized by rotational deformities of the spine that arise in the absence of obvious congenital or physiological defects. 3% of children develop IS, and 1 in 10 patients experience functional distress requiring physical or surgical intervention. Although genome-wide association studies detect IS-associated polymorphisms in divergent populations, genetic variability has precluded identification of causative mutations. A historical lack of animal models has also confounded our biological understanding of IS. As a result, treatment options remain limited to rigid brace wear and invasive corrective surgery. Previously, we generated the first genetically defined, developmental model of IS in protein tyrosine kinase-7 (ptk7) mutant zebrafish. These fish, deficient for an essential regulator of Wnt signaling, develop late-
onset spinal curvatures that model all defining attributes of human disease. Wnt signals control the biogenesis and polarized architecture of motile cilia, which are essential for proper cerebrospinal fluid (CSF) flow. Remarkably, interrogation of ptk7 mutants and additional zebrafish IS models revealed a critical role for motile cilia in normal spine development, and implicated defects in CSF flow as an underlying cause of IS. Here, I review our progress in further defining the pathobiological mechanisms underlying scoliosis in zebrafish IS models. Using next generation sequencing, lineage tracing and conditional genetic methodologies we demonstrate that focal neuroinflammatory signals, downstream of CSF flow defects, drive spinal curve formation. Furthermore, administration of non-steroidal anti-inflammatory agents can significantly impact the incidence of scoliosis in zebrafish models, providing intriguing evidence that simple immune-modulating therapies might prove effective in managing idiopathic-like spinal deformities. This work was funded by the Canadian Institutes of Health Research.

Program Abstract #65
Hedgehog signaling controls cell motility during vertebrate eye morphogenesis
Sarah Lusk, Hannah B. Gordon, Emily O. Wirick, Brooke Murray, Kristen M. Kwan
University of Utah, USA
During development, cells and tissues undergo dramatic movements to form 3-dimensional organs, the precise structures of which are critical for function. For the vertebrate eye, disruptions to these movements result in structural defects which can cause visual impairment. One such defect, uveal coloboma, results from failed development of a transient structure in the embryonic eye, the optic fissure. Mutations in the Hedgehog (Hh) receptor Patched lead to hyperactive Hh signaling and can cause coloboma in both humans and zebrafish. We set out to determine the cellular and molecular mechanisms by which hyperactive Hh signaling in the ptc2 mutant disrupts optic fissure development. Using multidimensional imaging and 4D cell tracking, we determined the morphogenetic events underlying normal optic fissure formation and pinpointed cells whose movements are disrupted by hyperactive Hh signaling. At the single cell level, motile behaviors are disrupted: instead of a normal migratory bipolar morphology, ptc2 mutant cells lose polarized morphology and fail to migrate directionally to their correct locations. To determine the underlying molecular mechanisms, we carried out genetic experiments and found that Gli1-dependent transcription is required. Using transplantation experiments, we found that overactive Hh signaling acts non-cell autonomously to disrupt cell morphology and migration. Taken together, we present a model in which a specific level of Hh signaling is critical for cell motility: we hypothesize that overactive Hh signaling leads to increased production of a secreted molecule or cell-surface protein that in turn disrupts migration behaviors. We are currently working to identify the critical secreted transcriptional target downstream of Hh signaling that regulates migration. This work was supported by grants from the NIH (EY025378, EY025780), a March of Dimes Basil O’Connor Starter Scholar Award, and the Knights Templar Eye Foundation.

Program Abstract #66
A tissue-scale pressure relief valve within the ear prevents pathological over-inflation
Ian Swinburne, Sean Megason
Harvard Medical School, USA
Management of water by specialized tissues is critical to both the development and healthy function of several organs. Understanding the mechanisms by which organs, such as the ear, eye, brain, and kidney, control water pressure is relevant to diseases such as glaucoma for the eye and Méniére’s disease for the ear. The inner ear is structured as a pressurized cavity bound by an epithelial sheet. Out of control water pressure within the ear can cause deafness and balance disorders—but it has been unclear how pressure is regulated within a healthy ear. Using live imaging, mathematical modeling, embryonic perturbation, and a newly developed pressure probe, we characterized how hydrostatic pressure is first used for size control of the embryonic zebrafish ear. Later, in larval zebrafish, we found a relief valve activity in the ear’s endolymphatic sac. Quantitative live imaging revealed cycles of slow pressure-driven inflation of the endolymphatic sac’s tissue followed by rapid deflation every 0.3-4.5 hours. Absence of these cycles in lmx1bb mutants leads to distended ear tissue that resembles pathologies associated
with hearing and balance disorders such as Ménière’s disease. Using serial-section electron microscopy, we found that thin lamellar projections underlie the relief valve function as the ultimate barrier to pressure release. Lattice light-sheet microscopy with adaptive optics revealed a lively and bubbling tissue where the overlapping lamellae dynamically extend and retract over one-another until they open under pressure. Current efforts aim to characterize the molecular basis of the relief valve formation and function. Our results identify a tissue-based mechanical valve activity in the ear’s mysterious endolymphatic sac and we speculate that similar strategies exist for other organ’s that manage water. This work was supported by Ménière’s Disease and Emerging Research Grants from the Hearing Health Foundation and NIH grants from the NIDCD.

Program Abstract #67
Electrical Membrane Potential shapes Early Embryonic Stem Cell Fates and Drives Gastrulation as revealed by Patient Driven Gene Discovery
Emily Sempou, Jie Zhu, David Zenisek, Mustafa Khokha
Yale University, USA

Exome sequencing is enabling candidate gene discovery in patients with birth defects. Excitingly, the vast majority of these candidate genes are novel to human embryonic development offering an exciting avenue for mechanism discovery. In order to model human development, we employed CRISPR based F0 screening in Xenopus and identified KCNH6, a voltage gated potassium channel from a patient with heterotaxy and congenital heart disease. Depletion of KCNH6 leads to global left-right patterning and cardiac defects in Xenopus, recapitulating the patient phenotype. In addition, depletion of KCNH6 affects gastrulation which we correlate with LR patterning defects indicating that gastrulation is the primary defect. Potassium channels regulate outward K ion flux that alters membrane electrical polarization, but the bioelectric properties of embryonic cells have been controversial. Using chemical inhibitors of ion channels, manipulation of extracellular ions, and direct electrophysiological measurement of membrane potential, we demonstrate that membrane potential is essential for gastrulation by unexpectedly defining germ cell layer cell fate. In addition, similar to excitable cells such as neurons and cardiac pacemaker cells, depletion of KCNH6 depolarizes the early embryonic cells and activates voltage gated calcium channels defining an upstream regulator of calcium in the early embryo, which had previously been undefined. Our results define membrane potential as a critical regulator of intracellular calcium in very early embryonic stem cells that defines germ cell fate.

Program Abstract #68
Cellular control of mammalian cranial neural tube morphogenesis
Eric Brooks1, Jennifer Zallen1,2
1Sloan Kettering Institute, USA; 2Howard Hughes Medical Institute, USA

Neural tube closure is a dynamic morphogenetic process that produces a closed and hollow tube from an initially flat epithelium through the concerted and regionalized actions of individual cells. Anencephaly, the failure of closure in the presumptive brain, accounts for 30-40% of human neural tube defects. However, the cellular mechanisms driving cranial neural tube closure remain largely unknown. We have conducted a quantitative analysis of cranial neural plate morphogenesis at cellular resolution using both fixed and live imaging approaches. This analysis reveals the distribution of dynamic cell behaviors—including cell-shape change, cell division, and cell rearrangement—during progressive stages of neural tube closure. Additionally, we are investigating the distribution and activity of the molecular machinery governing cell shape and rearrangement in this tissue. Together, these analyses will help to elucidate the cellular basis of cranial neural tube closure and provide a foundation for understanding the cellular phenotypes underlying failures in cranial closure. This work is supported by an NIH/NINDS fellowship to ERB (F32NS098832) and the Howard Hughes Medical Institute.

Program Abstract #69
Super-Mendelian inheritance mediated by CRISPR/Cas9 in the female mouse germline
Hannah Grunwald, Valentino Gantz, Gunnar Poplawski, Shannon Xu, Ethan Bier, Kimberly Cooper
A gene drive biases the transmission of a particular allele of a gene such that it is inherited at a greater frequency than by random assortment. Recently, a highly efficient gene drive was developed in insects, which leverages the sequence-targeted DNA cleavage activity of CRISPR/Cas9 and endogenous homology directed repair mechanisms to convert heterozygous genotypes to homozygosis. If implemented in laboratory rodents, this powerful system would enable the rapid assembly of genotypes that involve multiple loci (e.g. to model multigenic human diseases). Such complex genetic models are currently precluded by time, cost, and a requirement for a large number of animals to obtain a few individuals of the desired genotype. However, the efficiency of a CRISPR/Cas9 gene drive system in mammals has not yet been determined. Here, we utilize an active genetic “CopyCat” element embedded in the mouse Tyrosinase gene to detect genotype conversions after Cas9 activity in the embryo and in the germline. Although Cas9 efficiently induces double strand breaks in the early embryo and is therefore highly mutagenic, these breaks are not resolved by homology directed repair. However, when Cas9 expression is limited to the developing female germline, resulting double strand breaks can be resolved by homology directed repair that copies from the homologous chromosome and leads to super-Mendelian inheritance of the CopyCat allele. This work was supported by the Pew Biomedical Scholars Program, the Searle Scholars Program, and a Packard Fellowship for Science and Engineering.

Program Abstract #70
Understanding the Genetic Causes of Human Congenital Lymphatic Anomalies
Ajit Muley1, Jessica Giordano1, Sitharthan Kamalakara1, Colin Malone1, Jessica Kandel2, Russel Miller1, Ron Wapner3, David Goldstein1, June Wu1, Carrie Shawber1
1Columbia University, USA; 2University of Chicago Medical School, USA
Congenital lymphatic anomalies (CLA) are due to defects in lymphatic development, phenotypically heterogeneous and categorized empirically. The lymphatics are responsible for tissue fluid homeostasis, immune surveillance, and intestinal lipid absorption. In CLAs, lymphatic dysfunction leads to significant morbidities, including hemorrhage, bony deformities, respiratory compromise, intestinal dysfunction and sepsis. Subtypes of CLAs may arise from inherited and de novo germline mutations, while somatic mutations have been detected in the lymphatic endothelium of lymphatic malformations. However, the genetic causes of most CLAs remain unknown. We hypothesized that affected lymphatic endothelial cells isolated from CLA specimens and parental-fetal germline specimens can be used to comprehensively screen for mutations in CLAs. Whole exome sequencing was performed on maternal-paternal-fetal trio germline specimens (n=92) and lymphatic endothelial cells isolated CLA specimens (CLA-LECs; n=24), and variant analysis performed. CLAs were euploid and did not have large deletions or insertions, suggesting CLAs were mostly due to small base pair alterations. Previously reported and new mutations in CLA-associated genes (Pik3ca, Ptpn11, Rit1, Sos1, Rasa1, Ltzr1, Gjc2, Pik3r3, Tsc2, Piezo1) were observed in 10% of germline tissues and 59% of CLA-LECs. Rasa1, Piezo1 and Gjc2 mutations were identified in both fetal CLAs and CLA-LECs. Pathological variants were also seen in genes necessary for murine lymphatic development (Cpt1a, Cdkn1c, Pkd1, Nr2f2). Novel pathological mutations were detected in genes of neurogenic and cell metabolism pathways. CLA-LECs often had multiple mutations suggesting CLAs arise from compound genetic variants. The genetic information gained will increase our understanding of CLAs which is necessary for improved genetic testing and the development of novel therapies. Studies supported by a 2017 Million Dollar Bike Ride Pilot Grant and NIH/NICHD R03.

Program Abstract #71
Binary Transcriptional Control of Pattern Formation in Development
Hernan Garcia1, Nicholas Lammers1, Vahe Galstyan2, Armando Reimer3, Sean A. Meddin1, Chris Wiggins3
1University of California Berkeley, USA; 2California Institute of Technology, USA; 3Columbia University, USA
During embryonic development, tightly choreographed patterns of gene expression specify cell fate. Output transcriptional activity is characterized by bursts of gene expression, where promoters stochastically transition between transcriptional ON and OFF states. Here, we quantitatively tested the hypothesis that transcriptional
bursts are the main drivers of pattern formation. We quantified the transcriptional activity that leads to the formation of the widely studied stripe 2 of the even-skipped gene in living embryos of the fruit fly at the single cell level. We developed a novel memory-adjusted hidden Markov model to extract the parameters governing transcriptional bursting and show that that promoter switching dynamics cannot quantitatively explain pattern formation in the embryo. We discovered that, in addition to bursting, the window of time over which genes engage in transcription is also regulated along the embryo, and that this digital regulation of when promoters become competent for transcription is the main driver of pattern formation. Thus, in order to reveal the molecular rules behind the transcriptional control of pattern formation and reach a predictive understanding of development, a non-steady-state and quantitative description of both the regulation of promoter bursting and the transcriptional time window needs to be adopted.

Program Abstract #72
Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis
Jeffrey Farrell1, Yiqun Wang1, Aviv Regev2, Alexander F. Schier1,3
1Harvard University, USA; 2Broad Institute, USA; 3Biozentrum, Switzerland
During embryogenesis, cells acquire distinct fates by transitioning through transcriptional states, but the underlying trajectories and gene expression cascades have not been comprehensively identified. Here we combine large-scale single-cell RNA sequencing with a novel simulated diffusion based computational approach (URD) to uncover the branching transcriptional trajectories during zebrafish embryogenesis. Application of URD to 38,731 transcriptomes identified the trajectories of 25 cell types from the onset of zygotic transcription through early somitogenesis. Gene expression analyses associated developmental trajectories with known and candidate regulators, classic and novel marker genes, and their spatial origin in the blastula. Some developmental branchpoints contained intermediate cells expressing genes characteristic of multiple cell fates; unexpectedly, in situ gene expression analysis suggested that these cells switched specification from one fate to another. These findings reconstruct the cell fate trajectories during the embryogenesis of a vertebrate, highlight the plasticity of cell type specification, and provide a broadly applicable framework to reconstruct complex developmental trajectories from single-cell transcriptomes. This research was supported by the NIH (A.F.S., J.A.F., A.R.), the Allen Discovery Center for Cell Lineage Tracing (A.F.S.), Jane Coffin Childs Memorial Fund (J.A.F.), Charles A. King Trust (J.A.F.), Howard Hughes Medical Institute (A.R.), and the Klarman Cell Observatory (A.R.).

Program Abstract #73
Two distinct regulatory codes exist in promoters during the egg to embryo transition
Christine Reid, Hui Zhu, Qin Li, Julie Baker
Stanford University, USA
Within the first day of development the amphibian embryo undergoes dramatic cell proliferation, morphological changes, and activates numerous pathways for cell fate specification. Each of these changes is driven by alterations in gene expression, but a comprehensive understanding of the promoters that control gene expression remains elusive. To create a high-resolution map of transcription start sites (TSS) in the early embryo, I generated CAGE-Seq datasets for four critical time points: egg, blastula, gastrula and neurula stages. TSS in the egg and blastula are highly similar, suggesting that maternally specified promoters are maintained during early embryogenesis. In the egg, blastula and gastrula, promoters contain a TATA-binding protein (TBP) motif, suggesting that TBP is driving gene expression during early embryogenesis. Unexpectedly, neurula stage promoters contain the Initiator (INR) element, which binds TFIID family members, demonstrating that tissue specification during neurulation includes a fundamental change in the sequences driving gene expression. I find that a number of consistently expressed promoters shift TSS usage as embryogenesis progresses. Specifically, many genes use an egg promoter containing a TATA box, then shift to a promoter containing the INR element at the neurula stage. This suggests that the basal transcriptional machinery used to drive gene expression is fundamentally changing as the egg transitions to an embryo. The motif for YY1, a transcription factor essential for neurulation, is commonly found near shifting promoters, suggesting YY1 drives a shift from TATA based gene
expression to INR based gene expression. This work demonstrates that a shift from maternal to embryonic gene expression involves fundamental changes in basal transcription machinery.

**Program Abstract #74**

**Regulatory principles governing enhancer specificity during development**

Katrina Olson\(^1,2\), Granton Jindal\(^1,2\), Fabian Lim\(^3\), Jessica Grudzien\(^1,2\), Benjamin Song\(^1,2\), Emma Farley\(^1,2\)

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The human genome contains on the order of a million enhancers. These segments of the DNA act as switches to regulate where and when the approximately 20,000 genes are expressed. As such, enhancers provide the instructions for tissue specific gene expression, thus enabling successful development. Numerous studies have demonstrated that mutations in enhancers can alter tissue specific expression and cause phenotypic variation and disease. For example, a single mutation in a limb bud enhancer leads to aberrant expression of the gene SHH and results in extra fingers and toes. Genomewide analysis suggests that the majority of mutations associated with disease are located within enhancers. Despite the fundamental importance of enhancers for organismal integrity, we lack a broad understanding of how enhancer sequence encodes tissue specific expression. As a result we do not understand which changes in enhancer sequence are simply inert variation between individuals and which mutations lead to phenotypic diversity and disease. These fundamental questions remain unsolved because we cannot relate enhancer sequence to gene expression patterns and phenotype on a scale sufficient to identify the overarching regulatory principles. The two main challenges in deciphering the relationship between enhancer sequence and tissue specific gene expression are: 1) the complexity of enhancers, and 2) the complexity of organisms. To address these problems, we have developed high-throughput functional assays to test millions of enhancer variants in millions of whole developing embryos. The model organism that enables such in-depth functional approaches is the marine chordate *Ciona intestinalis*. I will discuss our recent findings using these high-throughput functional approaches to identify regulatory principles governing enhancer function and how violations in these principles can help us pinpoint mutations associated with disease.

**Program Abstract #75**

**Transcriptional control in time and space**

Nadine L Vastenhouw

*Max Planck Institute of Molecular Cell Biology and Genetics, Germany*

Upon fertilization, the genome of animal embryos is transcriptionally inactive until the controlled onset of transcription during the maternal-to-zygotic transition. We focus on this genome-wide onset of transcription in zebrafish, with the aim to understand how the transcriptional machinery and chromatin template are brought together in time and space to robustly regulate transcription in hundreds of blastomeres during genome activation. We analyze transcriptional regulation quantitatively (quantification of repressors, activators, transcripts) and at high spatial resolution (imaging transcripts, chromatin architecture, and transcriptional machinery in single cells) in the context of the developing embryo. This allows us to address questions about the timing of transcription initiation during development, synchrony between cells, and the function and establishment of nuclear architecture. I will present our latest results.

**Program Abstract #76**

**Dynamic identification of the dosage-compensated *Drosophila* male X-Chromosome during early embryogenesis**

Leila Rieder\(^1\), Matthew Booker\(^2\), Guray Kuzu\(^3\), Michael Tolstorukov\(^2\), Erica Larschan\(^1\)

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Heterogametic species face a chromosome imbalance. In species with the XY system of sex determination, including humans and *Drosophila*, males have half the X-linked gene dosage compared to females. Dosage compensation mechanisms evolved to restore balanced expression of X-linked genes between the sexes. The
Drosophila zinc-finger transcription factor Chromatin-Linked Adaptor for MSL Proteins (CLAMP) localizes to X-linked High Affinity Sites (HAS), which contain GA-rich cis-elements. CLAMP facilitates Male Specific Lethal (MSL) complex recruitment, which increases transcription by depositing the H4K16ac active chromatin mark. Previous investigation into these interactions has largely been confined to steady-state systems. To capture the dynamics of male X-chromosome identification in the early embryo, we used a meiotic drive system to generate pools of male embryos, precisely staged them by nuclear cycle, and performed small scale ChIP-seq for CLAMP, MSL complex, and the H4K16ac chromatin mark. Our results reveal that CLAMP localizes to HAS on the male X-chromosome long before MSL complex is recruited. MSL complex localizes at the time of widespread zygotic genome activation (ZGA), but not before. Although previous hypotheses suggest that MSL complex arrives at HAS and then spreads to nearby active genes, there is no observable time point when MSL is confined to HAS. Instead, MSL complex coats X-linked active gene bodies, although it is enriched at HAS. Moreover, we demonstrated that CLAMP is an early transcription factor that can open chromatin in a large domain surrounding its GA-rich binding sites, thereby establishing a more open environment on the male X-chromosome compared to autosomes. We propose a new model in which CLAMP localizes to HAS very early during embryogenesis and establishes a chromatin environment that is permissive for MSL complex recruitment, which occurs during ZGA. This work was supported by F32GM109663 and K99HD092625 to LER.

Program Abstract #77
A histone-based memory of embryogenesis in development and disease
Rebecca Resnick, Chao-Jen Wong, Pete Skene, Stephen Tapscott
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The double-homeobox transcription factor DUX4 is expressed in a short burst during embryonic genome activation in humans and establishes the cleavage-stage transcriptional program that persists through early embryogenesis, long after DUX4 itself is gone. We hypothesized that DUX4 expression leads to epigenetic changes that maintain this program in the absence of the pioneer factor, and that histone variants H3.X and H3.Y may play a role. H3.X/Y are induced by DUX4 and expressed in pre-implantation embryos, iPS cells, testis, and some cancers, as well as FSHD, a muscular dystrophy caused by de-repression of DUX4. H3.Y binds DNA less tightly than H3.3, and uses the HIRA chaperone complex which deposits H3.3 into active regions of the genome. Nucleosomes with H3.Y also exclude the chromatin condensing linker histone H1. Specific differences between H3.X/Y and canonical H3 reduce DNA methylation or repeat silencing, and many DUX4 targets are in typically silenced or repetitive regions. Upon DUX4 induction, we found that H3.X/Y localize to transcribed regions including DUX4 target genes. Using pulses of DUX4 to mimic the embryonic context, we see a synergistic effect on DUX4 target expression with a second DUX4 pulse when H3.X/Y are incorporated. Knockdown of H3.X/Y reduces this synergy, suggesting that H3.X/Y mediate this effect. Additionally, we see perdurance of target expression when H3.X/Y are incorporated. We propose a model whereby H3.X/Y act as an epigenetic memory of DUX4 expression in the embryo: during the DUX4 burst, H3.X/Y are expressed and incorporated at newly-transcribed zygotic genes targeted by DUX4, maintaining an open chromatin conformation and enabling activation of these genes throughout the cleavage stage. In a disease context, this epigenetic memory would accumulate in postmitotic muscle rather than dissipate as in the rapidly-dividing embryo, leading to over-activation of the program and development of FSHD. Funding: NIH F31NS101773, R01AR045203

Program Abstract #78
Zygotic genome activation in flowering plants
Stewart Gillmor
Langebio-CINVESTAV, MX
In animals, it is well accepted that early embryogenesis is under control of transcripts and proteins inherited from the egg. Regulation of development is transferred from the maternal to zygotic genome during the maternal to zygotic transition, which includes degradation of maternal transcripts, and the onset of zygotic transcription. In flowering plants, we have evidence both for and against maternal control of early embryogenesis. The current
debate on the nature of zygotic genome activation revolves around two key questions: How soon after fertilization does large-scale zygotic transcription begin? Does this transcription initially have a maternal bias? In my talk, I will present functional and genomic experiments from my laboratory which support the hypothesis that genome activation in Arabidopsis occurs soon after fertilization, with a bias toward transcription of maternal alleles. I will also describe effects of hybridization on parent-of-origin gene expression in early Arabidopsis embryogenesis, which may be of interest for allele-specific expression studies in animals.

Program Abstract #79
Adult pattern formation and regenerative ability in planarians
Christian Petersen
Northwestern University, USA
Animals capable of tissue regeneration can perfectly re-establish their form after diverse injuries, suggesting that systems enabling robust tissue patterning could be central for regenerative ability. Planarians have emerged as a powerful model organism to study whole-body regeneration mediated by adult pluripotent stem cells. Our studies have used the planarian *Schmidtea mediterrantea* to identify the cell signaling and regulatory principles that allow restoration of a body axis truncated by injury. Using the head-to-tail body axis of planarians as a model, we identified a canonical Wnt/beta-catenin signaling pathway mediated by asymmetric expression of the Wnt inhibitor *notum* that responds to tissue orientation at the wound site and polarizes the identity of the axis termini in regeneration. Downstream of this early decision step, a specialized stem-cell-dependent pathway is responsible for the differentiation of a signaling center that drives head outgrowth after decapitation. These and other injury-induced responses intersect with constitutive cues that define axis regionalization and enable reversible body scaling over a wide range, and the re-establishment of their domains likely accounts for the cessation of regenerative outgrowth. The ability of mature organs to absorb migratory progenitors buffers against alterations to body-wide patterning that occur after amputation, allowing seamless integration of pre-existing and new tissues. Together, these analyses seek to uncover the factors and regulatory logic underlying regenerative growth.

Program Abstract #80
The Legacy of Experimental Embryology: Is It Relevant Today?
Drew M. Noden
Cornell College of Veterinary Medicine, USA
Experimental Embryology came “of age” in the late 19th century, when concepts such as preformation and vitalism were being challenged for the first time by science-based data derived from direct intrusions into early stages of embryonic development. New terms such as induction, morphogenesis, differentiation, fields, and determination were invoked to provide a framework for understanding how cells and tissues cooperate in the making of a body with specialized organ systems. Then came genetics, biochemistry, imaging, and molecular biology – each with its own concepts and methods, and Experimental Embryology as a discipline became obsolete, along with farriers, steel erector sets, and (some would opine) anatomists. This presentation shares stories about some of the people and ideas that defined Experimental Embryology, from Driesch through Spemann and Harrison and then later 20th century proponents including my mentors, Viktor Hamburger and Rita Levi-Montalcini, and other colleagues I have been privileged to know. Is what they did and, more importantly how and why they came to make fundamental discoveries, is relevant and useful to students, teachers and researchers of modern developmental biology? The answer will be the listener’s decision.

Program Abstract #81
Choose Development!: An inclusive approach to enrich mentoring skills of junior scientists
Graciela Unguez1, Karen Bennett2, Carmen Domingo3, Marsha Lucas4, Ida Chow4
1New Mexico State University, USA; 2University of Missouri, USA; 3San Francisco State University, USA; 4Society for Developmental Biology, Bethesda, MD, USA
Since 2013, the Choose Development! Program from the Society for Developmental Biology (SDB) has provided a
welcoming research community to 29 undergraduate Fellows from across the country who belong to underrepresented (UR) communities in STEM. Fellows spend at least one full summer in the lab of established SDB members doing research under the tutelage of both Faculty and Lab (graduate student, postdoctoral fellow or technician) Mentors. They continue working as a 3-member team after the summer to prepare their research findings for presentation at the following year’s SDB national meeting. To date, our 5-year outcomes strongly support our hypothesis that long-term advocacy (by SDB) and mentoring is successful in promoting the continued education/training of UR students in developmental biology or related biomedical fields. Another innovation of Choose Development! is the concurrent mentoring training of Lab mentors along with their Faculty mentors – a different approach to the professional development of these early career scientists. Choose Development! is committed to valuing and recognizing the contribution of the Lab mentors by supporting their participation in mentoring workshops that address laboratory management skills essential for their careers as independent investigators. We will provide an overview of the mentoring training received by 26 lab mentors to date, highlighting the work we have done over the past four years, and new activities implemented last summer. To support all Choose Development! teams year round, we have established an active online community in Trellis (https://www.trelliscience.com), and set up video conferencing for both the mentors and the fellows. These approaches increasingly facilitate dialogue, relationship building, resource sharing, networking, navigation through career decisions and benchmarks, and personal and professional support for Fellows and Lab Mentors alike. Supported by grant NSF-IOS 1239422 (2013-2017) and SDB.

Program Abstract #82
Helping Students SOAR: Engaging Underrepresented Minority Undergraduates in Developmental Biology
Robert Kao
Heritage University, USA
Engaging and gauging (engaging) first-year, under-represented minority undergraduate general biology students through processes of inquiry, critical thinking, and affective learning is vital as they develop their scientific identity. An important challenge is how we can establish communities of practice and instill in our first-generation students self-awareness and reflection as they apply, analyze, and evaluate data on biological principles. In my presentation, I will describe an innovative and flexible SOAR framework that provide an active learning space for students to integrate inclusive student-centered, in-class discussions and longitudinal lab inquiries in developmental biology through metacognition and reflection-in-action. SOAR stands for Spiral curricula and process of inquiry (spiralquiry); Observations from experiments to Evaluation of Data; Affective Learning in active learning settings; and Research proposals as platform before and after summer research experiences. This transformative, culturally responsive mentoring approach encourages first-generation undergraduates to bring self-awareness to unclear or confusing topics that are clarified at the start of class or lab settings, and engages undergraduates in authentic course-based undergraduate research experiences (CUREs). In summary, the innovative and flexible SOAR framework provides students to develop their self-confidence and persistence in pursuing graduate PhD programs in molecular biology and developmental genetics.

Program Abstract #83
CourseSource: Evidence-Based Teaching Resources for Undergraduate Biology Education
Michelle K. Smith¹, Jessamina Blum², Erin L. Vinson¹
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Fostered by reports such as Vision and Change and Engaged to Excel, changes in the way colleges and universities are approaching their undergraduate STEM courses can be observed nationwide. Change has come in the form of initiatives dedicated to advancing evidence-based science education practices, research-based undergraduate courses, and other efforts that aim to provide educators with the tools and strategies needed to transform their classrooms. One stumbling block in the process of this transformation is the time and energy commitment, which can be substantial, needed to produce evidence-based active-learning materials. In response to this need, and recommended in the Vision and Change report, an effort was undertaken to create a peer-reviewed, open access...
journal of student-centered biology education resources: CourseSource. CourseSource is an open-access, online journal of peer-reviewed undergraduate biological teaching materials that:

- Incorporate student-centered, evidence-based pedagogy;
- Focus on professional society-developed learning goals and objectives for the major subfields of biology (developmental biology, genetics etc.);
- Are organized and formatted so that transfer and use in other classrooms is easily done.

CourseSource provides biology educators with tested, evidence-based activities designed by fellow educators that make difficult concepts accessible to undergraduates using a variety of strategies. Conversely, CourseSource provides authors with the opportunity to publish teaching materials in a high-quality, peer-reviewed format that documents their scholarly teaching efforts, accomplishments, and innovations. A key feature of CourseSource is the alignment of articles with learning goals and objectives developed by scientific professional societies, including the Society for Developmental Biology. This work is supported by the National Science Foundation grants DUE 1725130 and DUE 1725129.

Program Abstract #84
National Xenopus Resource – serving the Xenopus research community
Marcin Wlizla, Sean McNamara, Nikko Shaidani, Marko Horb
Marine Biological Laboratory, USA
Since its establishment in 2010, the National Xenopus Resource (NXR) has focused on development of three facets in particular: resources, services, and research, all of which promote advancement of Xenopus research. Our resources include wild type and inbred strains of both *Xenopus laevis* and *Xenopus tropicalis* as well as more than 150 distinct mutant and transgenic lines. The NXR services include generation of custom mutant and transgenic lines for *Xenopus* researchers who may not have the means or the expertise to do so on their own, laboratory space available for visiting scientists, and promoting dissemination of cutting edge techniques in *Xenopus* research by hosting the biennial ‘Xenopus Resources and Emerging Technologies’ meeting as well as through on-site held workshops giving hands-on instruction in bioinformatics, genome editing, and imaging. The research that the NXR does in-house focuses on developing techniques that will be of considerable use to the *Xenopus* community. As we grow, we continue to develop these facets further with the goals of serving the *Xenopus* community and promoting the use of *Xenopus* in the study of developmental processes and disease. Here, I describe the recent progress we have made in expanding and optimizing our operations as well as provide information on how investigators can best take advantage of the resources and services we provide. The NXR is supported by a grant from the National Institutes of Health (P40 OD010997).

Program Abstract #85
Analysis of primary literature in a Master’s-level course: effects on critical thinking and on what students perceive to be challenging in scientific papers
Ella Tour¹, Chris Abdullah¹, Richard Lie¹, Wenliang He²
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Primary literature offers rich opportunities to communicate the excitement of science and to teach students to think like a scientist: to understand experimental design, analyze original data, design follow-up experiments. We describe a Master’s-level course that offers a structured analysis of four papers: one flawed paper, one exemplary paper, and a pair of conflicting papers. Students who took this course reported significant perceived increases in critical thinking skills associated with reading of primary literature (e.g., analyze paper’s data, independently draw conclusions, propose a follow-up experiment). However, an objective increase in students’ skills was detected only in experimental design. We also examined the challenges students perceive when reading research articles. Understanding these challenges is important in developing effective teaching methods that utilize primary literature. We analyzed 69 pairs of pre- and post-course free responses to the question: “What aspects of reading and analyzing primary literature do you find most challenging?”. We describe six over-arching categories of challenges. Before instruction, “Unfamiliar techniques” presented the the most frequent challenge, while after
instruction “Paper’s conclusions” was identified as such. We also detected changes in the cognitive level of the identified challenges: the frequency of the challenges aligned with the Bloom’s Lower Order Cognitive Skills (Knowledge, Comprehension) decreased, while the frequency of the Higher Order Cognitive Skill challenges (Analysis, Synthesis, Evaluation) increased and both changes were statistically significant. We suggest that these changes are consistent with the transition from a novice to a competent reader and discuss the immediate implications of our findings to instruction that utilizes scientific papers. The funding for this project was provided by the grant by Academic Senate of the University of California, San Diego to E.T.

Program Abstract #86
Use of a simple, tactile modeling activity in a developmental biology class to visualize WT and mutant protein structure
Michelle McWhorter
Wittenberg University, USA
Students entering their first course that discusses molecular and/or cell biology often struggle with visualizing the central dogma and the resulting protein structure and function. In addition, they often also have difficulty visualizing how a mutation in a gene might impact the protein structure and ultimately its cellular function. As a way to help students begin to visualize these essential concepts, specifically in a developmental biology course, a simple modeling exercise has been designed using paper clips and a PVC pipe. Students are given a DNA sequence to transcribe and ultimately translate using a different colored paper clip to signify each different type of amino acid in the protein. After translating the WT sequence, students are also given a mutant DNA sequence to transcribe and translate. In order to give the paper clip protein some structure, it is taped to a PVC pipe. Students then examine the WT and mutant proteins to visualize the difference in the structure and function of the different proteins. This activity can be used to discuss any phenomenon in cell biology, but in a developmental biology class, it has been used to discuss paracrine signals and their corresponding receptor protein. The mutation occurs in the receptor- changing its ligand binding site- which allows for discussion of how this impacts the developmental processes in the cell and organism as a whole. In addition, the exercise allows for students to understand the difference between a synonymous and non-synonymous mutation- if several different mutant DNA sequences are given. This exercise has been used successfully in both an evolution class and a developmental biology class.

Program Abstract #87
A systematic approach to incorporate drawing into a developmental biology lab course
Matthew Beckman, Tara Sweeney
Augsburg University, USA
The use of drawing in the biological sciences has fallen out of fashion in the past half-century. Viewed historically, drawing has occupied a special place in zoology, embryology, and comparative anatomy. The move away from drawing can be attributed to general progress in methodology and instrumentation that has led to accessible high-quality imaging options. For cells, embryos and even fully developed organisms, modern fluorescence microscopy and scanning electron microscopy are now standard imaging modalities. Because modern developmental biology employs genetic strategies to mark or tag genes or proteins or to knockdown expression there is a requirement for imaging that permits quantitation of fluorescence signals or embryo dimensions. Drawing does not permit this level of analysis and is, therefore, no longer a practical method for data collection or even documentation of organisms in developmental biology research. We would not even imagine using a camera lucida today. But, does drawing still hold a place in developmental biology education? There is an emerging, though small, body of literature on the utility of drawing in science education. We believe that the general shift away from the incorporation of any artistic perspectives in most academic disciplines except in art is problematic. Seeing perspective and drawing as a way of knowing has great value in the study of developmental biology. For three consecutive iterations of a Developmental Biology course we introduced students to drawing through four lab-based, one-hour drawing lessons. Students also prepared sketches or drawings of the organisms being studied.
for a period during most of the lab sessions. The study of drawing in development culminated in each student producing a final drawing that represented a process or aspect of development that they found most interesting. We provide a general approach to integrating drawing into a developmental biology lab course.

**Program Abstract #88**

**Student engagement, assessed using heart rate, shows no reset following active learning sessions in lectures**

Diana Darnell, Paul Krieg  
*University of Arizona, USA*

Heart rate can be used as a measure of cognitive engagement. We measured average student heart rates during medical school lecture classes using wristwatch-style heart rate monitors. Analysis of 42 classes showed a steady decline in rate from the beginning to end of a lecture class. Active learning sessions within the class resulted in a significant uptick in heart rate, whereas short videos resulted in a precipitous drop. In both cases, the rate returned to the average level immediately following the interruption. This is the first statistically robust assessment of changes in heart rate during the course of college lecture classes. These findings suggest that the value of active learning within the classroom resides in the activity itself and not in an increase in engagement or attention during the didactics following an activity. Funded in part by an AMES grant from UACOM.

**Program Abstract #89**

**The Gene Expression Database for mouse development (GXD): fostering insights into the molecular mechanisms of development and disease**

Jacqueline Finger, Constance Smith, Terry Hayamizu, Ingeborg McCright, Jingxia Xu, Susan Bello, Cynthia Smith, James Kadin, Joel Richardson, Martin Ringwald  
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With over 1.6 million annotated expression results from 14,592 genes and 335,000 expression images, the Gene Expression Database (GXD) is a key resource for developmental biologists. GXD includes data from RNA in situ hybridization, immunohistochemistry, knock-in reporter, northern blot, western blot and RT-PCR experiments. Data are obtained from the scientific literature and by collaborations with large-scale data providers. GXD curators annotate expression data in standardized ways using genetic nomenclature, controlled vocabularies, and an extensive anatomical ontology. Because GXD is an integral part of the larger Mouse Genome Informatics (MGI) resource (www.informatics.jax.org), this curation work results in a deep integration of mouse expression, genotype, and phenotype data that, in turn, provides researchers with extensive search capabilities. We have now developed several new interface utilities that allow the direct correlation of expression and phenotype data and thus foster insights into the molecular mechanisms of development and disease. A new Gene Expression + Phenotype Comparison Matrix visually juxtaposes tissues where a gene is normally expressed against tissues where mutations in that gene cause abnormalities. The anatomy axis of the view can be expanded and collapsed, allowing users to interactively explore correlations between gene expression and phenotype at different levels of detail. The Mouse Developmental Anatomy Browser now provides not only access to expression data for a given anatomical structure (as before), but also to corresponding phenotype data. Conversely, MGI’s Mammalian Phenotype Browser now points to tissues affected by a given phenotype that in turn link to the wild-type expression data for these tissues. Improvements have also been made to the GXD Differential Expression Search. Visit the GXD Home Page at www.informatics.jax.org/expression.shtml to explore GXD. GXD is supported by NIH/NICHD grant HD062499.

**Program Abstract #90**

**A CRISPR/Cas9 Tissue-Specific Forward Genetic Screening Method in Danio rerio**

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Forward genetic screening in zebrafish has led directly to the identification of many genes involved in human congenital heart defects (CHDs). However, due to the cumbersome nature of these screens, many of the genes
responsible for developmental processes in the heart have not yet been identified or characterized. We have developed a rapid protocol to enzymatically create CRISPR/Cas9 libraries from arbitrary DNA substrates. Using this method we have generated a library that targets the genes expressed in the developing zebrafish heart at 48 hours post fertilization. The library is currently being used to carry out an F0 screen for heart defects. This method has several advantages over traditional forward genetic screens. First, mutations are limited to the exons of transcripts in a tissue of interest (e.g. the looping heart), frequently resulting in null mutations. Second, discrepancies between gene annotations and actual gene sequences are eliminated. Third, causative mutations can be identified without mapping by sequencing pools of sgRNA templates. Fourth, the cost of library generation is greatly reduced compared to synthesis technologies. By increasing the speed of gene identification, this project will provide useful tools for conducting efficient forward genetic screens in zebrafish and other species, providing greater insight into a number disease processes. This method could also be applied to other techniques that make use of CRISPR/Cas9 Libraries. NHLBI 2UM1HL098160

Program Abstract #91
Development of CRISPR/Cas9 strategies for gene disruption and functional Knock-In in the purple sea urchin
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The advent of widespread gene editing using CRISPR/Cas9 has created the opportunity to study the sea urchin with tools, such as genetic Knockouts (KO) and Knock-Ins (KI). We are attempting to adapt established CRISPR KI approaches for the purple sea urchin model. Given that the generation time and low survival rate through metamorphosis we want to evaluate approaches based on accuracy and efficiency. Here I present our approach for two CRISPR KI strategies, preliminary results on efficiency and accuracy, as well as a generalizable framework for approaching CRISPR KI in the sea urchin. In our first approach we used long ssDNA donors to target fluorescent proteins (FPs) to the genes encoding ABCB1a and β-Catenin. We first determined optimal tag position using FP overexpression and then designed single guide RNAs (sgRNAs) to the first (ABCB1a) or last (β-Catenin) coding exon. We screened candidate sgRNAs in vitro and in vivo, and cloned donor templates for in frame integration of an mCitrine FP based on the best sgRNA. Donor ssDNA was made using the Guide-IT Long ssDNA production kit, and subsequently microinjected under a variety of experimental conditions. In addition to traditional HDR mediated KI, we are developing a microhomology mediated strategy for short epitope tags using the BC2 epitope tag, a short linear peptide derived from human β-Catenin. This epitope can be detected with a FP fused to the BC2 nanobody. Targeted gene products can be visualized by co-injection of the nanobody with the donor and sgRNA. In this approach we have taken both published and lab validated sgRNAs and used 15-bp microhomologies to introduce the BC2-tag in frame with the gene of interest. While CRISPR/Cas has revolutionized gene editing, there are still many questions about how to best apply CRISPR in each model organism. The strategies I present here represent one of the first attempts to a generalizable schema for CRISPR/Cas9 knock-ins in the sea urchin embryo.

Program Abstract #92
Identification and characterization of microRNAs involved in ascidian larval metamorphosis
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Metamorphosis takes place within the life cycle of most marine invertebrates and urochordates. The marine ascidian is a classical model to study complex cellular processes and underlying molecular mechanisms involved in its larval metamorphosis. Large numbers of microRNAs (miRNAs) have been demonstrated to be involved in many developmental and metamorphic processes. However, the identification of miRNAs in ascidian larval metamorphosis has not yet been investigated. In this study, 165 miRNAs, including 59 novel ones, were identified from the embryo and larvae of Ciona savignyi. Twelve of them showed significant changes in expression before and during metamorphosis. In situ hybridization and northern blotting results revealed that three miRNAs (csa-miR-4018a, csa-miR-4018b, and csa-miR-4000f) are potentially involved in the signaling regulatory network for the
migration and differentiation of mesenchymal cells in larval metamorphosis. Furthermore, the luciferase reporter assay revealed that MAPK1 is a target of miR-4000f. Our results not only present a list and profile of miRNAs involved in Ciona metamorphosis but also provide informative cues to further understand their function in ascidian larval metamorphosis. This work was supported by the National Natural Science Foundation of China (Grant No. 31572352, 41706153, 31771649), Qingdao National Laboratory for Marine Science and Technology (QNL2016ORP0301), and the Taishan Scholar Program of Shandong Province, China (201502035).

Program Abstract #93
RNA-methylation-Dependent switch of Rncr3/miR124a regulates by MeCP2 induced alternative splicing mechanism
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Brain development requires strict coordination of neural progenitor proliferation and survival with differentiation and maturation of specific neural cell types, and there is a growing realization that non-coding RNAs contribute to the regulation of these events. miR124a is the most abundant brain miRNA and it has been implicated in neuronal maturation in the CNS, and IncRncr3 is the host transcript from which microRNA miR124a is derived through the microRNA processing machinery. Here we showed that Rncr3 interacts with the neurodevelopmental protein MeCP2, which binds to forms of DNA that have been methylated. Previous study demonstrated that MeCP2 physically interacts with and suppresses the nuclear DGCR8/Drosha miRNA processing complex, however, our evidence indicated that MeCP2 also can regulates an alternative splicing event of Rncr3 to control the miR124a processing and neural differentiation. Rncr3 undergoes cytosine methylation and treatment with increasing levels of folic acid, which provides methyl groups for cellular reactions, dramatically increases Rncr3 interaction with MeCP2 and inhibits miR124a processing, suggesting that folic acid through its effect on RNA cytosine methylation influences the binding of MeCP2 to Rncr3, serving as an epigenetic mechanism to control miR124a processing and neural differentiation. More importantly, our data showed that in the early neuroepithelial cell, a major alternative splicing version of Rncr3 does not contain miR124a sequence in exon-IV. MeCP2 knockdown in neuroepithelial cell induced an occurrence of another alternative splicing version of Rncr3 including miR124a, moreover, after 72h inhibition of MeCP2 expression, we clearly observed a significant increase of mature miR124a expression, suggesting that the alternative splicing mechanism of IncRncr3 RNA regulated by Rncr3-MeCP2 interaction is a major factor to control miR124a processing to fine-tune neural progenitor self-renewal and differentiation.

Program Abstract #94
Retinoic Acid receptors in hindbrain and spinal cord cell specification
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Tissue alignment during embryogenesis is controlled by cell communication signals. Our previous work has shown that Retinoic acid (RA) from the mesoderm is an essential signal to align the hindbrain and spinal cord to occipital and cervical somites. While RA represses spinal cord specification genes in the hindbrain, RA does not do this in the prospective spinal cord territory. A survey of the literature has revealed differential transcription of RA receptors in the neural tube, raising the possibility that different RA receptors mediate different RA functions in the hindbrain and spinal cord. To elucidate the function of each receptor in cell specification, we used reagents to inhibit each receptor individually. First, we characterized the effect that different isoform-specific inhibitors of RA receptors have on hindbrain and spinal cord cell specification. Then, we showed that different inhibitors against the same RA receptor isoform cause similar defects. Finally, we showed that inhibition of RARα or RARγ cause different hindbrain and spinal cord developmental defects. Together, our results suggest that different RA receptors regulate different RA functions in the posterior hindbrain and anterior spinal cord. (Support: Start Up funds, School of Arts and Sciences, University of Richmond)
Program Abstract #95
Mapping the Pax6 3’ untranslated region regulatory landscape: microRNA cooperation during neuronal cell-type specification

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PAX6 is a dosage-sensitive transcription factor essential for eye, central nervous system, and pancreas development, and is mutated in the haploinsufficiency disease aniridia. Though the mechanisms regulating precise PAX6 expression levels have not been elucidated, post-transcriptional regulation of Pax6 by microRNAs (miRNA) may represent an important mechanism for maintaining the correct dosage of PAX6. Several miRNAs have been implicated in regulating PAX6 in different developmental contexts. Notably, miR-7 has been implicated in regulating Pax6 in the brain and endocrine pancreas. Despite this, the physiological relevance of miRNAs in the fine-tuning and homeostasis of PAX6 in vivo remains poorly understood. We characterized an 876bp mouse Pax6 3’ untranslated region (3’UTR) and have identified 47 miRNAs predicted to target putative miRNA recognition elements (MREs) in the Pax6 3’UTR. Many of these miRNAs displayed distinct expression patterns in Pax6-expressing tissues and cells. Using in vitro RNA affinity purification, 25 of these miRNAs were found to interact with an exogenous Pax6 3’UTR-containing transcript in α-TC1-6 cells and are predicted to cooperatively regulate Pax6. miR-7a and b were among these, and we have identified two miR-7 MREs that are highly conserved between orthologous Pax6 3’UTR sequences. Pax6 3’UTR luciferase reporters suggest that both miR-7 MREs function additively to regulate PAX6. To explore the biological relevance of the miR-7 MREs in regulating neuronal subtype specification, we generated mice harboring mutations that disrupt one or both sites within the Pax6 3’UTR using CRISPR/Cas9 gene editing. Our findings suggest that, in vivo, microRNA regulation can be mediated through redundant interactions. Since aniridia is the result of insufficient PAX6 protein, understanding how miRNAs negatively regulate PAX6 may enable the development of therapies to block these interactions and de-repress PAX6. Funding: Sharon Stewart Trust

Program Abstract #96
Sall4 regulates maintenance and differentiation of neuromesodermal progenitor cells in mouse embryos

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It has been well established that the three germ layers (ectoderm, endoderm and mesoderm) develop through gastrulation in vertebrate embryos. However, recent findings support the presence and significant contributions of neuromesodermal progenitor cells (NMPs) to the development of postcranial body. NMPs are detected during the late gastrulation and post-gastrulation stages in the tailbud, and contribute to both paraxial mesoderm and neural tissues. Molecular mechanisms that regulate NMP differentiation and migration into either mesodermal or neural progenitors are active areas of investigation in developmental biology. We found that conditional knockout (cKO) of Sall4, a zinc finger transcription factor, using T-Cre caused posterior body truncation, suggesting early depletion of NMPs. Immunofluorescence analysis showed that Sall4 cKO embryos exhibit reduced mesoderm and expanded neural tube, suggesting that the balance of NMP differentiation into mesodermal vs neural progenitors is impaired. RNA-Seq analysis of posterior part of the embryos showed broad changes of expression of genes with mesodermal and neural differentiation gene ontologies. Consistent with the RNA-Seq results, we found reduced expression of NMP regulators (Fgf8, Wnt3a), reduced expression of regulators of paraxial mesoderm development (Msgn1, Tbx6) and elevated expression of neural genes (Sox2, Sox1) in Sall4 cKO embryos by in situ hybridization. SALL4 ChIP-Seq analysis showed an enrichment of SALL4 in neural differentiation genes. Moreover, analysis of expression of pre-neural genes, such as Nkx1.2 and Pax6, identified that Sall4 cKO mutants exhibit accelerated neural differentiation. These results provide genetic evidence that Sall4 regulates NMP maintenance and the balance of their differentiation into mesodermal versus neural tissues by repressing neural differentiation in mouse embryos. Funding sources: National Institutes of Health Grant AR064195 to YK.
Program Abstract #97
N-cadherin expression alters neuroectodermal cell fate choices during avian embryonic development
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Early developmental processes are tightly regulated and require tissues to segregate into three germ layers during gastrulation. After these tissues are specified, each germ layer is further determined, eventually differentiating into all the cells and tissues that will make up the adult organism. We are interested in processes controlling the specification and differentiation of the ectodermal layer. Ectodermal cells become the neural tube, non-neural ectoderm, and neural crest cells. Previous studies have shown that cell adhesion through cadherin proteins is important in regulating the cell fate of ectodermal derivatives. Neural cadherin (Ncad) is a calcium dependent cell-cell adhesion molecule and is required for the development of neural ectoderm, however its role in ectodermal cell fate specification remains understudied. We have performed in vivo loss of function experiments in stage 4 chick embryos using a translation blocking morpholino. In vivo microinjection of the morpholino followed by immunohistochemistry produced embryos with abnormal ectodermal derivative specification. Loss of Ncad increases proliferation, while expanding the non-neural ectoderm and neural progenitor cells. It simultaneously produces a reduction in neural crest and definitive neural tube cells. These results identify a previously unknown role played by Ncad during early tissue specification by showing that Ncad is necessary for proper ectodermal tissue specification. Recognition of the expanded role that Ncad plays may have implications for other aspects of development that involve Ncad such as gastrulation, neural crest migration and the formation of the heart and gut. This work was funded by the NIH Grant R15HD092170 and California State Northridge startup funds to CDR. The author gratefully acknowledges the support of Crystal D. Rogers, the Rogers lab and SDB for the opportunity to present this work.

Program Abstract #98
Cadherin-11 is required for the specification and cell survival of neural crest cells
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The neural crest (NC) is a multipotent embryonic population of stem-like cells that form various tissues in vertebrates including pigment cells, craniofacial bone, and the peripheral nervous system. NC cells are multipotent ectoderm-derived progenitor cells that begin as epithelial cells in the neural tube, but detach and migrate throughout the body after undergoing an epithelial to mesenchymal transition (EMT). NC cells express a variety of cell adhesion molecules, including cadherin proteins, that control their specification, EMT, and migration. Here, we identify the role of Cadherin-11 (Cad11) in early chicken NC development. Cad11 is a type II cadherin protein that is crucial for NC cell migration in amphibian embryos, and also regulates cell survival, proliferation, and migration in cancer cells. Using immunohistochemistry, we distinguished that Cad11 protein has previously unreported dynamic expression, which co-localizes with Sox2 in neural progenitor cells in early embryos. Then, as NC cells are specified in the dorsal neural tube and begin to undergo EMT, Cad11 becomes restricted to premigratory and migratory NC cells and is down regulated in the neural tube. Electroporation of a translation-blocking Cad11 morpholino at gastrula stages leads to a loss of Pax7 and Sox9-positive cells in the dorsal neural tube, but has no effect on more ventral neural tube cells marked by Sox2, suggesting that Cad11 is required for NC specification. We also establish that Cad11 is required for NC cell survival and that markers for p53-mediated programmed-cell death are increased after loss of Cad11. These findings show that Cad11 is necessary for normal NC development, and may increase our understanding of early NC-related developmental defects. This work was funded by an NIH R15HD092170-01 and CSUN Startup funding to CDR.

Program Abstract #99
Early segregation of neural crest cell fate from pluripotent state
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Neural crest (NC) is a multipotent stem cell population that gives rise to multiple derivatives in vertebrate embryos. Studies in chick and Xenopus embryos have eluded to NC specification during early gastrulation. However, the earliest specification of NC from a pluripotent stem cell state with well-defined molecular markers is unknown. We present here for the first time the earliest cell fate specification of avian NC from epiblast and of human NC derived from embryonic stem cells (ESCs). We demonstrate, in blastula stage chick embryos, a mesoderm and neural independent specification of NC from lateral epiblast explants. We further validate with fate mapping studies that cells in the lateral regions of epiblast are pre-specified to form NC. To interrogate the specification of human NC (hNC), we engineered a fast and robust model of hNC development based on Wnt-signaling activation in hESCs. Using this hNC model, we established a high-resolution temporal epigenetic and transcriptomic landscape of hNC specification from a pluripotent state. We identified highly specific changes in the epigenetic landscape and expression of ESC, prospective NC, and NC genes within 6hrs of NC induction from hESCs. Further, using mesendoderm differentiation assays, we found that unlike hESC, prospective NC (just 12hrs after Wnt-activation) are limited in their ability to respond to mesendoderm inducing signals. Taken together our data suggests that the hNC lineage departs from the ESC lineage 6-12hrs after Wnt-activation, and proceeds to form NC, suggesting they have been specified. Our two-pronged approach enables a strong cross-reference between state of the art omics analyses in our hNC model, and the powerful in ovo biology of classic chick embryology. Our data demonstrate for the first time the restriction in NC cell fate in avian and human NC that diverges from the epiblast/ESC fate at unprecedented early facets of development. Funding: NIH/NIDCR

A Tfap2-mediated molecular switch for neural crest induction and specification
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The neural crest is a multipotent stem cell population contributing to multiple vertebrate tissues. Neural crest formation begins with the induction of the neural plate border, which lies between the neural plate and the non-neural ectoderm and gives rise to the neural crest. Next, through the process of specification, neural crest cells acquire their specific molecular signature and come to reside in the dorsal neural tube. Several transcription factors are used reiteratively throughout neural crest formation; however, how they are able perform distinct functions at each step of this process is still unclear. One such factor, Tfap2a, has been shown to contribute to both induction and specification. Tfap2a belongs to the Tfap2 transcription factor family, consisting of paralogous proteins that act as dimers to regulate gene expression. Two other Tfap2 paralogs, Tfap2c and Tfap2b, are co-expressed with Tfap2a at discrete stages of this process, during induction and specification, respectively. Here, we examine the role of Tfap2 heterodimers in avian neural crest development. We propose a model in which these factors participate in a molecular switch underlying the transition from induction to specification based on different heterodimeric interactions between paralogs. First, we show that interactions between Tfap2a and Tfap2c occur during neural crest induction, while Tfap2a/Tfap2b interactions take place during specification. Next, using loss-of-function experiments, we clarify the extent of Tfap2 regulation in neural crest development. Lastly, global analysis of Tfap2 transcription factor binding reveals distinct targets of Tfap2a/c heterodimers during neural crest induction and Tfap2a/b heterodimers during specification. These results suggest that the dual roles of Tfap2a in the neural crest induction and specification programs are mediated by its heterodimeric interactions with Tfap2c and Tfap2b, progressively defining the presumptive neural crest.

Ronin (Thap11): A new transcriptional regulator of neural crest and craniofacial development
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Craniofacial malformations are a significant medical issue affecting 1 out of 3 babies born with a birth defect. A cell population called the neural crest (NC) gives rise to the majority of the craniofacial structures and thus defects during the development of this cell population underlie the majority of craniofacial malformations. The NC is a
transient, multipotent and migratory cell population that is considered a hallmark of vertebrate evolution. Besides giving rise to the craniofacial skeleton, the NC also contributes to a diverse array of tissues including sensory neurons and melanocytes. Although a gene regulatory network governing the various stages of NC development including specification, epithelial to mesenchymal transition, migration and differentiation has been revealed, a great deal is yet to be discovered regarding the factors essential for its multi-potential nature in mammals. Here we report for the first time a novel role for the transcriptional regulator and pluripotency factor Ronin (Thap11) in NC and craniofacial development in the mouse. Strikingly, conditional knock-out (CKO) of Ronin in the NC results in embryos with complete agenesis of the facial skeleton, whereas other NC derivatives remain unaffected. Moreover, Ronin has been implicated in a rare cobalamin (vitamin B12)-deficiency disorder, cblX. Consistent with our findings, these patients present with craniofacial abnormalities. This is the first study to reveal a role of Ronin and cobalamin in the NC and craniofacial development. Thus, continued investigation using these recently generated Ronin CKO models will be crucial in revealing the pathophysiology of the cobalamin-deficiency disorder as well as furthering our understanding of the mechanisms underlying the extensive multipotent developmental plasticity of the NC.

Program Abstract #102
Gcn5 regulation of craniofacial bone and cartilage development
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Craniofacial bone and cartilage development begins with cranial neural crest cells (cNCC), which after migrating from the neural tube, undergo terminal differentiation into osteoblasts, chondrocytes, or other cell types. Given the multipotent capacity of cNCC, scientists have long questioned how this terminal cell fate choice is regulated? Several master regulators of osteoblast and chondrocyte specification have been identified. For example, Runx2, is required for osteoblast specification in the cranial bones. With respect to cartilage development, Sox9 is required for chondrocyte specification in the head and neck. While Runx2 and Sox9 are required for osteoblast and chondrocyte specification, respectively, the regulation of their expression during craniofacial development is not well known. Epigenetic regulation is well known to influence development by controlling gene expression at the chromatin level. Epigenetic enzymes are well conserved and are required for embryonic development. One such histone modifying enzyme is the acetyltransferase Gcn5 (or Kat2a), which acetylates lysine 9 of histone H3 (H3K9ac). Mouse embryos that are homozygous for a mutant allele of Gcn5 in which only the enzymatic activity of Gcn5 is lost (referred to as Gcn5\textsuperscript{hat/hat}) die \textit{in utero}, suggesting that Gcn5 activity is critical for development. We have identified that Gcn5\textsuperscript{hat/hat} mutants have severe craniofacial defects, with skeletal abnormalities in both the bone and cartilage, suggesting the enzymatic activity of Gcn5 is required for proper neural crest development. We have observed disruption in the expression domains of Runx2 and Sox9. We now hypothesize that Gcn5 is recruited to the Sox9 locus and acetylates H3K9 at this location to enhance Sox9 expression. We aim to identify if Sox9 is a direct target of Gcn5 and if Gcn5 is required for activation of the chondrocyte lineage in craniofacial development. Project supported by NIH/NIDCR Grant Number: 3R01DE024034-01A1S1.
Program Abstract #103
Distinct genetic requirements underlie convergent pigment phenotypes in *Danio* fishes.

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How cellular diversity arises remains a fundamental question in biology. Zebrafish pigmentation has become a model for identifying mechanisms of pigment cell differentiation and their contribution to the diversity of form. Adult zebrafish have four pigment cell types: yellow xanthophores, black melanophores, iridescent iridophores, and non-iridescent “white cells.” Unlike other pigment cells, white cells occur only on the dorsal and caudal fins, and both their lineage and mechanisms of differentiation are unknown. White cells are absent in mutants that lack melanophores and some also contain melanin pigment. Accordingly, we hypothesized that white cells and melanophores share a common lineage, share common genetic requirements, or both. As an initial test of these hypotheses we labeled cells in the fin with photoconvertible Eos and imaged them daily to observe white cell differentiation. This demonstrated that white cells developed directly from melanophores. Chemical analysis revealed that white cells contain guanine-filled pigment organelles—like iridophores—but electron microscopy showed that these organelles are disorganized and shaped irregularly—unlike the highly ordered “reflecting platelets” of iridophores. To further understand pigment cell diversity we examined white pigmentation in the close zebrafish relative, *Danio aesculapii*. This species exhibits white cells on the dorsal and caudal fins, and yellow–white cells on the anal fin that contain xanthophore pigment. We hypothesized that *D. aesculapii* white and yellow–white pigment cells are distinct populations that arise from either melanophore or xanthophore lineages, respectively. *D. aesculapii* mutants that lack either melanophores or xanthophores are consistent with this hypothesis and also suggests that the two populations have distinct genetic requirements. Our work contributes to a mechanistic understanding of how pigment cell diversity arises in teleost fishes. Funding by NIH R35 GM122471 to DMP.

Program Abstract #104
Unraveling thyroid-hormone dependent cell fate specification in zebrafish pigment cells during post embryonic development

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During post-embryonic development, circulating endocrine factors are crucial for coordinating differentiation and morphogenesis of multiple adult stem cell derived lineages. One such factor, thyroid hormone (TH), helps to orchestrate the development and homeostasis of neural crest-derived pigment cell lineages in zebrafish. Different pigment cell types have strikingly different responses to TH signaling. TH represses melanophore population expansion, and fish lacking TH have twice the normal number of melanophores. By contrast, TH promotes xanthophore population expansion via increased proliferation and differentiation. To determine which stage of lineage progression is affected by TH as well as the mechanisms responsible for the divergent cellular responses, we assessed gene expression in thousands of individual cells using single-cell RNA-sequencing of all post-embryonic neural crest derivatives, including pigment cells, and we reconstructed lineage trajectories in silico. These analyses revealed TH-dependent changes in developmental progression of melanophore and xanthophore lineages, as well as candidate pathways contributing to the TH-associated phenotypes. Additionally, to understand how TH signaling differentially modulates gene-expression programs in these lineages, we investigated TH-receptor dependent chromatin remodeling using whole-epigenomic analyses and genetic knockouts. Our results suggest that ligand-independent repression by TH-receptors plays a role in xanthophore differentiation and other aspects of pigment cell development. Together, these studies provide novel insights into how TH defines and maintains diverse cell lineages during development and deepen our understanding of the pigment cells, progenitors and cryptic subpopulations that contribute to adult pigmentation. Funding sources: R01 GM111233, NIH R35 GM122471, NIH T32 GM007270.
Program Abstract #105
Gradual loss of pigmentation proceeds larvae death in the zebrafish mutant crasher
Samantha Neuffer, Lauren Clancey, Cynthia Cooper
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Giving rise to multiple distinct cell types, zebrafish neural crest cells offer a relevant model system for studying the mechanisms underlying cell fate and differentiation choices. We have isolated a zebrafish mutant, crasher, with stage specific defects in neural crest derived pigment cell differentiation and maintenance. crasher mutants are also homozygous lethal at 8-9 days post fertilization, suggesting that crasher promotes the development of life supporting systems. We have mapped the crasher mutation to a region containing seven candidate genes, including gna15. Highly expressed in cells of the hematopoietic and skin cell lineages, GNA15 (gna15 in humans) is a Gq class alpha subunit that pairs with G-Protein Coupled Receptors. Aberrant expression of GNA15 has been associated with tumorigenesis in black pigment cells (melanocytes), leading us to study melanocyte gene expression in crasher mutants. Analysis of crasher phenotypes via in situ hybridization experiments, suggest that silver cell (iridophore) and melanocyte specification genes are expressed as expected in crasher mutants. Conversely, expression of melanocyte differentiation genes, dct and tyrp1, is decreased as compared to wildtype siblings. Last, examination of neural crest gene marker, foxd3, indicates normal neural crest induction in crasher mutants. These data suggest that crasher regulates the differentiation and maintenance of neural crest derivatives. Ongoing work involves experiments to better define the reason for premature death in crasher mutants.

Program Abstract #106
Fate of the neural crest cells in inner ear development
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Neural crest cells (NCCs) are the transient migratory, multipotent population of cells that give rise to many cell types during embryogenesis. In the inner ear, NCCs give rise to the glial of the VIIIth ganglion, dark cells of the vestibular system, and the intermediate cells of the stria vascularis. These last two cell types, share about 95% of their transcriptome with melanocytes, which are a late-migrating subpopulation of NCCs. Skin melanocytes and glia originate from different subpopulations of NCCs. NCCs that will give rise to most derivatives in the embryo are early migrating, NCCs that originate melanoblasts migrate later and through a different path. Intermediate cells of the stria are essential for normal hearing; they contribute K+ to the endolymph, a specialized fluid that allows the function of the mechanosensory hair cells. Mutations that affect NCCs migration often result in syndromes that include deafness. The present challenge is to understand how and when these NCCs migrate and integrate into the inner ear to form the glial cells of the VIIIth ganglion and intermediate cells of the stria vascularis. In order to better understand the contribution of NCCs to the different cell types in the inner ear, we have used an inducible Wnt1-CreER transgenic line to label early and late-migrating subpopulations of NCCs. Our preliminary data suggests that an early migrating population of NCCs gives rise to glial cells of the VIIIth ganglion, while intermediate cells of the stria vascularis originate from a late-migrating subpopulation of NCCs. Funding Source: NIH- National Institute on Deafness and Other Communication Disorders (NIDCD)

Program Abstract #107
Transcriptome analysis of the cardiac neural crest reveals a critical role for MafB
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Neural crest cells are a multipotent cell population, unique to vertebrate embryos. They arise from dorsal neural tube along the body axis, migrate extensively throughout the body and differentiate to a variety of cell types. The ‘cardiac neural crest’ is a subdivision that arises from the neural tube at the post-otic to 3rd somite level. These cells migrate through pharyngeal arches 3, 4 and 6, and contribute to cardiovascular morphogenesis, including the
neurons of the cardiac ganglia, smooth muscles of the great arteries and septum of the outflow tract. We have used a FoxD3 neural crest enhancer to isolate a pure population of early migrating cardiac neural crest cells from chicken embryos to perform transcriptome analysis. This has led to the identification of numerous genes up-regulated in this cell population. For example, MafB was found to be a highly up-regulated transcription factor. While well-known for its role in hindbrain patterning, its expression and role in the cardiac neural crest was not previously explored. Here we show that MafB is specifically expressed in cardiac neural crest cells, but absent from other neural crest cell populations. Next we tested the functional role of MafB using knockdown, knockout and dominant negative approaches. The results show that MafB is necessary for cardiac neural crest development and functions as a regulator of sox10 expression in the cardiac neural crest. Our study reveals a novel role for MafB in a cardiac crest gene regulatory subcircuit. Taken together, our transcriptome analysis and perturbation studies provide a new molecular insight into what makes the cardiac neural crest population unique.

Program Abstract #108
Ascc2 as a novel regulator of cardiac progenitor specification and migration
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The cardiovascular system is the first functional organ system to form in vertebrate embryos and is essential for subsequent development. In humans, congenital heart defects are among the most common birth defects, often resulting in lethality. Congenital cardiovascular abnormalities and muscular dystrophies (CMDs) are one group of congenital disorders, presenting with muscle weakness and in a subset of cases, heart and respiratory failure. Recently, human loss of function alleles of Activating signal co-integrator 1 (ASC1) complex members, ASC1 and ASCC1 have been shown to lead to CMD with heart and respiratory failure along with musculoskeletal phenotypes indicating a novel role for the ASC1 complex in cardiovascular development and disease. The ASC1/Trip4 complex was identified in HeLa cells to consist of Asc1/Trip4, Activating signal cointegrator 1 complex subunit 1 (ASCC1), ASCC2 (subunit 2), and ASCC3 (subunit 3). We determined that during murine embryonic stages, the members of the Asc1 complex do interact with each other and that Ascc2 showed highest expression in the cardiac region. No prior model system mutants for any member of the ASC1 complex existed. Ascc2 mutants were identified through the Knockout Mouse Project and were lethal prior to embryonic day (E) 9.5. We found that by E8.5, mutant embryos were smaller with under-developed hearts, and in some cases, failure of heart tube formation from the cardiac crescent. Despite the disruption of cardiac development in Ascc2 mutants, examination of early cardiac mesoderm markers showed a significant increase in genes required for cardiac progenitor specification and migration, but no change in endothelial cell markers. Preliminary conditional knockout data indicate that Ascc2 is required in cardiac progenitors during embryonic development. These results suggest that Ascc2 plays an important role in regulating embryonic heart formation. T32 HL007676

Program Abstract #109
Molecular switches of differentiation from myogenic progenitors into myoblasts and satellite cells in the mouse developing tongue
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The transcription factor Nfix acts as the unique molecular switches from embryonic to fetal myoblast lineage and from myogenic progenitor to muscle satellite cell lineage in limb and trunk myogenesis. We focused on myogenic cell differentiation regulated by Nfix and their related factors during tongue development in embryonic mice. Tongue primordia including muscle tissues were dissected from ICR mouse embryos at E9.5-18.5. Analyses of myogenic gene expression and myogenic differentiation markers were carried out by mRNA/ microRNA microarray, qPCR, and immunohistochemistry. We confirmed that myogenic signaling molecules, including Nfix, were up-regulated in association with differentiation of myogenic cells from myogenic progenitors during E10.5 to E11.5. Nfix expression started at E10.5 and reached its peak level at E14.5. Nfix-positive myogenic cells were detected from E11.5. Nfix-associated embryonic myoblast-specific genes (e.g. Meox1, Meox2) were down-
regulated and fetal myoblast-specific genes (e.g. *Musculin, Eno3*) and muscle satellite cell differentiation-regulatory genes (e.g. *Pax7, Hesr3, Notch1*) were up-regulated after E14.5. Our analyses supported the involvement of multiple microRNAs (e.g. *miR-152, miR-378*) that target Nfix mRNA, in regulating myogenic gene expression. Immunohistochemical analysis revealed that Nfix-positive myogenic cells and MyoD-positive myoblasts were localized in tongue primordia after E11.5. Myogenic cells and surrounding vascular endothelial cells in tongue primordia were Cxcr4- and its ligand Cxcl12-positive, respectively. Collectively Nfix and its related gene/miRNA expression may play a pivotal role in myoblast and satellite cell differentiation during tongue myogenesis. Supposing that the embryonic and fetal myoblasts are coexistent at the early stage of tongue myogenesis, and satellite cell commitment in tongue muscle occurs earlier than that in limb skeletal muscle. Supported by JSPS KAKENHI Grant number 15K11024.

Program Abstract #110

**Hedgehog signaling controls progenitor differentiation timing during heart development**

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The balance between progenitor cell maintenance and differentiation is tightly regulated during embryonic development and adult tissue homeostasis. Hedgehog (Hh) signaling is known to function in the progenitor cell populations of several developing and regenerative organs, and in cancers with resident progenitor subpopulations. We investigated the role of Hh signaling in cardiac progenitors during mammalian heart morphogenesis. Removal of active Hh signaling from cardiac progenitors *in vivo* caused diminished progenitor gene expression, increased cardiac differentiation gene expression, precocious differentiation and Congenital Heart Disease. We generated a mouse embryonic stem cell model of tunable Hh signaling with inducible expression of the activating Hh transcription factor (TF), GLI1. When GLI1 was activated in cardiac progenitors, a progenitor regulatory network was maintained and the onset of the cardiomyocyte differentiation program was delayed, without altering differentiation potential. Progenitor-specific, but not cardiomyocyte differentiation-specific, accessible chromatin regions were enriched for GLI binding motifs *in vivo* and *in vitro*, and GLI1 expression promoted a global shift in chromatin accessibility towards a progenitor-like profile at GLI-bound distal regulatory elements. The shift from active to repressive GLI TF abundance experienced by cardiac progenitors entering the heart comprised a molecular switch that determined the activity patterns of distal regulatory elements. Thus, Hh signaling modulates a GLI switch at regulatory elements to activate a progenitor-specific gene regulatory network and inhibit premature differentiation, thereby determining cardiac progenitor differentiation timing. This provides a novel molecular paradigm for progenitor maintenance by signal-dependent TFs with implications for organ development, regeneration and cancer. Funding: R01 HL092153 (IM), R01 HL124836 (IM), F32 HL136168 (MR), AHA 17POST33670937 (MR).

Program Abstract #111

**Isolation of Ventricular-Specific Heart Progenitors from the Gastrulating Mouse Embryo Reveals a Role for NOTCH in Human Heart Development**

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Major advancements in the field of heart development have led to the description of multiple progenitor populations that contribute to the heart as it develops; however, little remains known about the earliest specification events of the mammalian heart, or the mechanisms that direct atrial versus ventricular specification and differentiation. In this study, we provide the first evidence of a population of cells that gives rise selectively to cardiovascular cells of the ventricles, and that is labeled by transient expression of Foxa2 during gastrulation. Our discovery that Foxa2 expression marks an early mesoderm population with ventricular differentiation potential
enables us to use this model to further explore the development of the ventricular lineage prior to the establishment of the four-chambered heart, with the goal of uncovering the molecular mechanisms that regulate ventricular specification and differentiation. Shortly before the differentiation of the four chambers of the heart, Foxa2 lineage tracing labels a subdomain of the cardiac crescent, presumably marking the immediate precursors to ventricular cells of the heart. We isolated and profiled Foxa2 lineage-traced cardiac mesoderm cells from the embryo and determined signature genes that may be relevant for the development of the ventricular lineage. Finally, using the human pluripotent stem cell differentiation model we showed that induction of a signaling pathway revealed through this analysis, the NOTCH pathway, increases commitment to the cardiac lineage. These findings not only advance our understanding of early cardiac cell fate specification to support better treatments for congenital heart disorders but have also allowed us to translate our findings to the human pluripotent stem cell system, which may ultimately lead to improved strategies for regenerative medicine approaches. Funding sources: NIH/NHLBI F31 HL136216 (EB); NIH/NHLBI R01 HL134956, R56 HL128646 (ND)

Program Abstract #112
The origin and function of the cardiac lymphatics: winning entry to the British Society of Developmental Biology’s advocacy writing competition
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Lymphatic biology has exploded over the last decade, and is now implemented in cancer, obesity, heart diseases, inflammatory disorders and beyond. Understanding lymphatic vessel heterogeneity is essential for developing targeted therapies for such conditions. Part of this heterogeneity may arise from distinct mechanisms contributing to lymphatic development in different organs. I describe the example of cardiac lymphatics, as part of the winning entry to the British Society of Developmental Biology’s advocacy writing competition. Klotz and colleagues used Cre recombinase-based lineage tracing to discriminate a non-endothelial origin of cardiac lymphatic vessels (1). Conditional deletion of Prox1 in lymphatics resulted in cardiac defects, and the lymphangiogenic vascular endothelial growth factor C improved myocardial function post-infarction, demonstrating the important roles of lymphatic biology in cardiovascular development and disease. I also describe the trajectory of research in lymphatic development, and its implications for novel lymphatic-based therapies across a range of organ systems. (1) Klotz et al. (2015) Nature 522: 62-67.

Program Abstract #113
Enhancing In Vivo Lineage Reprogramming of Skeletal-Muscle Cells into Endoderm-Like Cells
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Beta cells are pancreatic endocrine cells that regulate blood glucose levels through the production and release of insulin. In diabetes, high blood glucose levels ultimately result from the loss of beta cells, making their replacement a goal of cell based therapies. Although in vitro production of beta cells from stem cells holds great promise, their production and transplantation comes with critical safety risks and efficacy obstacles. To overcome these issues, our lab sought an alternative approach to generate replacement beta cells by directly converting other cells into beta cells, while they remain in the body. Because beta cells can function outside the pancreas, other cell types throughout the body can be considered for conversion into beta cells provided their loss is non-lethal. We used the zebrafish vertebrate model system to identify factors that could convert a range of differentiated cell types within a living fish into endoderm lineages, including pancreatic beta cells. Using this in vivo screening tool, we successfully identified two factors that, when expressed in the same cell, could reprogram muscle cell identity into endoderm cells that can develop into pancreas-like cells. As we continue to coax these reprogrammed muscle cells into beta cells, we tested strategies to increase lineage reprogramming efficiency. Here, we report on our efforts, using a combination of synthetic biology and chemicals, to enhance induced in
vivo lineage conversion. Funding was provided by the California Institute of Regenerative Medicine through San Diego State University.

Program Abstract #114
MicroRNA Regulation of Mesodermal Cell Fate in Early Sea Urchin Development
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microRNA-124 (miR-124) is a highly conserved microRNA (miRNA). The objective of this project is to investigate how miR-124 regulates mesodermal cell specification. The purple sea urchin, *Strongylocentrotus purpuratus*, is used to examine the function of miR-124 in regulating the Delta/Notch signaling pathway in early development. The sea urchin Delta/Notch signaling pathway specifies mesodermally-derived blastocoelar and pigment cells that perform immune functions. We bioinformatically identified two or more potential miR-124 binding sites in the 3'UTRs of *Notch*, *Ese* (blastocoelar cell transcription factor), and *Gcm* (pigment cell transcription factor). Previous literature indicated that both *Ese* and *Gcm* are positively regulated by Notch signaling. We hypothesize that miR-124 regulates mesodermal cell fates by suppressing Delta/Notch signaling pathway and its downstream targets. We have discovered that miR-124 inhibition resulted in ectopic misexpression of *Ese*. We will test the molecular mechanism of how miR-124 alters *Ese* expression. To test the direct regulation of miR-124 on *Notch*, *Ese*, and *Gcm*, we cloned their 3'UTRs downstream of *Renilla* luciferase reporter constructs. For *Notch*, one functional miR-124 regulatory site was identified. To test the impact of miR-124 suppression of Notch, we blocked miR-124 suppression of *Notch* with Target Protector Morpholino (TP MASO) that is designed to be complementary to the validated *Notch* miR-124 seed and flanking sequences. We are in the process of assessing the impact of blocking miR-124 suppression of *Notch* on mesodermal cell fate changes, by using blastocoelar and pigment cell markers in whole mount in situ hybridization experiments. Preliminary results indicated that *Notch* TP MASO-injected embryos have significantly increased spatial expression of *Gcm* compared to control-injected embryos. Results from this study will identify the regulatory mechanism of miR-124 in mesodermal cell fate determination.

Program Abstract #115
Revisiting Brachyury’s role in the Ciona notochord gene regulatory network
Wendy Reeves, Yuye Wu, Matthew Harder, Michael Veeman
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Although the transcription factor Brachyury has long been established as a key regulator of notochord development, the gene regulatory network underlying notochord differentiation and morphogenesis remains largely undefined. We have used FACS followed by RNAseq analysis to identify a set of >1300 genes enriched in the notochord of the simple chordate *Ciona* during key stages of morphogenesis. This gene set has been extensively validated by in situ hybridization, which also revealed several genes with regionalized expression within the notochord. Notochord enriched genes included both expected effector gene classes (ECM, cytoskeleton, solute transporters, etc.) as well as genes not previously associated with notochord morphogenesis. We also identified several new notochord-expressed transcription factors, in addition to expected notochord transcriptional activators. We used our extensive set of notochord enriched genes to examine the long-held model that Brachyury is a master regulator of the notochord gene regulatory network. To test Brachyury’s ability to induce notochord fate and gene expression, we ectopically expressed Bra using three different tissue-specific enhancers and quantified the effects genome-wide by RNAseq. We found that the majority of notochord-enriched genes (including most notochord enriched transcription factors) were not induced by ectopic Bra expression and that targeted tissues also continued to express many of their own unique markers. These results suggest that Bra is not a simple master regulator of notochord differentiation. Supporting this conclusion, preliminary analysis of gene expression in the Bra mutant *chobi* also found that expression of many enriched notochord genes was not affected by loss of Bra function. Instead of being a straightforward regulatory cascade entirely downstream of Brachyury, the notochord GRN appears to be more complex. Funding: NIH (1R01HD085909); KINBRE (NIH P20GM103418); CMADP COBRE (NIH P20GM103638).
Program Abstract #116
Identifying the Mechanism Driving Anterior-Posterior Differences in Gene Expression in the Ciona Notochord
Matthew Harder, Wendy Reeves, Mercedes Santiago, Chase Byers, Maia Carlson
Kansas State University, USA

Invertebrate ascidian chordates are an excellent group to study embryonic patterning because they have vertebrate-like embryonic anatomy but unusually small, simple embryos and compact genomes. The larval notochord of the ascidian Ciona robusta consists of two separate lineages; the primary notochord, comprised of the anterior 32 cells, and the secondary notochord, comprised of the posterior 8. The primary and secondary notochord cells both express the key notochord regulator Brachyury and intercalate into a single-file rod, but there are quantitative and qualitative differences in morphogenetic behavior between these two cell types. We have recently identified several genes with differential expression between these lineages, including a fibulin-like gene and a putative sodium/ascorbate symporter. Manipulation of upstream signaling pathways suggests that the expression of these genes is positively regulated by both Wnt and FGF signaling. We have identified cis-regulatory modules that largely recapitulate the expression pattern of these two secondary notochord markers and are dissecting these CRMs as an entry point towards understanding the regulatory basis for secondary-specific expression. The fibulin-like CRM shows extensive redundancy and considerable use of negative regulation. We have identified key regulatory subregions within the fibulin-like CRM, and have identified several putative transcription factor binding sites that affect reporter expression when mutated. These ongoing experiments will be integrated into a gene regulatory network for regionalized expression in the Ciona notochord.

Funding: “Morphogenetic effector networks in the Ciona notochord” NIH 1R01HD085909

Program Abstract #117
Arsi expression provides new insights on how sulfation levels are regulated during endochondral bone formation
Rafaela Grecco Machado, Patsy Gómez Picos, Devin Brown, Katie Ovens, Ian McQuillan, Brian Eames
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Sulfatases are a highly conserved family of enzymes in which deficiencies lead to diseases including mucopolysaccharidoses and its associated skeletal phenotypes. Sulfatases remove sulfate groups from sulfated proteoglycans (PGs), which are one of the main components of cartilage extracellular matrix. We hypothesize that sulfatases promote the maturation of cartilage during endochondral ossification. To test this hypothesis, sulfation in the developing chick humerus was assessed by synchrotron chemical-specific X-ray fluorescence. Indeed, sulfation levels were higher in immature than in mature cartilage. Looking for genes that could be regulating PG sulfation, laser capture and RNA sequencing of mature and immature chondrocytes in chick and mouse developing humerus were performed. Based on RNA sequencing results three expressed sulfatases are being studied: ARSB, GALNS, and ARSI. ARSB and GALNS were previously described to participate in chondroitin sulfate PG catabolism, while ARSI (significantly upregulated in mouse mature cartilage), has still unknown biochemical and biological functions. Preliminary ISH results show Arsi expression in mouse mature cartilage and in a transition zone between mature and immature cartilage in chick humerus. To test the biological function of ARSI, zebrafish overexpressing this sulfatase on their cartilage were created using Gateway-Tol2 technology. The development of endochondral bones in wt and transgenic zebrafish larvae will be analyzed and related to the level of sulfates and sulfatases. Acknowledgments: Government of Saskatchewan-SIOGS, CIHR

Program Abstract #118
A cell-autonomous role of TGF-beta signaling in the maintenance of the tendon cell fate
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Tendons transmit forces from muscle to bone to generate movement. Despite their importance to overall musculoskeletal function, little is known about the critical regulators of tendon development. Our group has previously identified transforming growth factor-beta (TGFβ) signaling as a potent inducer of tenocyte marker
scleraxis (Scx), and a critical player for tendon formation. To further explore the role of TGFβ signaling in tendon development, here, Tgfbr2 type II receptor gene (Tgfbr2) was disrupted specifically in Scx-expressing cells using a Cre-lox system. Our results show that the formation and maturation of most mutant (CKO) tendons was unperturbed. Surprisingly, the CKO tenocytes lost tendon markers in early post-natal stages and reverted to a progenitor-like state, as evidenced by increased clonogenicity and expression of common progenitor markers. To test if this was a cell autonomous role of TGFβ signaling, Tgfbr2 expression was reinstated in CKO tenocytes using adeno-associated viral vectors. We find that persistent presence of TGFβ signaling in individual CKO tenocytes was sufficient to prevent the loss-of-cell fate phenotype, indicating a cell-autonomous role of TGFβ signaling. Interestingly, ablation of TGFβ signaling in individual tenocytes was not sufficient to disrupt their differentiation status, implying that the disruption of cell fate in CKO tendons also involves extrinsic factors, e.g. the cellular microenvironment. Altogether, these findings reveal that tendon differentiation state may be dependent on continuous maintenance, and that TGFβ signaling plays an essential, cell-autonomous role in this process. This latter fact has opened an important avenue for investigating other critical regulators for maintenance of the tendon cell fate. Acknowledgement: This work was supported by grants from NIH (R01AR055973, R01DC014160) and Shriners Hospitals for Children (SHC 5410-POR-14).

**Program Abstract #119**

**Gpr182 is a novel regulator of hematopoietic stem cell specification**

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Hematopoietic stem cells (HSCs) generate all hematopoietic lineages over the life of an animal and are the basic units of bone marrow transplants used in the treatment of hematologic diseases. However, the availability of this therapy is limited by the supply of immunologically compatible donor cells. Thus, *in vitro* generation of autologous HSCs from induced pluripotent stem cells (iPSCs) for use in therapy is a major biomedical objective. Several challenges to generating HSCs capable of high-level engraftment and multi-lineage potential have yet to be overcome. In vertebrates, HSCs are specified from hemogenic endothelium (HE) in the floor of the embryonic dorsal aorta (DA). Inductive signals originate from nearby cells, but the identity of the full set of signals remains unknown. Difficulties in generating HSCs *in vitro* likely stem from a lack of knowledge of *in vivo* signals and receptors. Reproducing the full set of *in vivo* HSC specification signals might help overcome some of the barriers to *in vitro* directed differentiation but requires identifying the full set of inductive signals. We have identified a novel role for Gpr182 in the control of HSC specification. We show that *gpr182* is expressed in the DA and that HSC specification is disrupted when Gpr182 is knocked down. Importantly specification of the DA and HE cells, which is a prerequisite for HSC specification, is unaffected. Our results suggest that Gpr182 could be mediating a signal that is required proximally for initiation of the hematopoietic program. Recently we demonstrated that trunk neural crest cells (NCC) mediate HSC specification through an unknown signal(s). Similar to Gpr182, NCC derived signals are not required for DA or HE specification suggesting that Gpr182 could be mediating a NCC derived signal. Interrogating the role of Gpr182 in HSC specification will lead to novel insights into the signals regulating HSC specification in the vertebrate embryo. NHLBI R00HL097150, NIDDK R01DK113973

**Program Abstract #120**

**Gradual expansion of binucleation of male accessory gland cells regulated by Dpp**

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The Drosophila male accessory gland (MAG), which is an internal reproductive organ analogous to the mammalian prostate, has a unique binucleation state in all of its epithelial cells. During the development of the MAG, binucleation occurs in the middle of the pupal stage after a cell cycle arrest in the G2 phase. The binucleation starts around the central part of the primordium of the MAG and gradually expands toward both the distal and proximal ends of it. Prior to this binucleation wave, activation of a signal caused by Decapentaplegic (Dpp), a
member of the bone morphogenic protein (BMP) family of diffusible extracellular ligands, can be detected throughout the MAG primordium, although its highest intensity is found around the central part of it. These findings led us to predict that the binucleation itself or its timing is regulated by Dpp signal during MAG development. When somatic mosaics with elevated or depressed Dpp signaling strength were made in the MAG primordia, premature or delayed binucleation was observed, respectively. Thus, we concluded that Dpp signaling affects only the timing of the binucleation and does not affect whether binucleation will occur. After the cell cycle arrest in the G2 phase, String (Stg), a Drosophila homolog of the budding yeast G2-M phase regulator, Cdc25, is required to start binucleation. When the somatic mosaics had elevated or depressed Dpp signaling strength in the MAG primordia, premature or delayed expression of Stg was observed, respectively. Thus, we concluded that Dpp signal promotes the timing of binucleation by induction of the transcription of Stg.

Program Abstract #121
Vestigial-dependent recruitment contributes to patterning and growth of the Drosophila wing
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In the developing wing of Drosophila melanogaster, wing fate is defined by the expression of the selector gene vestigial (vg). The size of the Vg domain is determined by two mechanisms. First, through proliferation of vg-expressing cells near the dorsal-ventral boundary of the wing disc; and second, through the propagation of the Vg pattern to neighboring cells through a recruitment process. Although the molecular factors that determine the progression of recruitment have been recently uncovered, little is known about the spatio-temporal propagation and contributions of these mechanisms to the final size of the wing. We quantitatively measured the spatial pattern of Vg at different times during wing disc development and show that the width of the Vg gradient increases more than what would be expected from scaling by homogeneous tissue growth. We show that this overscaling phenotype can be explained by a mathematical where the Vg concentration expressed in each cell is assumed as a function of its difference with the neighboring cells. Moreover, recruitment-impaired animals have adult wings that are well proportioned, but smaller than control wings, suggesting that recruitment contributes to organ growth. In support of this hypothesis, we performed a lineage-tracing experiment that directly demonstrates the contributions of cell proliferation and recruitment in this system.

Program Abstract #122
RSG1 and Its Partners Regulate Cell-type Specific Primary Cilia initiation
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Primary cilia are essential for intercellular signaling that promotes normal development and tissue homeostasis. Our previous work showed that small GTPase RSG1 functions in a final step of ciliogenesis. In Rsg1 mutant mice, cells have fewer cilia of normal length; despite fewer cilia, the Rsg1 mutant mother centriole recruits centriole appendage, IFT and ciliary vesicle-associated proteins normally. Removal of the capping protein CP110 from mother centriole is important to initiate ciliogenesis. We find that RSG1 can bind to CP110, although CP110 removal does not require RSG1. We find that the few cilia in Rsg1 MEFs have less stable axonemes with reduced microtubule acetylation and detyrosination. Knockdown of two microtubule-depolymerizing kinesins, KIF24 and KIF2A, partially rescues cilia formation in Rsg1 cells. These data suggest that RSG1 acts at a late step in cilia initiation that regulates the stability of axonemal microtubules. Cilia are present in most, but not all, cell types in mammals. However what determines whether cilia form in a particular cell type is not clear. We are investigating the hypothesis that RSG1 and its partners play important roles in cell-type specific cilia formation during mouse embryo development. To test this, we are examining yolk sac endoderm and extra-embryonic endoderm stem
(XEN) cells; both cell types lack cilia but have mature basal bodies and can form cilia when the AURKA–HDAC6 cilium disassembly pathway is inhibited. We find that their mother centrioles retain CP110 and fail to recruit RSG1. In addition, TEM data reveal that their basal bodies fail to dock apically and the axonemal microtubules fail to elongate. We suggest that removal of CP110, recruitment of RSG1 and apical docking are coordinately regulated during cell-type specific cilia initiation, which determines the ability of cells to respond to specific developmental signals. The work is supported by the NIH grants (R37 HD03455 and R01 GM126124 to K.V.A).

Program Abstract #123
Max's Giant Associated Protein (Mga) acts as a molecular switch during early Zebrafish development
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In early embryos, cells gradually transition from a dividing, pluripotent state into a non-dividing, differentiated state. A large number of transcription factors and signaling pathways are involved in mediating this process, but the key triggers are still unknown. We propose that the transcription factor, Max's Giant Associated protein (Mga), acts as a switch between proliferation and differentiation in embryos. Mga is a dual-specificity transcription factor with two DNA binding domains (T-box and bHLHZip), and was originally identified as a member of the Max-interacting network of transcription factors. These transcription factors all act to antagonize Myc activity by competition for Max protein and E-box binding sites, but the role of Mga in vivo is poorly understood. In cell culture, Mga promotes exit from the cell-cycle by acting as a transcriptional repressor and antagonizes Myc-dependent cell proliferation. We previously showed in zebrafish embryos that Mga acts in a tissue specific manner to control Dorsal-Ventral Pattering by regulating Bmp expression. Here we show that depletion of Mga is required in the whole embryo for formation of the neural crest. Embryos depleted of Mga using a specific morpholino display malformation of a variety of tissues derived from neural crest cells. Furthermore, a marked decrease in neural crest markers was observed during gastrulation. Mga depleted embryos also show a marked decrease in Bmp signaling activity and an increase in Myc activity, as revealed by analysis of a Myc responsive transgene and endogenous Myc targets. This is the first demonstration that Mga acts as a Myc antagonist in vivo. Collectively, our data support the hypothesis that Mga acts to control the switch from a proliferative, pluripotent state, to a differentiated state.

Program Abstract #124
Insights into the role of pax-3 and the gene regulatory network that specifies hypodermal cell fates in C. elegans
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The formation of the C. elegans skin (the hypodermis) is an excellent model to study how general categories of cell types acquire a specific cell fate. Early events in C. elegans embryogenesis induce hypodermal precursor cells to differentiate into either dorsal, lateral, or ventral hypodermal cells. Our work suggests that the specification of the three major hypodermal cell fates may involve cross-regulation between important transcription factors, many of which have homologs in humans and other model organisms. Previous work from our lab showed that the Paired-box transcription factor PAX-3 is involved in specifying the ventral (P cell) hypodermal fate during embryogenesis. We have shown that PAX-3 acts in the ventral hypodermis by repressing the expression of genes required to adopt a lateral hypodermal (seam cell) fate, specifically the GATA factor egl-18 and ceh-16/engrailed. In addition to our work on PAX-3, other authors have shown the involvement of REF-2, VAB-15 and HLH-3 in the specification of the ventral hypodermis. Our data from reporter expression analysis as well as RNAi experiments suggests that PAX-3 acts earlier during embryogenesis, upstream of these additional transcription factors. Finally, we have found through a yeast one-hybrid approach, that the T-box factor TBX-8 binds a cis-regulatory region of the pax-3 promoter. Combinatorial RNAi of tbx-8 and its redundant factor tbx-9 caused ectopic expression of pax-3 and other ventral reporters in the dorsal hypodermis. Our results indicate that the T-box factors TBX-8 and TBX-9 act in the dorsal hypodermis by repressing, either directly or indirectly, the expression of genes required to
specify the ventral hypodermal fate. With our current data and ongoing experiments, we aim to extend the knowledge on the regulatory interactions that control these cell fate decisions earlier during development.

Program Abstract #125
The Ldb1 co-regulator is required for pancreatic endocrine progenitor appearance and differentiation
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Pancreatic β-cells are indispensable for glucose homeostasis and their dysfunction is central to diabetes mellitus. β-cell development is regulated by transcription factor (TF) cascades, mediating differentiation of embryonic progenitors into mature β-cells. TF roles have been highly characterized yet the role of interacting co-regulators is underappreciated. Our prior studies showed that the essential TF, Islet-1 (Isl1), interacts with a broadly-expressed transcriptional co-regulator, Ldb1, to regulate late β-cell maturation and function. However, Ldb1 is also expressed early in development, before Isl1 is expressed, including in embryonic multipotent progenitor cells (MPCs) and endocrine progenitors expressing the TF Neurogenin-3 (Ngn3). MPCs populate the endocrine and exocrine pancreas, while Ngn3+ endocrine progenitors (Isl1-) delaminate from the ductal epithelium to specifically form islets. We hypothesize that Ldb1 has Isl1-independent roles in maintaining progenitor identity and survival. To test this, we generated a pancreas-wide Ldb1 knockout (Ldb1Δpanc) and observed severe developmental and postnatal defects. At embryonic day 13.5 (E13.5) Ldb1Δpanc embryos exhibited disorganized progenitor pools, suggesting impacts on endocrine cell identity. At E15.5 Ldb1Δpanc mice had significant reductions of Ngn3+ progenitors and Pdx1hi, a marker of new β-cells. Ldb1Δpanc mice were born as expected but died by postnatal day 7 with severe hyperglycemia, hypoinsulinemia, and drastically reduced islet cell numbers. Considering that Ldb1Δpanc defects were endocrine specific, we generated a model of Ldb1 deletion in Ngn3+ progenitors, termed Ldb1Δendo. We confirmed loss of Ldb1 in islets and observed hyperglycemia, with severe reduction of islet cells in Ldb1Δendo neonates. We are assessing the developmental phenotype, specifically examining markers of apoptosis, altered cell identity, and delamination. This project is funded by NIH DK111483, DK105209, and ADA 1-16-JDF-044.

Program Abstract #126
Using mRNA electroporation to understand how avian germ cells are specified
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Primordial germ cells (PGCs) are the embryonic precursors of oocytes and spermatozoa, which fuse to form zygotetes in animals that sexually reproduce. We study PGC development in the quail system, which is capable of being imaged during gastrulation when PGCs proliferate and mature. We have generated multiple transgenic quail lines with distinguishable fluorescence in PGCs, allowing for dynamic studies into their development. Using these transgenic quail lines, we developed a novel method of transfecting PGCs in vivo by messenger RNA (mRNA) electroporation. This method allowed fluorescent proteins to be expressed faster, more uniformly, and more efficiently than standard DNA electroporation. Interestingly, PGCs were efficiently electroporated through use of earlier stage embryos (blastoderm to primitive streak formation), yet PGCs were completely refractory to electroporation through use of later stage embryos (during primitive streak regression). We hypothesize the presence of epiblastic stem cells in early stage embryos that can express fluorescent proteins through electroporation and later receive inductive signaling molecules to differentiate into PGCs that are refractory to electroporation. We are now repeating these experiments to obtain publication quality results and interpretations. Through mRNA electroporation of PGCs in vivo, our preliminary data suggests that inductive signaling pathways may be active in germline specification, a dissent from previous studies in the avian system. These findings will inform future functional experiments focused on perturbing this putative induction event through mRNA electroporation of activators and inhibitors of inductive signaling molecules.
Program Abstract #127

Huntington’s Disease impairs germ fate acquisition in isogenic human embryonic stem cells
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Huntington's disease (HD) is a fatal neurodegenerative disease caused by expansion of CAG repeats in the Huntingtin gene (HTT). Neither its pathogenic mechanisms nor the normal functions of HTT are well understood. To model HD in humans, we previously engineered a genetic allelic series of isogenic human embryonic stem cell (HD-hESC) lines with graded increases in CAG repeat length. We have also recently developed a highly quantitative technology that allows high-resolution analysis of human embryonic stem cell (hESC) differentiation, by simply confining their geometry in disc-shaped colonies, or “gastruloids”. When cultured on these micropatterns, and stimulated by BMP4, hESC self-organize to generate radially symmetrical patterns of discrete embryonic fates, which model human gastrulation. Surprisingly, hESCs containing HD displayed alterations of the relative thickness and position of the rings, when grown as gastruloids. Interestingly, this phenotype was CAG-length dependent: the higher the CAG length, the smaller the SOX2+ (ectoderm) inner circle became. By dissecting the molecular mechanisms that govern self-organization in gastruloids, we demonstrate that HD mutations induce an increased signaling through the BMP pathway, due to a decreased production of BMP inhibitors. Therefore, these results demonstrate that Huntington’s disease, contrary to general belief, is not just an adult-onset disease, but its effects may appear as early as gastrulation.

Program Abstract #128

Novel Cellular Compartments Transiently Utilized Prior to the Refractory Period of Xenopus Regeneration
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Many vertebrates lack the ability to effectively regenerate tissues in response to wounding, though embryonic tissues retain some degree of regenerative capacity. *Xenopus* tadpoles are capable of full, scarless tail regeneration, but gradually lose regenerative competency through metamorphosis. During a brief stage of development, known as the refractory period, tadpoles temporarily lose regenerative competency. The refractory period is also concurrent with changes in nutrient acquisition, suggesting that metabolic shifts could be responsible for the loss of regenerative capacity, though the link between metabolism and the refractory period is poorly understood. Here, we utilize the regenerating fin margin epithelia to probe this relationship further by characterizing microtubule-associated cellular compartments identified in the developing tail fin. These compartments appear to be surrounded by microtubules and disruption of tubulin polymerization leads to significant changes in the size of these structures. Immediately following tail amputation, preliminary data indicates that these compartments rapidly decline in abundance, suggesting that they may be nutrient storage compartments which are utilized to facilitate the increased resource demand during regeneration. Indeed, preliminary evidence suggests a role in glycogen storage for these compartments. Further studies into the composition and mechanism of utilization for these compartments will enable us to probe the metabolic requirements for regeneration. PHS NRSA T32GM007270 from NIGMS, R01NS099124 from NINDS.

Program Abstract #129

The many roles of strain cues in morphogenesis
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We prove that a population of cells, each of which uses only strain cues for timing and positional information, can form a variety of key morphogenic patterns. Invading cells form closed loop networks; cells on curved surfaces devolve into sliding layers or patchwork 2D domains; strain-cued cell transformations generate very stable solitary-wave-like timing signals; and, when a solitary wave propagates across it, an initially homogeneous population can spontaneously form periodic segments. Cell actions are described by one or more simple
“response functions” including: the tendency of cells to maintain a preferred area density; kinematic feedback, where a pair of cells that detect relative sliding motion enhance that motion; strain-cued strain transformations, e.g., the onset of growth or a change in the preferred cell shape; and some obvious constraint responses (also based on strain cues). Demonstrated solutions are very robust. The response functions are presumed switchable but remain active and constant throughout a morphogenic process. The response functions contain fewer parameters than available observables, permitting successful tests of the theory. Quantitative results favor the depiction of cell motions and deformations as “automatous”, i.e., sustained by internally-powered cell action, in several systems studied. Much of the life-cycle movement of pattern-forming ameloblasts engaged in amelogenesis is accountable by strain cues, including: the complex trajectories in 3D space of individual cells during amelogenesis; the maintenance of steady-state conditions in the continually erupting mouse incisor (mechanism for maintaining constant distance between events); and the appearance and spatial wavelength of “cohorts” of ameloblasts, which are an instance of spontaneous segmentation into a periodic structure. The theory is also relevant to somitogenesis, convergent extension, innervation and angiogenesis, and collective cell migration including metastasis.

Program Abstract #130
A unique mechanism of simultaneous guidance of migration and patterning of Drosophila tracheal epithelial tubes by a single FGF signal
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Elegant branched tubular networks of epithelial cells constitute diverse types of animal organs. Commonly, gradients of the soluble growth factors are hypothesized to guide migration of the growing epithelial tubes. Here we show a different mechanism by which a Drosophila FGF, Branchless (Bnl), simultaneously patterns and guides the migration of tracheal epithelial cells to form tubular branches. Bnl is dynamically expressed ahead of each tracheal branch expressing its receptor, Breathless (Btl). Using endogenously expressed Bnl:GFP and Btl:Cherry from genomic knock-in alleles, we uncovered that Bnl:GFP does not form pre-patterned gradient, rather it dynamically forms only receptor-bound gradients within the recipient branches. Moreover, the tracheal cells project cytonemes to directly contact the bnl-source and receive Bnl. Bnl gradient formed in trachea acts as a morphogen to induce concentration-dependent multiple gene activities, which in turn differentially feedback-control the Bnl-receiving cytoneme formation, making the signal gradient self-generating. To investigate how tracheal cells find direction for migration without a chemotactic gradient, we developed a bnl-specific expression driver, bnl-LexA, using genome editing and simultaneously marked the bnl-expressing cells along with the trachea. Live imaging of such embryos revealed a unique morphogenetic movement of the bnl-sources that spatiotemporally guides coordinated migration of tracheal branch. These results show that while the migration of bnl-expressing cells ahead of tracheal branches guides migration of tracheal cells, the target-specific Bnl dispersion by the cytonemes generates branch-specific gradients to pattern the cells into the growing tubular branches. In the future, it will be important to investigate whether the paradigm of simultaneous guidance and patterning of cells by contact-dependent exchange of the same signal can be generalized in all other branched tissue patterning.

Program Abstract #131
Wnt and BMP signaling interact to promote formation of the upper airways of the respiratory tract
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Tracheomalacia is a congenital disorder characterized by the underdevelopment of the trachea in which the cartilaginous rings usually located ventrally are either extraordinarily flaccid or absent. The Wnt Signaling Pathway is critical for the development of the respiratory tract. Deletion of Wls, a key regulator of Wnt signaling, abolished expression of Sox9, resulting in tracheomalacia. RNA sequencing studies revealed effects on gene expression,
notably a down-regulation in BMP signaling molecules. We hypothesize that BMP signaling promotes tracheal cartilage formation downstream of Wnt signaling. Methods: Genetically engineered mice were utilized to conditionally delete Wls and Bmp4 in developing trachea. Whole-mount immunofluorescence was used to determine differences in Sox9 patterning between control and mutant samples. In vitro and ex vivo studies using primary mesenchymal cells isolated from developing trachea, were performed to test the effects of activators and inhibitors of Wnt and Bmp signaling on mesenchymal gene expression. Results: Bmp4 is a target of Wls-induced signaling in developing trachea. Deletion of Bmp4 from tracheal mesenchyme caused phenotypes affecting the trachea ranging from tracheal atresia, truncation to tracheas with poorly differentiated mesenchyme, lacking mesenchymal Sox9 expression and presenting ectopic muscle. Bronchi emerged from esophagus wherein Sox9 was present but abnormally patterned. Wnt/β-catenin target genes including Notum, Lef1, and Axin2, were induced in vitro after treated with BMP4 while downregulated in vivo after mesenchymal deletion of Bmp4. Conclusions: BMP4 is critical for tracheal cartilage development by promoting Sox9 acting downstream of Wnt signaling. Simultaneously, Bmp4 differentially induces expression of Wnt/β-catenin target genes in tracheal mesenchyme likely to further support cartilaginous lineage development. This work was partially supported by NIH NHLBI R03HL133420 to DS.

Program Abstract #132
MicroCT Analysis Reveals Reproductive Duct Defects in Zebrafish wnt4a Mutants
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The reproductive ducts connect the gonads with the genital orifice, and are therefore essential for gamete expulsion. However, few genes have been identified that are required for the development of the reproductive ducts. While analyzing the role of Wnt4a in zebrafish female sex determination, we made the surprising discovery that wnt4a mutants produced normal gametes but were nonetheless sterile. Histological analysis, using standard hematoxylin and eosin (H&E) staining revealed that wnt4a mutants appeared to lack reproductive ducts. To analyze this apparent defect in more detail we used microcomputed X-ray tomography (microCT) to comprehensively compare the morphology of the zebrafish reproductive ducts in wild-type and wnt4a mutants. Unlike standard H&E histology, microCT produces high-resolution 3D images of internal structures. MicroCT was previously limited to hard tissue because soft tissues lacked sufficient X-ray absorption. However, low toxicity contrast agents such as iodine solution have been found to add contrast to softer tissues, such as gonads and reproductive ducts. Using these methods, our analysis has revealed the reproductive ducts in wild-type zebrafish in unprecedented detail, and has confirmed that wnt4a mutant zebrafish lack reproductive ducts, thus explaining their sterility. Bruce Draper (NSF IOS-1456737); Michelle Kossack (T32ES007059); Samantha High (T32HD007348)

Program Abstract #133
Multiciliated Cell Dynamics in the Oviduct
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In females, the oviducts (also called Fallopian tubes) serve as the conduit between the ovary and uterus and are the site of mammalian fertilization. While the oviduct is a critical site for female fertility, how oviduct physiology is regulated at the genetic, molecular, and cellular level is almost completely unknown. The oviduct is lined by a single layer of epithelium, which is composed of multiciliated and secretory cells. The multiciliated cells (MCCs) project hundreds of motile cilia from their apical surface, where they beat together and are hypothesized to capture the ovulated oocyte and sweep it down the oviduct. Interestingly, the oviduct displays proximodistal patterning – at the proximal oviduct (near the uterus), there are few MCCs, while at the distal oviduct (near the ovary) there is a high percentage of MCCs. While this proximodistal patterning is known, it is completely unclear how it is regulated or if it is required for fertility. How are MCCs specified along the proximodistal axis of the oviduct? Do stem cells participate in this patterning? How do physiological changes in the oviduct (i.e. estrous cycle, sperm and oocyte entry, fertilization) change the proximodistal patterning? We are combining quantitative
high-resolution imaging, mouse genetics, and transcriptomic analysis to begin answering these questions and provide a better understanding of the role of the oviduct in female fertility. This work was funded by NIH grants awarded to JBW.

Program Abstract #134
Characterizing Dystrophin’s organization and functional network in Drosophila egg chamber development
Miranda Villarreal, Srishti Goel, Nick Seitz, Andres Vidal-Gadea, Kevin Edwards
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Mutations in the giant actin-membrane linker protein Dystrophin (Dys) are the cause of Duchenne Muscular Dystrophy (DMD). Detailed understanding of Dys protein’s basic cellular and developmental roles is important for designing effective muscular dystrophy therapies. In Drosophila, Dys mutations are viable and produce two visible phenotypes: wing crossveins are detached from the longitudinal veins, and in oogenesis the developing eggs fail to elongate. We found these phenotypes are genetically separable, and focused on the egg chamber morphogenesis functions of Dys. A GFP protein trap in the endogenous Dys locus shows that Dys is localized at the basal side of epithelia such as the egg chamber follicle cells, where it makes a plane-polarized striated pattern. Dys-GFP also accumulates on membranes of the egg chamber nurse cells, especially near the actin ring canals. However, in both cases Dys-GFP maintains a pattern distinct from nearby actin bundles. Loss of Dys leads to failure of circumferential banding of f-actin in the follicle cells, but promotes excess cell surface projections. Dys-RNAi eliminates Dys-GFP, confirming both constructs function as expected. Germline RNAi against Dys does not trigger the typical Dys phenotypes, indicating Dys is instead required in the follicle cells and/or ovary muscle. The beta-Heavy spectrin ("betaH" encoded by karst) is structurally related to Dys, and so we checked for redundancy between them. betaH, Dys double mutants have severe ovary defects and high lethality, unlike the single mutants, suggesting the two spectrin family members overlap in their developmental functions. The sequence of fly Dys was re-analyzed in light of newer structural data from the mammalian protein; we find that Dys is structurally well-conserved except that spectrin repeats 14-15 (of 24) are degenerated, possibly forming an extra hinge region. Supported by NIH AR068583, NSF instrumentation grant, and Illinois State University.

Program Abstract #135
Src-mediated junctional remodeling drives acinar elongation in response to basement membrane stiffness cues
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A comprehensive understanding of how organs acquire their proper shapes is an ultimate goal of developmental biology. The Drosophila egg chamber (follicle) serves as a simple yet tractable 3D model to approach this goal. The acinar follicle initially grows isotropically, and later elongates two-fold along the anterior-posterior axis en route to becoming an ellipsoid egg. Follicle elongation relies on both cell-intrinsic and ECM extracellular matrix (ECM) stiffness cues, but the morphogenetic behaviors that actually elongate the organ are not known. Here we apply an advanced image analysis workflow to map these behaviors in toto. Through morphometric data from WT and elongation-deficient mutants, we find that a major contributor to follicle elongation is the reorientation of anisotropically-shaped cells at the organ anterior. An ongoing genetic screen indicates that this reorientation is specifically regulated by the Src tyrosine kinase. Src may transduce stiffness cues from basal cell-matrix junctions to remodel apical cell-cell junctions and instruct this polarized cellular behavior. This process has striking similarities to that documented in Drosophila trachea, pointing to a conserved mechanism utilized to elongate ‘edgeless’ tubular epithelia that is distinct from mechanisms that elongate bounded, planar epithelia. This work is supported by NIH RO1 grants GM068675 and GM111111 to D.B. and NIH grant 4R00HD088708-03 to S.J.S.

Program Abstract #136
Investigating the role of Innexin in stretched cell morphogenesis in the Drosophila ovary
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In multi-cellular organisms, epithelial morphogenesis is vital for proper development of tissues and organs, where sophisticated cell-cell communication is involved. Among signal transduction, cell adhesion, and direct cell-cell communication, instant cellular responses are usually mediated by Gap junctions. Gap junctions are conserved structures essential for intercellular exchange of small molecules. Dysfunction of gap junctions leads to severe developmental defects and disruption of physiological functions such as heart diseases. Thus, gap junctions may play roles in epithelial morphogenesis. The *Drosophila* ovary is an excellent model to study functions of gap junction proteins. There are eight Innexin (Inx) proteins or gap junction proteins in *Drosophila*. During oogenesis, a group of follicle cells, called stretched cells, flatten dramatically over the nurse cell compartment. Knockdown of Inx led to abnormal stretched cell morphogenesis. Surprisingly, blocking gap junctions did not affect stretched cell morphogenesis, indicating that Inx controls stretched cell flattening in a gap-junction-independent manner. I am currently investigating how Inx regulates stretched cell morphogenesis. This research is funded by the National Science Council Grants and Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University.

Program Abstract #137
Hedgehog signaling controls gene regulatory networks for cardiac mesoderm morphogenesis.
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The roles of cell signaling during early heart development and in the etiology of cardiac morphogenesis defects resulting in congenital heart disease (CHD) are poorly understood. We report a requirement for Hedgehog (Hh) signaling during gastrulation that is essential for the generation of cardiac mesoderm required for heart morphogenesis at later stages. Germline inactivation of Hh signaling through deletion of the membrane-bound signal transduction component Smo (Smo) results in severe CHD, characterized by cardiac hypoplasia, failed cardiac chamber formation, and embryonic lethality. Cre-mediated conditional deletion of Smo indicated that Hh signaling is required in the Mesp1Cre mesoderm domain and surprisingly, not in the Nkx2.5Cre cardiac progenitor domain. Furthermore, genetic inducible fate mapping for Hh-receiving cells from E5.5-7.5 revealed no marking of cardiac progenitors, consistent with an indirect effect on early cardiogenesis. RNA-seq on Hh-deficient Mesp1Cre-positive mesoderm during late gastrulation (E7.5) and validation by in-situ-hybridization demonstrated Hh-dependence of multiple genes critical to the production, ingression, and migration of gastrulating mesoderm—especially those involved in canonical FGF signaling. Additionally, treatment of gastrulating chick embryos with the Smo antagonist, cyclopamine, caused migratory defects in primitive streak mesoderm and resulted in severe allocation defects towards the anterior embryo, reminiscent of FGF signaling defects. Differential chromatin accessibility profiling revealed candidate regulatory elements harboring Gli binding sites near critical Hh-dependent Fgf pathway loci, providing evidence for direct regulation by Hh signaling. These studies uncover a Hh to FGF molecular pathway, establishing a novel network for the generation of anterior cardiac mesoderm during gastrulation and providing new insights into developmental mechanisms for CHD.
Grants: F30: HL136200-01A1; R01: HL092153-09

Program Abstract #138
Identifying the signals underlying endoderm-cardiac communication during heart tube assembly
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Organ formation is critically regulated by inter-tissue communication. During cardiac development the adjacent endoderm is required for bilateral populations of cardiac cells to move to the midline and form a single heart, a process known as cardiac fusion. However, the molecular mechanisms that underlie this relationship have remained an enduring mystery. We have found that mutations in the platelet-derived growth factor receptor
alpha (Pdgfra) leads to cardiac fusion defects in both zebrafish and mice. Quantitative live imaging in zebrafish revealed that pdgfra mutant cardiomyocytes are not properly directed towards the midline. Although there are no evident defects in the anterior endoderm of pdgfra mutants, directional cardiomyocyte movement appears to occur in response to a localized source of the PDGF ligand pdgf-aa. Expression studies reveal that pdgf-aa is expressed in the lateral regions of the anterior endoderm, medially adjacent to the anterior lateral plate mesoderm where pdgfra is expressed. Altogether, these findings suggest that PDGF signaling is likely an important component of the molecular architecture underlying the endoderm-myocardium relationship. Our future studies are directed towards testing the hypothesis that paracrine PDGF signals from the endoderm direct the collective movements of cardiomyocytes towards the midline. Using tissue-specific genetic techniques we plan to determine where pdgfra and pdgf-aa function to regulate cardiac fusion. Furthermore, we plan to elucidate the cell behaviors and downstream pathways activated by pdgfra in order to steer the collective movements of cardiomyocytes towards the midline. Finally, in the long-term we plan to extend our studies to elucidate the biophysical forces that underlie the collective movement of cardiomyocytes towards the midline.

Program Abstract #139
Regulation of Cardiac Outflow Tract Size by the Cadm Family of Cell Adhesion Molecules
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Vertebrate heart formation requires input from two temporally distinct phases of cardiomyocyte differentiation. During the initial phase, progenitors from the first heart field (FHF) form the primitive heart tube. Then, during the subsequent phase, progenitors from the second heart field (SHF) contribute additional cells to both poles of the heart. Recruitment of SHF progenitor cells to the arterial pole creates the cardiac outflow tract (OFT), but the precise mechanisms responsible for controlling OFT progenitor proliferation and differentiation remain to be elucidated. Members of the Cadm family of cell adhesion molecules participate in both homophilic and heterophilic binding in order to regulate intercellular interactions and signaling. Our recent studies have demonstrated that Cadm4 plays an important role in restricting the size of the zebrafish OFT. Knockdown of cadm4 results in a dramatic OFT expansion, whereas overexpression of cadm4 results in a diminished OFT. Furthermore, cadm4 activity limits both the production of OFT progenitor cells and the perdurance of progenitor cells near the arterial pole. Our data suggest a cell adhesion-based mechanism that regulates OFT cardiomyocyte differentiation by restricting SHF progenitor proliferation and restraining the deployment of SHF progenitor cells to the OFT. Our current analyses are focused on employing loss-of-function mutations in cadm4, cadm2a, and cadm3 in order to elucidate which specific heterophilic interactions between Cadm family members influence progenitor cell behavior and thereby limit OFT size. This work is supported by NIH R01 HL108599.

Program Abstract #140
Spine Patterning Is Guided by Segmentation of the Notochord Sheath
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The spine is a segmented axial structure made of alternating vertebral bodies (centra) and intervertebral discs (IVDs) assembled around the notochord. Here, we show that, prior to centra formation, the outer epithelial cell layer of the zebrafish notochord, the sheath, segments into alternating domains corresponding to the prospective centra and IVD areas. This process occurs sequentially in an anteroposterior direction via the activation of Notch signaling in alternating segments of the sheath, which transition from cartilaginous to mineralizing domains. Subsequently, osteoblasts are recruited to the mineralized domains of the notochord sheath to form mature centra. Tissue-specific manipulation of Notch signaling in sheath cells produces notochord segmentation defects that are mirrored in the spine. Together, our findings demonstrate that notochord sheath segmentation provides a template for vertebral patterning in the zebrafish spine. Funding sources: NIH grants R01 AR065439-04 and R01
Radial WNT5A-guided Post-mitotic Filopodial Pathfinding is Critical for Midgut Tube Elongation

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The early midgut undergoes intensive elongation, but the underlying cellular and molecular mechanisms are unknown. The early midgut epithelium is pseudostratified. Its nuclei travel between apical and basal surfaces in concert with cell cycle, a process termed interkinetic nuclear migration (IKNM). Using 3D confocal imaging and 2D live imaging, we profiled behaviors of individual dividing cells. As the nucleus moves apically for mitosis, the cell remains connected to the basal surface via a basal process (BP). During mitosis, the BP splits in two, but one BP is generally lost. The remaining BP is always inherited by one daughter cell; the other nascent daughter cell is born without a basal connection but with an apical link to its sister. Interestingly, during G1, some daughter cells directly use the inherited BP as a “conduit” to transport the nucleus back to the basal side, while >50% of daughter cells generate a new basal filopodium and then use that filopodial path to return the nucleus. Importantly, we found that efficient filopodial pathfinding depends on mesenchymal WNT5A. In the absence of WNT5A, post-mitotic filopodial pathfinding is impaired; some cells fail to tether to the basal surface and their nuclei remain at the apical side for a prolonged period. These cells eventually undergo apoptosis, leading to a shortened midgut. Thus, these studies reveal previously unrecognized strategies for efficient post-mitotic nuclear trafficking, which is critical for early midgut elongation.

Investigating the mechanism of CAV3 regulation of neural tube closure in Ciona

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Neural tube closure (NTC) is an important morphogenetic process in which the flat neural plate transforms into the hollow neural tube that will go on to give rise to the brain and spinal cord. In humans, failure to undergo proper NTC leads to a number of common and disabling birth defects. Our previous work has revealed a role for T-Type Calcium Channels (CAV3s) in NTC that is conserved among chordates, Ciona and Xenopus. Loss of CAV3 leads to improper NTC characterized by an open brain phenotype. The molecular mechanism leading to the phenotype remains to be elucidated. RNA-Seq analysis of a CAV3 mutant line of Ciona, termed bugeye (bug) revealed misregulation of several transcripts, including several implicated in cell adhesion. One of particular interest is the aberrant upregulation of the transcript for FLRT. In wildtype Ciona embryos FLRT is highly expressed during NTC, but then declines precipitously. In bug embryos FLRT transcript does not appear to be properly downregulated following NTC. FLRT is best known for its role in neurite outgrowth, and in a context-dependent manner can act to either to encourage adhesion or repulsion. We speculate that FLRT may play a role in tissue separation and adhesion during NTC, and that the failure to downregulate FLRT in bug leads to an open neural tube. Consistent with this we find that overexpression of FLRT recapitulates the bug phenotype. Moreover, we speculate that CAV3 plays a role in signaling the completion of NTC, and thereby leading to changes in the transcriptional profile. Additionally, our preliminary results suggest that an ascidian-specific member of the IgCAM family (ACAM), as well as neural cell adhesion molecule (NCAM), may act as intermediaries to activate CAV3 as NTC comes to completion. Loss of either ACAM or NCAM recapitulates the bug phenotype and, in the context of neurite outgrowth, NCAMs are known to signal through T-type calcium channels. This work supported by an award from the NIH (HD038701)

The Ajuba LIM protein Wtip regulates actomyosin contractility during vertebrate neural tube closure

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Ajuba protein has been implicated in actomyosin contractility in Drosophila embryos, however the mechanisms remain unclear. Here we examine the localization and function of Wtip, a member of the Ajuba LIM family, in Xenopus early embryos. In vivo depletion of Wtip inhibited apical constriction in neuroepithelial cells and elicited neural tube defects. Wtip was localized to cell junctions, but this localization was punctate in the epidermis, and linear in the neural plate. In cells undergoing Shroom3-induced apical constriction, the punctate distribution was reorganized into linear. Conversely, the linear junctional pattern of Wtip in neuroectoderm changed to puncta in cells with reduced myosin II activity. Suggestive of a potential mechanism, the C-terminal fragment of Wtip physically associated with Shroom3 and interfered with Shroom3 activity and neural fold formation. We propose that Wtip is a tension-sensitive cytoskeletal adaptor that regulates apical constriction during vertebrate neurulation. This study was supported by a NIH grant to S. S.

Program Abstract #144
Claudins uniquely regulate multiple phases of neural tube closure
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We identified unique roles for members of the claudin family of tight junction proteins in regulating epithelial remodelling during neural tube closure. Although claudins are best known for their roles in regulating paracellular permeability and maintaining epithelial integrity, they also bridge the apical tight junction to the actin cytoskeleton. We showed that selective removal of Cldn4 and 8 causes open neural tube defects in chick embryos due to defective convergent extension and failure of apical constriction at the neural plate midline. Although other tight junction proteins localize normally, apical accumulation/localization of Par3, Rho-GTPases and PCP signalling components were altered, as was the shape of the apical cell surface. Mutation analysis revealed that distinct domains within the Cldn8 cytoplasmic tail are required for convergent extension versus apical constriction. We also discovered that Cldn3 is uniquely required in the non-neural ectoderm for fusion of the lateral edges of the neural folds to form the closed neural tube. SEM analysis of Cldn3-depleted embryos revealed changes in the cell membrane at points of neural fold contact and a loss of the extracellular mesh that joins the apposing neural folds. We hypothesize that individual claudins direct changes in epithelial remodelling through their unique interactions with protein complexes at the apical cell surface.

Program Abstract #145
Vangl1 controls polarity and Vangl2 is important for cell shape during neural tube formation.
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In the mouse embryo, morphogenesis and elongation of the neural tube involves convergence and extension (CE), driven by epithelial cell intercalation, as well as bending and closure of the neural plate into a tube, driven by cell shape change and cytoskeletal reorganization. At the genetic and molecular level, components of the planar cell polarity (PCP) pathway have been shown to regulate these polarized cell behaviors. Mutations of the Van Gogh like 2 (Vangl2) gene, lead to mice with a short body axis and open neural tube from the hindbrain to spinal cord regions (craniorachischisis). Previous data from our lab showed that the Vangl2 Loop tail (Lp) mutant, affects cell intercalation efficiency but not polarity of intercalation in the neural plate cells. As the close homolog Vangl1 has a similar localization to Vangl2 in the neural plate, our hypothesis is that Vangl1 may compensate to maintain normal polarity in the absence of Vangl2. In wt embryos, the cell pair separation occurs mostly in the anterior-posterior direction. Using a line carrying knockout alleles of both Vangl genes we find that loss of Vangl1 and 2 (Vangl1^gt/gt; Vangl2^ko/ko) affects the polarity of cell pair separation, while the knockout for Vangl2 with one copy of Vangl1 (Vangl1^gt/+; Vangl2^ko/ko) does not. The polarity of intercalation is also lost in embryos lacking Vangl1 and one copy of Vangl2 (Vangl1^gt/+, Vangl2^ko/+), and in mutants for Vangl2 only (Vangl1^gt/; Vangl2^ko/ko), showing that Vangl1 is important for polarity and not Vangl2. In absence of Vangl2, the apical area of neural cells is significantly increased and the actin and myosin filament organization is disrupted, suggesting a role of Vangl2 in apical
constriction. These data show that the polarity of CE is primarily regulated by Vangl1 while Vangl2 is responsible for regulating the apical changes that drives neural tube bending and closure.

Program Abstract #146
The role of Notch signaling during ommatidial rotation in Drosophila eye
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The Drosophila eye consists of ~800 regularly arranged ommatidia and each ommatidium consists of 8 photoreceptors. During late larval and pupal stages, photoreceptors are recruited into ommatidial clusters, differentiate and rotate 90° towards the equator in dorsal and ventral halves of the eye to generate a mirror image pattern across the dorsoventral midline. During this process, R3/R4 cell fate specification is instructed by an interplay between Fz/PCP and Notch signaling pathways and this specification is crucial to determine the orientation of rotation. The mechanism of Notch involvement in R3/R4 specification has been well understood. Here we present a subsequent role of Notch signaling to regulate ommatidial rotation (OR). Analysis of rotation-specific Notch alleles suggests that Notch signaling modulates the execution of OR, independently of its role in R3/R4 specification. Furthermore, Notch genetically interacts with multiple known regulators of OR, such as argos (aos), prickle (pk) and Abelson tyrosine kinase (abl). By employing an R3/R4 cell-specific driver to perturb Notch signaling only during OR, we show that Notch transcriptional activity is required in R3/R4 pair to control this process. In particular, Notch regulates aos and fmi expression levels in R4 to modulate the activity of EGFR and PCP signaling pathways during OR. Notch signaling is also required for Abl apical localization in R4, suggesting a potential role for the Notch receptor to provide direct cues into cytoskeletal and/or junctional remodeling pathways in addition to its well-known role as a transcriptional co-activator. NIH NEI R01 Project # 5R01EY013256

Program Abstract #147
Mechanisms of skull expansion
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The neurocranium forms from sheet-shaped mesenchymal condensations that rapidly expand over a growing brain. Despite the importance of the neurocranium little is known about the cell behaviours that drive early expansion of these skeletal elements. We use ex vivo imaging to identify cellular mechanisms that drive the distal expansion of the skull toward the top of the head. Proliferation and spatiotemporally controlled progressive differentiation concertedly drive this collective spread of osteoblasts. Our ex vivo imaging system provides insights into mechanisms of morphogenesis in a mesenchymal-derived, sheet-shaped tissue.

Program Abstract #148
Prevention of holoprosencephaly by hmmr-mediated regulation of canonical and non-canonical Wnt signaling
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Separation of the telencephalon into bilateral hemispheres is fundamental for forebrain function. Hemisphere separation is mediated by the floor plate (FP) and roof plate (RP), i.e. the ventral and dorsal midline of the neural tube, respectively. Both are established during neural tube closure, and defects in their morphogenesis or signaling lead to hemisphere misseparation, a condition known as holoprosencephaly (HPE). While FP-derived HPE manifests with phenotypes as severe as cyclopia, the external phenotype of HPE caused by RP defects is generally milder. Internally, however, roof plate-derived HPE is characterized by malformation or absence of the hemisphere-separating septum pellucidum and choroid plexus. We have identified hmmr (hyaluronan-mediated motility receptor) as a gene crucial for establishment of the forebrain RP. hmmr loss of function (LOF) in Xenopus laevis led to RP malformation and loss of hemisphere separation by the septum. The defect was traced back to the time of neural tube closure, when RP precursors did not reach their dorso-medial destination. This was due to a failure in radial intercalation (RI), a mesenchymal-to-epithelial transition essential for neural morphogenesis.
Endogenous *hmmr* suppressed canonical Wnt signaling but cooperated with Wnt / PCP signaling to facilitate cell polarization and migration required for RI. Interestingly, the zinc-finger transcription factor *zic2*, mutations in which cause HPE, also suppresses canonical Wnt signaling. Indeed, *Xenopus zic2* LOF phenotypes were similar to those of *hmmr*. Together, our results strongly suggest that roof plate formation requires a transient attenuation of canonical Wnt to permit Wnt / PCP-mediated morphogenetic movements, a mechanism regulated by *hmmr*. Supported by a MvW fellowship from the MWK BW and the BW foundation’s elite programme for postdocs to KF, an LGF graduate student fellowship to TO and DFG-funded grant SCHA965/6-2 to AS.

**Program Abstract #149**

**Dissecting the genetic basis of head morphology and evolution using haplodiploid wasps**

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Complex traits such as shape and size are known to be multigenic in nature. Gene interactions can be exposed by the phenomenon of epistasis, where two alleles produce a phenotype significantly different from the expected sum of their individual effects. Detecting interactions between loci becomes rapidly intractable as the number of genes increases. This has caused a serious deficit in our understanding of the complex genetic basis of morphological evolution. Use of a naturally haploid system, however, completely eliminates dominance effects and thus considerably reduces the difficulty in detecting intricate webs of genetic interactions. The *Nasonia* clade of parasitic wasps is fitting for studies in developmental genetics and molecular evolution due to their haplodiploid genetics and the ability to make interspecies crosses that result in fertile hybrid offspring. Crosses between two closely related species reveal novel hybrid phenotypes visible in the recombinant F2 males, many of which appear to be due to negative epistatic interactions among several loci. In order to identify the loci causing disrupted development, we have generated targeted introgression lines containing intervals affecting head development, and are using Multiplexed Shotgun Genotyping of hybrids to identify additional contributing loci. We are also combining our genetic tools with a method of 3D imaging that allows for high-throughput phenotyping and quantitative analysis of geometric morphometrics. Combined, these techniques allow us to identify loci responsible for complex traits, developmental incompatibilities and the nature of their epistatic interactions. Our preliminary results confirm that *Nasonia* is a uniquely powerful system with which to probe the role of complex gene interactions in the evolution of form. Funding: University of Illinois at Chicago

**Program Abstract #150**

**Using zebrafish as a model to understand the role of HSPG2 in early craniofacial development**

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We present a patient diagnosed with a multiple congenital anomaly syndrome characterized by scoliosis, skeletal abnormalities, intellectual disabilities, and craniofacial defects. Trio-based whole exome sequencing identified compound heterozygous variants in the *HSPG2* gene: a maternally inherited c.5998-7A>G variant in the promoter region and a paternally inherited c.4916C>T variant in the coding sequence. *HSPG2* encodes for perlecan, a large proteoglycan that plays an important role in cartilage formation, cell adhesion, and basement membrane stability. Mutations in *HSPG2* have been associated with Schwartz-Jampel syndrome and idiopathic scoliosis, two disorders characterized by skeletal abnormalities. However, the mechanisms by which *HSPG2* regulates cartilage development are not completely understood. Therefore, we performed morpholino-mediated knockdown of *hspg2* in the developing zebrafish. Knockdown of *hspg2* disrupted craniofacial development causing a truncation of the Meckel’s cartilage and a reduced number of ceratobrachial cartilages, two neural crest derived structures. Further examination of neural crest cell specification and differentiation demonstrated decreased expression of *sox10* and *dlx2a* at 3 and 1 day post fertilization, respectively. Our data suggests that *HSPG2* regulates cranial neural crest cell specification and differentiation whereby mutations in the human ortholog of *HSPG2* likely result in craniofacial abnormalities. This work was supported by NIMHD Grant no. 2G12MD007592 and NINDS Grant no. NS099153-01A.
Program Abstract #151
Convergent Thickening: a newly characterized morphogenetic machine that generates force for blastopore closure in *Xenopus laevis*

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Gastrulation in amphibians involves the internalization of the prospective endoderm and mesoderm through the blastopore, thereby shaping the basic body plan. These movements are driven by forces generated by embryonic cells, integrated at the tissue level as “morphogenetic machines” that give rise to embryonic shape change and reorganization. Here we describe our recent characterization of one such machine, Convergent Thickening (CT), including its pattern and timing, morphogenic movements, cellular mechanism, and biomechanics. CT is expressed by cells in the involuting marginal zone (IMZ) laying around the blastopore, from the earliest stages of gastrulation, until cells roll around the blastopore lip and are internalized (“involute”), when they transition to different cell behaviors. Explants of the IMZ in culture converge and thicken to a greater extent and more rapidly than do explants of other tissues, and generate tensile force along their long axis. In the intact embryo, CT generates tensile force around the blastopore, driving its closure. Our evidence suggests that CT is driven by a change in the interfacial tension between the presumptive mesodermal cells lying in the IMZ and the overlying superficial epithelial layer. This is supported by observations of tissue behavior, measurements of tissue surface tension, and measurements of the force of thickening. CT is clearly distinct from the other morphogenetic machine that operates within the cells beginning in the IMZ, Convergent Extension (CE), which also generates tension around the blastopore, but after involution. CT continues to operate in embryos that do not express CE, and depends on different patterning and effector molecules. CT appears to be conserved across the anurans and different species vary in their dependence on it to close their blastopore.

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Program Abstract #152
PCP signaling regulates protrusion formation required for convergent extension

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Convergent extension (CE) is a collective cell movement that drives axis elongation through cell intercalation. CE is deeply conserved and necessary for gastrulation and neural tube closure in frogs, fish, mice, and humans. In each organism, CE movements are governed by Planar Cell Polarity (PCP) signaling, however how PCP proteins function during this dynamic process remains unclear. A key feature of PCP signaling is that asymmetric localization of core PCP proteins establishes a cellular asymmetry which in turn guides polarized cell behavior. Despite the requirement for PCP proteins during CE in the *Xenopus* mesoderm, this classical, dynamic asymmetry has not been observed in this tissue. Additionally, how PCP proteins coordinate behaviors stereotypic of CE is unclear. For example, protrusions extended from gastrula mesenchyme cells make stable attachments to neighboring cells and exert traction, allowing cells to interdigitate. These events are dependent on PCP signaling, however, the process by which the cells extend stable protrusions, and how this protrusion machinery interacts with PCP proteins, is unknown. Here, we use in vivo, time lapse imaging of *Xenopus* mesoderm to identify how dynamic PCP localization is established and coordinates specific behaviors required for CE. Further, we have used TIRF microscopy to identify how actin dynamics control protrusion formation with the goal of understanding how this process is regulated by PCP signaling. These experiments will further our understanding of the molecular processes governing cellular movements in CE. This work is supported by NICHD.

Program Abstract #153
Actin flows engage cadherin microclusters to remodel cell junctions during convergent extension

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

Program Abstract #154
Chondroitin sulfate proteoglycan 4 (Cspg4) regulates convergent extension during zebrafish gastrulation
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Convergent extension is a key process in body axis elongation. During gastrulation, the morphogen gradient controls the direction of cell movement. Accumulating evidences demonstrated that pericellular and extracellular proteoglycans play an important role in regulating gradients and signal transductions of morphogens. Chondroitin sulfate proteoglycan 4 (Cspg4) is a membrane spanning proteoglycan modulating proliferation, migration, and cytoskeleton rearrangement of cancer cells and many progenitor cells such as oligodendrocyte progenitor cells and progenitor mesenchymal cells. Apart from the chondroitin sulfate glycosaminoglycan side chains, Cspg4 core protein also acts as a co-receptor for FGF and PDGF signaling, and interacts with extracellular matrix such as collagen and fibroectin. In this study we evaluated the roles of Cspg4 in zebrafish embryogenesis by morpholino-based gene knockdown and mRNA overexpression. The results showed that knockdown of cspg4 significantly suppressed convergent extension movement. In addition, when globally overexpressing cspg4 mRNA, there were about 10% of embryos with cyclopia. Furthermore, we generated a mutant fish line that lacks the transmembrane domain of Cspg4, and this mutant zebrafish line phenocopies the mRNA overexpression embryo including 5% of cyclopia. In addition the mutant embryos were insensitive to cspg4 knockdown and the convergent extension movement were normal in the mutant morphants. Our findings indicated that Cspg4 is necessary for convergent extension and the temporal and spatial expression pattern is important for midline development. We would like to acknowledge the financial, technical and material support for this work from Ministry of Science and Technology in Taiwan (grant number: 105-2628-B-002-005-MY4 and 105-2917-I-002-033), and National Institute of Genetics, the National BioResource Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Program Abstract #155
Keller explant open-face development recapitulates natural morphogenesis of dorsal marginal zone
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During Xenopus laevis gastrulation, dorsal involuting marginal zone (DIMZ) converge and extends into notochord and dorsal non-involuting marginal zone (DNIMZ) converge and extends into neural plate. The autonomous capacity of Xenopus laevis dorsal marginal zone to remodel itself has been attested, and extensively studied, through ‘Keller sandwich explant’ procedure. Which consists of two explanted dorsal marginal zone cultured together face to face and provide a unique spatial-temporal view of the notochord and the neural plate being shaped. On the other hand, ‘Keller explant open-face’ (KE-OF), which consists of an explanted dorsal marginal zone cultured alone, is thought to provide a scenario in which the notochord is shaped by convergent extension, but the neural plate is not. We challenged this characterization by performing in situ hybridizations in order to distinguish endoderm, mesoderm and ectoderm. Our molecular results point out for a novel interpretation upon KE-OF development: 1. DIMZ cells do converge and extend into a notochord like structure, but they do it as they roll towards the ventral side of the explant in an involution like behavior; 2. DNIMZ cells also take part in the
convergent extension cell sorting behavior and a neural plate like structure is shaped. In contrast with the previous interpretation, it is shown that the explanted dorsal marginal zone recapitulates both involution and convergent extension morphogenetic movements.

Program Abstract #156
Dynamic analysis of apical constriction during embryonic development
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Apical constriction is a process used reiteratively throughout embryonic development to bend tissues by shrinking the apical surfaces of epithelial cells, but little is known about its dynamic execution during vertebrate development. Here we study apical constriction behaviors during both gastrulation and neural tube closure in live *Xenopus* embryos to understand the kinetics of both apical cell surface shrinking and actomyosin dynamics during this process. To do this, time-lapse imaging of cell junctions and actin localization is followed by segmentation and lineage tracing to track constrictive behaviors and actin dynamics in hundreds of individual cells over hours of development. Using these techniques we have found both similarities and differences in constrictive behaviors during gastrulation and neural tube closure, and we have begun to map specific constrictive behaviors to different zones of the neural plate. Using these maps of constrictive behaviors, we will create a framework for investigating the dynamic localization of other components of the cell’s constrictive machinery across tissues as well as investigating the role of neural tube defect mutations in apical constriction. Funded by NIH Ruth L. Kirchstein NRSA F32

Program Abstract #157
Investigating the formation of spatial and directional patterns in the mammalian epidermis
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Organ morphogenesis involves a complex interplay between epithelial-mesenchymal interactions, cell fate induction, and tissue polarity. However, it remains unclear how this interplay coordinates morphogenesis in the developing skin epithelium. The mammalian skin, which is decorated with spatially patterned, globally aligned hair follicles (HFs), is an excellent system to explore how spatial and directional cues coordinate epithelial morphogenesis. HFs develop from multicellular placodes that emerge from the embryonic epidermis in waves of evenly spaced epithelial clusters. Epithelial-mesenchymal crosstalk specifies HF progenitors, while planar cell polarity (PCP) orients the direction of HF growth and the asymmetric positioning of early HF lineages. Recently, we developed methods to perform long-term live imaging of epidermal development to capture the individual and collective cell behaviors that drive polarized morphogenesis of mammalian HFs. We discovered an unanticipated and novel pattern of collective cell movements that generates both morphological and cell fate asymmetry of developing follicles. Shortly after placode formation, HF progenitors undergo dramatic cell rearrangements coordinated in a counter-rotational pattern of cell flow that repositions placode cells within the epithelial plane. To generate the pattern of cell flow, spatial patterning of radial cell fates cooperates with PCP to direct polarized cell neighbor exchanges through myosin-dependent junction disassembly. Using a combination of live imaging, genetic manipulations, and transcriptional profiling, I will investigate how the spatial patterning of distinct placode lineages instructs these collective cell movements. I hypothesize that spatial patterning establishes two radially-arranged lineages that differ in motility, contractility, and cell-cell adhesion properties, and that these differences are essential for the generation of counter-rotational cell flow. Supported by NIH-NIAMS.

Program Abstract #158
Identifying long-range polarity cues using spontaneous mutations in mammals
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A key molecular pathway that coordinates local cell behaviors into tissue-scale patterns is the Planar Cell Polarity
The ‘core’ PCP proteins are transmembrane proteins that are asymmetrically localized within an epithelial plane where they act on downstream cytoskeletal regulators to generate the structural polarity of a cell. PCP asymmetry is propagated to neighboring cells by the core pathway itself through extracellular protein interactions and intracellular negative feedback. However, the mechanism that coordinates PCP asymmetry across great distances remains elusive. The mammalian skin provides an ideal system to uncover long-range polarity cues. The expansive tissue is decorated with thousands of HFs that are oriented in the same direction through a PCP driven process. Recently, we showed the murine back skin is compartmentalized into regional domains that influence the direction of PCP signaling. This data suggests that region specific long-range polarity cues cooperate to coordinate PCP and HF orientation across the entire tissue.

In support of this idea, spontaneous mutations in small mammals have produced animals that are prized for their distinct, region-specific disruptions in HF orientation. One of the mutants inhibits tissue-level PCP asymmetry in the embryonic epidermis without altering local PCP asymmetry, a phenotype that is expected in the absence of a long-range polarity cue. By mapping and characterizing mutants with disrupted HF patterns we will reveal novel mechanisms that coordinate cell polarity across tissues.

Program Abstract #159
Characterizing Early Developmental Defects in an Avian Model of Maternal PKU
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Maternal phenylketonuria [MPKU] is a syndrome of multiple congenital anomalies including cardiovascular malformations [CVMs], brain and growth restriction when a mother with Phenylketonuria [PKU] does not control her dietary intake of Phenylalanine [Phe]. In this study, we aim to establish and characterize an avian model of MPKU. We focused on early developmental defects. METHODS: We investigated the effect of 2500μM Phe exposure by in-ovo yolk injection. Following the injection, the embryos underwent further development for 48 hours until dissection was performed. At HH14-17, India ink was injected into the yolk as a contrast dye. Images were taken of embryos and they were scored based on Drake et. al (2006.) RESULTS: Embryos exposed to high Phe displayed gross morphological changes including developmental and growth delays, anterior and posterior abnormalities, and torsion defects. FUTURE STUDIES: Histological analysis is underway to determine changes in heart development. Currently there is no data interrogating the mechanism by which Phe causes heart defects. We plan to utilize this model to define the mechanism of Phe cardiac teratogenicity which is critical for improving MPKU treatments and outcomes.

Program Abstract #160
Cellular analysis of ear pinna development in murid rodents
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Models of epimorphic regeneration, such as zebrafish fins and salamander limbs, have long been studied through the lens of development. Deconstructing how specific cell lineages contribute to regenerated tissue requires an understanding of cell lineages during organ formation. African spiny mice (Acomys cahirinus) have emerged as a bonafide model of epimorphic regeneration as they are capable of re-growing excised body skin and regenerating musculoskeletal tissue in the ear pinna while closely related murids such as mice heal identical injuries via fibrosis. Since most mammals have external ear pinnae, our 4 mm ear punch assay is particularly useful for assaying regenerative ability across species. Not surprisingly, our understanding of ear pinna development is almost non-existent. The few studies that have characterized ear pinna development have focused specifically on embryonic time points and neglect postnatal development. This study provides a cellular characterization of late gestational and postnatal ear pinna development in Acomys and Mus. Our analysis demonstrates that ear pinna development is largely similar in these species, allowing us to use Mus as a general developmental model. Previous studies have suggested that the entire mesenchyme of the pinna is neural crest derived. Using Wnt1-Cre;ROSA26Sgs reporter mice to lineage trace neural crest we found that muscle is not of neural crest origin. Finally, we used the naturally
occurring short ears mouse mutant which have a mutation in Bmp5 to investigate how Bmp-signaling regulates normal pinna development. Although short ear mice have truncated ear pinnae, cellular analysis shows normal tissue architecture with regions devoid of elastic cartilage that is replaced with adipose tissue. Interestingly, Oil Red O lipid and Fabp4 adipocyte staining of heterozygous and null mice suggest that Bmp5 may play a role in differentiation of an adipochondrocyte precursor. Funding: UK Biology, NSF and OISE (IOS-1353713)

Program Abstract #161
Uncovering a spatiotemporal profile of FGF action in early Xenopus laevis development
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Many studies show that FGF signaling plays a critical role in the induction of somitic mesoderm and notochord in early development and that inhibition or absence of wild-type FGFR in Xenopus laevis inhibits the induction of somites and notochord. Others have demonstrated the existence of FGF8 gradients in chick and mouse embryos which may contribute to the so-called segmentation clock. However, the spatiotemporal aspects of FGF function in early Xenopus development remain poorly characterized. Our work uses varying concentrations of an FGFR tyrosine kinase inhibitor (SU5402) applied at specific stages during early development to compile a more complete spatiotemporal profile of the role of FGF in somite and notochord induction. Immunofluorescence microscopy was used to assay morphological perturbations which result from FGFR inhibition in early X. laevis development. Our preliminary results show that SU5402 blocks mesodermal induction most significantly when administered between stages 8 and 9.5, indicating crucial FGF action during this period. Additionally, inhibition at progressively earlier stages 8-16 interval correlates with increasingly truncated notochord and reduced somite patterning along anteroposterior axis with downstream effects on neurulation and blastopore closure. In aggregate, these findings provide a strong foundation for the further spatiotemporal characterization of FGF activity in Xenopus development. We will also discuss future possibilities, including optogenetic control of FGFR function and use of halo-tagged FGF to study the spatiotemporal dynamics of specific FGF ligands.

Program Abstract #162
Programmed Variations of Cytokinesis Contribute to Morphogenesis in the C. elegans embryo
Joshua Bembenek1, Xiaofei Bai1, Bi-Chang Chen3, Ryan Simmons1, Lindsay Uehlein1, Diana Mitchell1, Eric Betzig2
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Cytokinesis involves several regulated steps including cleavage furrow specification and ingression, midbody formation and abscission. How various aspects of cytokinesis are regulated and deployed in different cell division contexts during development is not well understood. To address this, we investigated cytokinesis in the invariant lineage of the C. elegans embryo. We observed several markers that label the furrow, central spindle and different structures within the midbody. Several parameters of cytokinesis are reproducibly altered in different stages of the lineage. During the first two divisions, cells undergo consistent patterns of furrow ingression asymmetry and midbody inheritance, suggesting specific regulation of these events. Cytokinesis patterns change substantially during morphogenesis. In the intestine and the pharynx, midbodies form after symmetric furrowing and migrate across the cell to the nascent apical midline. This midbody migration event coincides with polarization during a mesenchyme to epithelial transition. Interestingly, midbody ring components are internalized, while other midbody components including the Aurora B kinase (AIR-2) remain on the apical surface. In amphid sensilla precursor divisions a midbody migration event that leads to a focal aggregation of AIR-2, which persists along the leading edge of extending dendrite structures well after cytokinesis is complete. Inactivating temperature sensitive cytokinesis mutants during morphogenesis causes defects in lumen formation and dendrite formation. Therefore, the proper execution of cytokinesis and specific cytokinetic regulators such as AIR-2, may regulate the final interphase architecture of a terminally dividing cell during morphogenesis. Funded by NIH R01GM114471.
Program Abstract #163
Genetic and Environmental Perturbation Of Tbx6 Leads To Predictable Segment And Vertebral Malformations
Stephen Devoto, Kevin Serra, Katherine Clifford, Iris Chipendo, Christina Vyzas
Wesleyan University, USA
During vertebrate embryonic development, early skin, muscle, and bone progenitor populations organize into segments known as somites. Defects in this conserved process of segmentation lead to skeletal and muscular deformities, such as congenital scoliosis, a curvature of the spine caused by vertebral defects. The t-box transcription factor Tbx6 is required for segmentation in zebrafish and mice. We use inducible transgenes to increase or decrease Tbx6 expression transiently during the segmentation period. We show that either a transient increase OR a transient decrease causes segment defects and subsequent vertebrae defects. These defects are highly reproducible, and include missing and fused vertebral spines, arches, and ribs, as well as an increase in the variability of the length of the centra. The position of defects along the axis depends on whether Tbx6 protein levels were increased or decreased. Moreover, we show that temperature or osmotic stress during the embryonic segmentation period leads to segmentation and subsequent vertebrae defects in zebrafish, and that the effects of environmental stress are dependent on Tbx6 gene dosage. Funded by NIH grant 1R15AR066284

Program Abstract #164
Specification of mesodermal lineages was altered in the evolution of the lecithotrophic sea urchin Heliocidaris erythrogramma
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A striking feature of sea urchin development is early specification of molecularly, morphologically, and behaviorally distinct types of mesoderm prior to gastrulation. The echinoderm gene regulatory network (GRN) for mesoderm specification and differentiation has become a key system for understanding GRN evolution. Its details – including early signaling, several layers of sub-circuits for cell fate commitment, and terminal differentiation genes – are known in detail for euechinoid sea urchins and comparative data for other echinoderm groups have recently proliferated. The euechinoid Heliocidaris erythrogramma exhibits lecithotrophy, a derived mode of development in which embryogenesis and juvenile metamorphosis co-occur, entailing extensive changes to axial patterning, gastrulation, and its fate map. Among these changes, H. erythrogramma has lost the typical euechinoid trait of an asymmetric cell division at specification of skeletogenic mesenchyme (SM), which also forms the embryonic organizer. We have performed the first detailed GRN analysis of H. erythrogramma mesoderm, which exhibits extensive changes from that of planktotrophic euechinoids in cell signaling, transcription factor expression patterns, and cell behavior. The role of Delta signaling has changed extensively. In other euechinoids Delta signaling is necessary for specification of all non-skeletogenic mesoderm (NSM) and SM is specified independently of Delta. In H. erythrogramma NSM pigment cells are specified independently of Delta and skeletogenic markers are repressed by Delta signaling. SM cells behave differently in H. erythrogramma, with skeletogenic markers restricted to the archenteron and prospective coelomic pouch and excluded from ingressed mesenchyme, unlike other euechinoids. Surprisingly, these changes appear not to have arisen directly from equalization of early cleavages.

Program Abstract #165
Creating a toolkit for multi-vertebrate biochemical analysis
Camilo Echeverria
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Developmental biologists use various model organisms to answer scientific questions about the mechanisms that control developmental processes. While multiple models allow range, use of such varied animals can lead to organism-specific understanding of gene regulation and protein function. Our lab is focused on creating a biochemical toolkit identifying antibodies that recognize conserved and divergent proteins in multiple vertebrate organisms as well as identifying proteins that maintain conserved functions across vertebrates. To analyze the
similarities and differences, we use the axolotl (*Ambystoma mexicanum*) and the chicken (*Gallus gallus*), to find cross-species functional tools. Our goal is to create a molecular and biochemical toolkit for the developmental biology community by using bioinformatics to characterize sequence similarities and authenticating antibodies that recognize endogenous axolotl and chicken proteins. Here, we perform western blot and immunohistochemistry to determine size and spatiotemporal localization of the proteins. To identify evolutionarily conserved developmental and functional similarities and differences between the organisms, we perform gain and loss of function experiments and perform comparative analysis. Our research will identify tools that can be used in multiple organisms and will clarify the similarities and differences between the molecular mechanisms that control the development of multiple vertebrate species. Ultimately, we hope to identify whether the conserved proteins also share function between organisms, and we will perform cross-species rescue experiments to demonstrate. This research contributes to understanding the role of these proteins in the development of vertebrate embryos, while creating a list of molecular tools that can be used throughout the community for direct comparative analysis. This work was funded by CSUN Startup Funding and NIH 1R15HD092170-01 to CDR.

**Program Abstract #166**  
**Dissecting the relationship between Sox10 and the neural crest GRN in two vertebrate species**  
**Alexis Avila, Crystal Rogers**  
*California State University, Northridge, USA*

Neural crest (NC) cells are embryonic stem-like cells that give rise to many derivatives including craniofacial bone and cartilage, pigment cells and most of the peripheral nervous system. These cells arise in the dorsal neural tube, but then undergo an epithelial to mesenchymal transition (EMT) to migrate to numerous destinations.

Understanding the molecular mechanisms that regulate how these cells are created and the proteins that control their development is crucial to identify possible causes for abnormal vertebrate development. Several transcription factors have been identified as important mediators of NC specification and EMT. Here, we perform comparative analysis of NC development in two divergent vertebrate species, the salamander, *Ambystoma mexicanum* (axolotl), and the amniote, *Gallus gallus* (chicken). We characterized multiple NC transcription factors and identified that while the spatiotemporal expression of the factors is conserved, the NC gene regulatory network (GRN) may not be. Pax7 is expressed in the dorsal neural tube and pre- and post-migratory NC cells, and Sox9 is only expressed in a subset of Pax7-positive cells in both organisms. Gain and loss of function studies were used to determine if the function of the NC transcription factors is conserved. We identified that in chick embryos, overexpression of Sox10 is sufficient to induce ectopic expression of Sox9, but not Pax7. However, in axolotls, chicken Sox10 can activate Pax7 as well as Sox9 suggesting possible species-specific developmental differences. These studies will increase our understanding of the similarities and differences that vertebrate species use to regulate NC development, and will provide new insight into potential therapeutic approaches by highlighting new tools that can be used across the vertebrate spectrum. This work was funded by NIH 1R15HD092170-01 and CSUN Startup Funding to CDR.

**Program Abstract #167**  
**The Development of the Neural Crest and peripheral glia in a Holocephali, the Elephant shark**  
**Miranda Luque¹, Alizain Lalani¹, Nellie Manoukian¹, Catherine Boisvert², Maria Elena de Bellard¹**  
¹California State University Northridge, USA; ²Curtin University, Australia

The Neural crest emerges after delamination from the dorsal neural tube and gives rise to the wide variety of neuronal, glial, and non-neuronal derivatives are the progenitor cells for many head structures and the peripheral nervous system in osteichthyans. In vertebrates, these cells give rise to a variety of cranial and facial features, and to the development of electroson sensory receptors. The class Chondrichthyes is usually divided into two sub-classes. The sharks and rays belong to the subclass Elasmobranchii and the chimaeras (also known as ratfishes and ghost sharks) to the subclass Holocephali. Here we studied the chimera Callorhinchus *millii*, in a more detailed manner the development of peripheral nervous system in the chimera. Specifically the trunk neural crest by using in situ
and antibody labeling for neural crest cells, glia and neurons. We found that chimera Sox9 positive cells labeled a thin migratory population of neural crest cells as well as a disseminated group of cells under the skin, possibly melanocytes. We also did a more detailed labeling of the peripheral nervous system using TuJ1 antibody as well as other well known neural markers (GFAP, S100, FoxD3, etc.). From these studies, our data suggests that Holocephali C. Milii, and Elasmobranchii embryos share a similar cell migration pattern in the neural crest. This work was partly supported by an NIH/NINDS AREA grant 1R15-NS060099-01 and NIH-MBRS SCORE-5S06GM048680-13 to MEdB and a MARC scholarship to M.L.

Program Abstract #168
The development of the Trunk Neural Crest in the Turtle, *Trachemys scripta*
Maria De Bellard, Catherine Dombroski, Catherine M. Campbell, Sophia Goldberg
CSUN, USA
The neural crest is a group of multipotent cells that emerges after an epithelial-to-mesenchymal transition from the dorsal neural tube early during development. These cells will give rise to a wide variety of cells that comprises the peripheral nervous system as well as craniofacial cartilages and bones and endocrine organs. While much is known about these cells in mammals, birds, amphibians and fish, relatively little is known in another type of amniote as snakes or lizards. In this study, we show for the first time ever trunk neural crest migration in turtle and gecko by vital-labeling live neural crest cells with Dil and by immunofluorescence. As in birds and mammals, we find that early migrating trunk neural crest cells use both a ventromedial pathway and an inter-somitic pathway in the snake. However, unlike birds and mammals, we also observed large numbers of late migrating neural crest cells under the developing skin. On the other hand, gecko trunk neural crest migration was not distinguishable from birds, a closer relative than snakes. Our results show for the first time the migration of neural crest cells in early evolved reptiles like turtle and gecko. The similarities and differences among birds, snake and gecko reinforces that these organisms evolve separate pathways early in evolution. This work was partly supported by an NIH/NINDS AREA grant 1R15-NS060099-03.

Program Abstract #169
Characterization of premigratory NCCs during development of turtle (*Trachemys scripta*) embryos
Moira Dougherty¹, Andrew Schiffmacher², Lisa Taneyhill³, Scott Gilbert³, Judith Cebra-Thomas¹
¹Millersville University, USA; ²University of Maryland, USA; ³Swarthmore College, USA
The bones of the plastron, the turtle’s ventral shell, develop through intramembranous ossification, in the same manner as facial bones, suggesting they are produced by the same type of cells, known as neural crest cells (NCCs). Previous work has demonstrated the existence of a unique second migration of NCCs away from the neural tube of turtle embryos; these migrate ventrally, appear to be skeletogenic and may contribute to the bones of the plastron. The hypothesis being tested is that these late-emerging cells result from premigratory NCCs that are not depleted at the end of first wave of migration and remain dormant until the second migration commences. These premigratory NCCs may be unable to migrate from the neural tube during the period between migrations, either due to the environmental conditions or intrinsically. The goal of this project is to determine whether the neural crest specifiers Sox9, Sox10 and FoxD3 are expressed in premigratory NCCs that are not depleted at the end of first wave of migration and remain dormant until the second migration commences. Premigratory NCCs may be unable to migrate from the neural tube during the period between migrations, either due to the environmental conditions or intrinsically. The goal of this project is to determine whether the neural crest specifiers Sox9, Sox10 and FoxD3 are expressed in premigratory NCCs that are not depleted at the end of first wave of migration and remain dormant until the second migration commences. This set of genes has been shown to be required for premigratory NCCs to emigrate, and the lack of expression of one or more of these specifiers could be preventing them from exiting the neural tube. Elucidating the molecular control of migration in this unique population of trunk NCCs would further our understanding of its role in the development of the turtle shell. Funding sources: National Science Foundation, Millersville University

Program Abstract #170
Late-migrating neural crest cells in turtle embryos display strong osteogenic potential
Seth Martin¹, Jennifer Spengler¹,², Rebecca McLennan³, Matthew Smith¹, Paul Kulesa², Scott Gilbert³, Judith Cebra-
Turtle plastron bones develop by intramembranous ossification, suggesting that they are derived, like the facial bones, from neural crest cells. Using cell-labeling and neural tube explant cultures, we have shown that cells expressing neural crest markers emerge from the trunk neural tube in the turtle *Trachemys scripta* in two migratory phases over a greatly extended period. These findings were confirmed by electroporation of plasmids driving expression of fluorescent proteins. The early phase of migration (comparable to the period of neural crest cell (NCC) migration in other amniotes) extends until stage G10-11. These cells give rise to typical NCC derivatives. The NCCs that emerge late (beginning in stage G15-16 turtle embryos, well beyond the stage of neural crest migration in chick or mouse embryos) appear to migrate ventrally to form an ectomesenchymal dermis that gives rise to the bones of the plastron. Thus, there appear to be two distinct migratory phases in vivo. To test the hypothesis that the second wave of trunk NCCs in turtle embryos is capable of differentiating into bone, *T. scripta* neural tubes were cultured. The resulting NCCs were allowed to differentiate, and the differentiated cell types produced were analyzed by immunofluorescence. A subset of the cultures produced melanocytes, a typical trunk NCC-derived cell type, while virtually all of the cultures produced a substantial number of 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 between the bones of the skull. A better understanding of intramembranous ossification, and analysis of an enriched population of osteogenic NCCs, could result in improved treatment options. Funding sources: National Science Foundation, Millersville University, Stowers Institute

Program Abstract #171

**Orphan nuclear receptor, GCNF, is required for early neural crest cell induction and survival**

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Neural crest cells (NCC) are considered to be a vertebrate innovation that significantly contributed to the evolution, predation, radiation and adaptation of vertebrates to most niches of the planet. NCC comprise a unique vertebrate cell population that is frequently termed the “fourth germ layer” because they form in conjunction with the other germ layers and give rise to a diverse array of cell types and tissues including most of the craniofacial skeleton, peripheral nervous system, and pigment cells amongst many others. NCC development is dependent on gene regulatory network control of several cellular mechanisms including formation, migration and differentiation with defects resulting in clinical manifestations termed neurocristopathies. In contrast to aquatic and avian species, we currently have a very poor understanding of the factors that regulate mammalian neural crest cell formation. Here we describe molecular analyses of a mouse neurocristopathy model that revealed a critical role for the orphan nuclear receptor, *Germ cell nuclear factor (Gcnf/Nr6a1)*, in mammalian neural crest cell formation and survival. Comparison of null and conditional *Gcnf* mutant embryos indicates that *Gcnf* is required prior to E8.0 for proper neural crest cell formation. Furthermore, *Gcnf* functions as a bimodal switch to repress neural stem cell fate and promote the acquisition of neural crest cell identity. Thus our findings have identified a novel regulator of mammalian neural crest cell development and defined a temporal window for mammalian neural crest cell formation which is earlier than previously thought and raises important questions regarding the appropriateness of *Wnt1Cre* in studies of mammalian neural crest cell formation. Work in the Trainor lab is supported by the Stowers Institute for Medical Research and the National Institute for Dental and Craniofacial Research (DE016082). WAM was supported by the American Association of Anatomists Postdoctoral Fellowship.
Program Abstract #172
Characterization of a Dauer Constitutive Mutant with Dauer-Specific Molting Defect in the Nematode Pristionchus pacificus
Heather Carstensen, Reinard Villalon, Ray Hong
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Under harsh conditions, nematode larvae develop into a non-feeding, dispersal stage known as the dauer larvae (dauer). While several key genetic components controlling dauer development are conserved between the free-living C. elegans and parasitic nematodes, the pathways that coordinate dauer formation with host availability may have diverged. Specifically, unlike the C. elegans ‘naked’ dauer, the dauer of parasitic nematodes often retain their old J2 cuticles, likely to protect them from environmental stress between hosts, but is shed when a new host becomes available. These ensheathed dauer are also found in Pristionchus pacificus, a necromenic nematode associated with beetles. To explore the genetic link between the dauer-specific molt in parasitic nematodes and developmental and behavioral responses to host cues, we characterized the developmental phenotypes and genetic interactions of the P. pacificus dauer mutant tu391. We found that tu391 is a dauer-constitutive allele that not only forms dauers inappropriately, but the dauers also show an additional molting defect in which the old cuticle incarcerates the living dauer rather than binding closely with the new cuticle. Unexpected, this incarcerated phenotype lasts for only a day. Unlike non-dauer stages, the tu391 mutant dauer larvae are attracted to the beetle pheromone and are able to suppress the paralyzing effect of the host pheromone in a sensitized genetic background, suggesting that normal tu391 function mediates the development of key sensory neurons. These phenotypes are supported, in part, by defects in tu391 sensory neurons’ ability to take up the lipophilic dye Dil. Because P. pacificus development resembles both free-living and parasitic nematodes, further characterization of its dauer development may give us insight into how nematodes acquire pre-adaptations to parasitism. (NIH SCORE SC3 GM105579, 5R25GM063787-15)

Program Abstract #173
Evidence of developmental niche construction in dung beetles: effects on growth, scaling and reproductive success
Daniel Schwab, Sofia Casasa, Armin Moczek
Indiana University, USA
Much thinking in evolutionary biology is dominated by a mindset that views traits and trait variants as emergent properties of genes and genomes, and environments as strictly external to, separable from, and acting in a unidirectional manner on the organisms that develop within them. However, a growing literature has begun to demonstrate that organism-environment interactions are a well-integrated feature of normal development. Furthermore, it is now apparent that many organisms can actively and reciprocally modify their environments via their physiology and behaviors through the process of niche construction. During development, niche constructed modifications have the potential to bias phenotypic variation and enhance organism–environment fit. Yet, few studies exist that experimentally assess the degree to which environmental modifications shape developmental and fitness outcomes, how their influences may differ among species, and identify the underlying proximate mechanisms. Here, we experimentally eliminate environmental modifications from the developmental environment of three Onthophagus dung beetle species. We show that these modifications (1) differentially influence growth among species, (2) consistently shape scaling relationships in fitness-related traits, (3) are necessary for the maintenance of sexual dimorphism, (4) influence reproductive success among females of at least one species and (5) implicate larval cultivation of an external rumen as a possible mechanism for environmental modification. Our results present evidence that Onthophagus larvae engage in niche construction, and that this process is a fundamental component of normal beetle development and fitness. This study was carried out while DS was supported by a National Science Foundation Graduate Research Fellowship. Additional support was provided by the National Science Foundation IOS grants 1256689 and 1120209 to AM and through a grant from the John Templeton Foundation.
Program Abstract #174
Plasticity through canalization: The contrasting effect of temperature on trait size and growth in *Drosophila*
Samuel Gascoigne¹, Jeanne McDonald¹, Shampa Ghosh², Alexander Shingleton¹
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In most ectotherms, a reduction in developmental temperature leads to an increase in body size, a phenomenon called the temperature size rule (TSR). In *Drosophila melanogaster*, temperature affects body size primarily by affecting critical size, the point in development when larvae initiate the hormonal cascade that stops growth and starts metamorphosis. However, while the thermal plasticity of critical size can explain the effect of temperature on overall body size, it cannot entirely account for the effect of temperature on the size of individual traits, which vary in their thermal sensitivity. Specifically, leg and male-genital size show reduced thermal plasticity, while the wings show elevated thermal plasticity, compared to overall body size. Here we show that these differences in thermal plasticity among traits reflect differences in the effect of temperature on the rates of cell proliferation during trait growth. Counterintuitively, the elevated thermal plasticity of the wings reflects canalization in the rate of cell proliferation across temperatures. The opposite is true for the legs. These data reveal that environmental canalization at one level of organization may explain plasticity at another, and vice versa. This research was supported by NSF grants IOS-1406547 and IOS-0919855 to AWS.

Program Abstract #175
High water temperature during development disrupts morphogenesis, but not cell type specification in *Ciona intestinalis*
Steven Irvine, Rose Jacobson, Evelyn Siler
University of Rhode Island, USA

The range of temperatures at which marine animals can produce normal offspring is a major factor in their ecology, and can influence such parameters as their geographic range. Normal embryogenesis in the tunicate *Ciona intestinalis* falls off steeply at incubation temperatures above 23°C, which is within the normal range of water temperature experienced by animals in Rhode Island. In order to understand which aspects of development are most sensitive to high temperature, we have examined the defects that appear during development at high water temperatures. Notably, even in larvae that are severely deformed, pigment cells of the ocellus and statocyst, which appear late in embryogenesis, are present, indicating that this complex cell type specification pathway is not disrupted. Histochemical assays show that acetylcholinesterase and alkaline phosphatase expression are also present in the proper tissues. On the other hand, examination of the morphology of embryos deformed due to high incubation temperature reveals that the construction of the tail is particularly prone to disruption by high temperature, whereas the sensory palps resist disruption. These observations suggest that mechanisms involved in morphogenetic processes, such as cytoskeletal remodeling or assembly of extracellular matrix, are more sensitive to high temperature than cell type specification pathways. They also show that not all larval structures are equally sensitive to temperature during development. This study may point towards aspects of development that are either targets of, or constraints on, selection for successful reproduction at the high limit of environmental temperature.

Program Abstract #176
The SHARK gene cymric is truncated in the ascidian *Molgula occulta*
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Ascidians share several developmental features with the vertebrates including pharyngeal slits, an endostyle, and a notochord inside a larval tail. During typical ascidian development notochord precursor cells converge and extend to create a 40 cell notochord that becomes flanked by muscle making a swimming tail. The molgulids are a monophyletic clade of ascidians in which a tailless phenotype has independently evolved multiple times. We are searching for the molecular basis of this tail loss by investigating the differential gene expression of two species,
Molgula oculata and Molgula occulta. M. oculata has the tailed phenotype, but M. occulta has lost the tail and notochord, developing twenty notochord cells that do not converge or extend. We have sequenced the genomes and developmental transcriptomes of both species and are observing differential gene expression of notochord related genes to identify the developmental mechanisms responsible for tail loss. We first investigated cymric, a SHARK tyrosine kinase located in the myoplasm in M. oculata. Cymric is altered in M. occulta (Swalla et al. 1993); M. oculata makes the full transcript, but the M. occulta copy is missing the tyrosine kinase domain. Genome analysis confirms the transcriptome findings; the M. occulta cymric gene is on its way towards becoming a pseudogene like the pigment gene tyrosinase (Racioppi et al. 2017) and tail muscle structural genes (Kusabe et al. 1996). Our results suggest that the altered cymric is not the original mutation causing taillessness in molgulid ascidians, but rather a secondary effect observed in M. occulta so we are now investigating the differential effects of upstream genes such as T-box transcription factors and ERK 1 & 2 to identify the initial change causing the tailless phenotype. We would like to thank the BEACON: An NSF Center for the Study of Evolution in Action, and the University of Washington RRF grant for funding.
functional perturbation of genes expressed in the shell gland. The cell lineages that give rise to the shell gland are known, and we have screened transcriptomes for genes involved in shell patterning and biomineralization in embryos and larvae. Expression of the genes within the shell gland may reveal putative roles in biomineralization. These data form the basis for constructing a biomineralization gene regulatory network in a gastropod mollusc, allowing for comparative studies to be taken among other calcifying members in the Metazoa. They also provide a starting point for identifying how mollusc biominerals can respond and adapt at a developmental level to the inevitable environmental changes that the world oceans face in the coming century. Funded by U.C. Regents.

Program Abstract #179
The genetic programs underlying the fin-to-limb transition
Tetsuya Nakamura
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Understanding the evolutionary transformation of fish fins into tetrapod limbs is a fundamental problem in biology. The search for antecedents of tetrapod digits in fish has remained controversial because the distal skeletons of limbs and fins differ structurally, developmentally, and histologically. Moreover, comparisons of fins with limbs have been limited by a relative paucity of data on the cellular and molecular processes underlying the development of the fin skeleton. We provide a functional analysis, using CRISPR/Cas9 and fate mapping, of 5′ hox genes and enhancers in zebrafish that are indispensable for the development of the wrists and digits of tetrapods. We show that cells marked by the activity of an autopodial hoxa13 enhancer exclusively form elements of the fin fold, including the osteoblasts of the dermal rays. In hox13 knockout fish, we find that a marked reduction and loss of fin rays is associated with an increased number of endochondral distal radials. These discoveries reveal a cellular and genetic connection between the fin rays of fish and the digits of tetrapods and suggest that digits originated via the transition of distal cellular fates. We are currently discovering the genetic programs that had transformed distal fin rays into tetrapod digits and wrists by using functional genomics and genetics.

Program Abstract #180
The limb development program is restricted to the mesenchymal compartment in salamanders
Sruthi Purushothaman, Ashley W Seifert
University of Kentucky, USA
Limb organogenesis is an ideal model to investigate how cellular and molecular networks have developed during tetrapod evolution. Comprehensive studies in chicken and mouse embryos have led to an integrative limb development model for vertebrates. Urodeles, however, appear to deviate from key parts of this model. For instance, they undergo pre-axial skeletal specification and do not develop an AER. Expression analysis of key limb development genes has not been extensively studied and where it has been, results from the literature are conflicting. The present study explores the axolotl limb with regards to spatiotemporal expression of key developmental genes that set up the anterior-posterior and dorsal-ventral axes. Whole mount in-situ hybridizations (WISH) were done on developing axolotl limb buds at 6 morphologically defined stages. Sectioned limbs were used to define cellular expression in ectodermal and mesenchymal compartments along with regionalization along the anterior-posterior and dorsal-ventral axes. We also used real time PCR to validate the observed spatial expression patterns in the limbs. Almost all genes examined were restricted to the mesenchyme in developing limbs, including Fgf ligands normally expressed in the mouse and chicken AER. Shh expression, which is normally restricted to the zone of polarizing activity in the posterior margin of tetrapod limbs, exhibited bipolar expression in an anterior and posterior zone. We also tested the role of mesenchymally restricted Fgf8 during limb outgrowth using the Fgf-receptor inhibitor SU5402 and found the resultant limb phenotype closely resembled Msx2cre;Fgf8 knockout mice. While our findings support conservation of a common genetic limb program in tetrapods, they also demonstrate that this molecular machinery has shifted in Urodeles such that it resides predominantly in the limb mesenchyme. Funding sources: University of Kentucky
Program Abstract #181
Uncovering Genetic Regulators of Hindlimb Morphology in the Domestic Pigeon
Emily Maclary, Elena Boer, Rhiannon Turner, Michael Shapiro
University of Utah, USA

Morphological variation has major impacts on biodiversity and health, affecting behavior, mate choice, and evolutionary fitness. Despite this importance, the genetic basis of morphological diversity is largely unknown, and remains a central question in developmental biology. Both wild and domesticated birds show extraordinary morphological diversity, from variation in color and size to the development of novel constructive traits. The domestic pigeon, *Columba livia*, is an exceptional species for genetic analysis of morphological changes. Extensive selective breeding has given rise to hundreds of breeds prized for unique anatomical traits. One such trait, foot feathering, is seen in both wild and domestic avian species. The conversion from scaled epidermis to feathered epidermis on the foot in the domestic pigeon is mediated in large part by cis-regulatory changes at two genes, *Pitx1* and *Tbx5*. Intriguingly, foot feathering is also associated with changes in musculoskeletal patterning in the hindlimb. Here, we identify and characterize putative cis-regulatory variants at the *Pitx1* and *Tbx5* loci and assess phenotypic variation in musculoskeletal patterning, using diverse pigeon breeds and F2 offspring from founders with divergent foot feathering phenotypes.

Program Abstract #182
Developmental mechanisms underlying forelimb heterochrony and reduction in the emu *Dromaius novaehollandiae*
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The salient feature of birds is powered flight, however, flight has been lost multiple times in the evolution of the avian lineage, frequently in the Ratites. Accordingly, flight-loss results in multiple changes to the avian body such as robust legs, digit loss, and reduced wings. While there is a wealth of comparative anatomical knowledge among these animals, the underlying genetic mechanisms that result in these changes remain unclear. The emu (*Dromaius novaehollandiae*) has a reduced wing with heterochronic development and outgrowth. This delay means that tissues need to be coordinated differently in the emu embryo relative to other amniotes to build a limb. Here, we use a combination of embryological, genetic, and genomic techniques to determine the mechanisms that result in the emu forelimb heterochrony. We find that *Tbx5* and *Fgf10* are expressed in the lateral plate mesoderm (LPM), yet *Fgf8* fails to be expressed in the ectoderm. A precocial limb can be induced in the emu via transplantation of chick LPM or exogenous FGF8b. Furthermore, over-expression of *Fgf10* is sufficient to drive precocial *Fgf8* expression and result in limb outgrowth. These results suggest a mechanism where *Fgf10* levels in the emu are sufficient to drive an epithelial to mesenchymal transition to allow muscle cells to migrate, but insufficient to induce limb growth at equivalent stages as the chick. Recently, it was reported that the heart marker *Nkx2-5* is ectopically expressed the emu forelimb (Farlie et al. 2017). RNA-seq and ATAC-seq analyses revealed several additional heart markers and a signature of cardiac differentiation in the forelimb. Subsequent experiments suggest that deficient signaling in the LPM results in insufficient induction of somatopleure in the emu forelimb resulting in an activation of the cardiac program. Taken together, these results present a molecular mechanism underlying the observed cardiac signature of the emu forelimb and the resulting forelimb heterochrony.

Program Abstract #183
3D morphological analysis of the developing spiral intestine in the little skate, *Leucoraja erinacea*
Nicole Theodosiou, Emmanuela Oppong
Union College, USA

The organs of the digestive tract have evolved differences in function and shape depending on an animal’s diet and habitat. While the stomach appeared as an adaptation to store food in intermittent feeders, the morphology of the intestines has also changed. Unlike most vertebrates that have long coils of intestines to assist in absorbing...
nutrients from high protein diets, elasmobranchs have short intestines that form an internal spiral valve. The spiral valve structure allows elasmobranchs to have a large surface area for absorption encased within a short run of intestine. The spiral valve intestine is unique to all basal fishes including the lamprey of the jawless superclass Agnatha, the jawed cartilaginous fishes Chondrichthyes, and the sarcopterygians (including coelacanths, lungfish) of the Osteichthyes. Teleosts, however, lack a spiral valve intestine. Thus, the spiral intestine represents an early feature in the evolution of vertebrates, and provides a great model for investigating the evolution of morphology.

To better understand formation of the spiral valve intestine, we have generated a developmental time series of 3D models from micro-CT scan sets of a cartilaginous fish the little skate, *Leucoraja erinaceae*. The spiral initiates as a single indentation after the gut tube has formed. The number of spiral turns or folds increases during development in a reiterative and reproducible pattern. The spiral pitch and helical angles differ from rostral to caudal ends within the intestine, and these profiles change with developmental stages. Analysis of the 3D structure of the spiral valve intestine during development provides insights into possible mechanisms that facilitate spiral formation. This work was supported by NSF-MRI award #1531850 and the Union College MakerWeb Consortium.

**Program Abstract #184**

**Development and evolution of pigmentation in Betta splendens**

*Belinda Sly*

*Transylvania University, USA*

Perhaps one of the most striking features of animal morphology is the presence of diverse colors. Among vertebrates, teleost fish exhibit some of the most varied and brilliant pigmentations. Five different pigment cell types have been identified in teleosts: brown to black melanocytes, yellow xanthophores, red erythrophores, reflecting iridophores, white leucophores, and blue cyanophores. The genetics of pigmentation via melanocytes has been well-studied in zebrafish and shows many similarity to that in mammals. Less is known about the genetics of the other pigmentation cell types. In an effort to learn more about the evolution of the molecular and cellular basis of coloration we have begun a research program that employs *Betta splendens* as a model system.

We have determined that pairs of *B. splendens* can be reliably bred to produce adequate numbers of large, transparent larvae for pigmentation analysis and that differential pigmentation phenotypes show up as early as two days post fertilization. Furthermore, our identification of melanophores, iridophores, xanthophores, and erythrophores in *B. splendens* juveniles support our assertion that this organism is a good model for studying the development of a wide variety of colorations. The complex patterns we observe during adult pigmentation development could shed light how such varied colorations are achieved. We are in the process of sequencing and annotating the *B. splendens* genome as well as analyzing RNAseq data comparing RNA expression in blue, white, and red *B. splendens*. Current results will be presented. Funding: David and Betty Jones Faculty Development Fund, Dean Laura Bryan of Transylvania University, and GCAT-SEEKquence Consortium.

**Program Abstract #185**

**How to paint a butterfly? The developmental basis of butterfly color pattern variation**

*Erick Bayala, Nicholas VanKuren, Darli Massardo, Kenneth Barr, Marcus Kronforst*

*University of Chicago, USA*

The association of color patterns with the biology of animals has been an important topic of research in genetics, ecology, and evolution for decades. Extensive work in butterflies has yielded a list of candidate genes that are associated with specific color patterns and the evolution of color polymorphism (Van Belleghem, et al., 2017). However, despite the identification of numerous candidate genes, few studies have focused on how these loci actually produce color patterns during development. Furthermore, the developmental mechanisms responsible for establishing color patterns and the developmental basis of color pattern diversity remain obscure. *Heliconius* butterflies are an ideal clade in which to explore the developmental mechanisms of color patterning based on the multiple genes that have been identified as candidates associated with specific colors (Van Belleghem, et al., 2017). The yellow/white wing patterns and switches between these colors have been crucial for speciation events...
via mate preference differences and also to the mimicry of other non-palatable butterfly models (Chamberlain, et al., 2009). Studies in the Kronforst lab on Heliconius cydno identified aristless1 (al1) as the candidate gene and functional switch between yellow and white variants. Here I want to discuss my work on identifying the pattern of expression of al1 in white and yellow Heliconius butterflies. In addition, I will present functional results of al1 knockdowns. Finally, I will describe my preliminary work focused on understanding the regulatory environment and possible upstream pathways of al1. Coupling this information together will lead to understanding the developmental events associated with al1 and how these events lead to the specification and formation of yellow and white color patterns in Heliconius butterflies. Funding: Initiative for Maximizing Student Development (NIGMS R25GM109439), Developmental Biology Training Grant (T32 HD055164) and NSF (IOS-1452648).

Program Abstract #186
Inspecting the role of the trans-regulatory landscape to the origin, diversification, and loss of a sexually dimorphic fruit fly pigmentation trait
Jesse Hughes
University of Dayton, USA
A major goal for evolutionary-developmental biology research is to identify the genetic changes underlying the origins, diversification, and loss of morphological traits. Such traits are built by the spatial and temporal regulation of gene expression, and thus the evolution of gene expression is often involved in their evolutionary histories. Gene expression is under the control of a network of transcription factors (trans-landscape) that ultimately impinge on the cis-regulatory elements (CREs) that control the expression of differentiation genes whose encoded proteins produce particular traits. Transcription factor genes are often highly pleiotropic, and therefore it seems reasonable to expect that evolutionary changes in gene expression are more frequently due to mutations altering the CREs for differentiation genes than the trans-landscape. Our research aims to test whether this expectation for a conserved trans-landscape applies to the origin, diversification, and loss of a well-studied fruit fly pigmentation trait in the Sophophora subgenus. The origin of a male-specific pattern of abdominal pigmentation involved the gain of CREs controlling the expressions of pigmentation enzyme genes responsive to the prevailing trans-landscape of body plan patterning and sexual dimorphism transcription factors. Here, we share our results from tests of these CREs in transgenic hosts that represent the ancestral sexually monomorphic trait, diverse forms of the derived dimorphic trait, and a secondary loss of the dimorphic trait. The outcomes will reveal the extent to which this particular trans-landscape has remained conserved while the pigmentation phenotype has widely evolved. The results are likely to bear upon the evo-devo of animal form. This project has received funding from the National Science Foundation as grant to TMW (IOS-1555906) and a Graduate Research Fellowship to JTH (DGE-1439647).

Program Abstract #187
The regulation of the tan gene in the evolution of complex wing and abdominal color patterns in Drosophila guttifera
Komal K. B. Raja, David Trine, Catrina Latuszek, Nathaniel Maki, Timothy Massoglia, Rebecca Hobmeier, Jackson Waugh, Thomas Werner
Michigan Technological University, USA
How changes in gene expression drive the evolution of complex morphological patterns is an intriguing question in developmental biology. However, the mechanisms that generate these complex patterns remain largely unknown. Here we sought to identify the genetic mechanisms that induce the pigmentation gene tan in complex expression patterns on the wings (16 spots) and abdomen of Drosophila guttifera. The abdominal color pattern of this species consists of four distinct sub-patterns: one pair of dorsal, median, and lateral rows of black spots, plus a dark dorsal midline shade on a brownish background. The pigmentation gene tan is necessary for the formation of brown pigment in insects. Using in situ hybridization, we show that tan is expressed in patterns resembling the spotted adult wing and body color pattern of D. guttifera. Using a transgenic reporter assay in D. guttifera, we found two important cis-regulatory elements (CREs) of the tan gene: one being active where all the black spots
will form on the wing, and one where the six longitudinal spot rows will form on the abdomen. We have previously shown that the yellow gene, which is responsible for making black melanin, also contains two CREs that mimic the tan gene CRE expression patterns on the wings and abdomen. In a sequence comparison of the yellow and tan wing and abdominal CREs, we found that the CREs of both genes contain similar putative transcription factor binding sites for predicted regulators of color patterns. These results provide a first example of how complex morphological traits may be regulated through the parallel coordination of two downstream target genes. This project is funded by NIH grant 1R15GM107801-01A1.

Program Abstract #188
The regulation of the yellow gene in the evolution of a complex abdominal color pattern in Drosophila guttifera
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Michigan Technological University, USA
Changes in the expression of developmental genes play an important role in morphological evolution. However, the genetic and molecular mechanisms that generate complex morphological traits during animal development remain largely unknown. Here we sought to identify the molecular mechanisms underlying the formation of the complex abdominal color pattern of Drosophila guttifera. The color pattern of this species consists of four distinct sub-patterns: one pair of dorsal, median, and lateral rows of black spots, plus a dark dorsal midline shade on a brownish background. The pigmentation gene yellow is necessary for the formation of black pigment in insects. Using in situ hybridization and immunohistochemistry, we show that yellow mRNA and Yellow protein are expressed in patterns very closely resembling the adult color pattern. Using a transgenic fluorescent reporter assay, we identified one cis-regulatory element (CRE), which drives gene expression comprising all six rows of spots in the epidermis of the developing abdomen. Further sub-divisions of this CRE and subsequent transgenic reporter analyses revealed the core CRE to be a stripe-inducing element, when a cluster of putative repressor (Engrailed-binding) sites was excluded from the CRE. We compared the activity of the D. guttifera CRE to the orthologous sequence from Drosophila deflecta, a species that shows a similar spotted abdominal color pattern.
We found that the core CREs of both species drive abdominal stripe expression patterns in the transgenic D. guttifera background, thus suggesting that spot patterns on fruit fly abdomens may have evolved from an ancestral stripe pattern through partial repression of portions of the stripes. Adult abdominal pigment patterns of closely related species confirm this mechanism by displaying color pattern variations from stripes to spots with all intermediate stages. This project was funded by NIH grant 1R15GM107801-01A1.

Program Abstract #189
Signal to shape: how alterations to signaling pathway activity generates newly evolved morphology
Sarah Smith, William Glassford, Winslow Johnson, Mark Rebeiz
University of Pittsburgh, USA
The development of anatomical form is multifaceted, involving both the patterning of gene expression and the morphogenesis of tissues at the cellular level. However, our understanding of how these two processes are integrated remains unclear. Studies of rapidly evolving anatomical structures address this question by providing examples of genetic alterations that can be connected to their naturally occurring cellular effects on morphogenesis. We examined the posterior lobe, a recently evolved appendage-like structure on the genitalia of members of the Drosophila melanogaster clade. During posterior lobe development, expansion of unpaired (upd), a ligand of the JAK/STAT pathway, is observed in species that develop this structure. We characterized the regulatory region of upd and uncovered a posterior lobe enhancer. Through CRISPR/Cas9 deletion of this enhancer, we found that it is vital for expression of upd in the posterior lobe and required for proper lobe development. To investigate how expansion of JAK/STAT signaling contributed to posterior lobe development, we measured its cellular morphology and found that the posterior lobe forms through elongation of cells along their apico-basal axis. We have identified a cellular effector, short stop (shot), which is highly patterned during posterior lobe development and may directly contribute to the elongation of cells within the posterior lobe. In
addition, we have determined that shot is regulated by the JAK/STAT pathway in the cells of the posterior lobe. This work highlights the complexity of development by linking the expanded expression of a signaling pathway ligand with a novel morphogenetic process through the activation of a cellular effector. Funding provided by the NIH.

Program Abstract #191
A Sox gene is a key player in the spider segmentation gene regulatory network
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The Sox gene family encodes a HMG-containing domain similar to the SRY (Sex-Determining Region Y) of eutherian mammals. These genes regulate many processes during embryogenesis in metazoans. In insects, Dichaete is the only Sox gene known to be involved in segmentation. To determine if similar mechanisms are used in other arthropods, we investigated the role of Sox genes during segmentation in the spider Parasteatoda tepidariorum. While Dichaete does not appear to be involved in spider segmentation, RNAi knockdown of Sox 21-B1 (a Drosophila Sox 21-B orthologue) perturbed the sequential addition of opisthosomal segments and the expression of segmentation genes. Thus, we have found that segmentation in other arthropods is also regulated by a Sox gene, but that spiders employ a different gene from insects. Our work provides new insights into the function of an important and conserved gene family across arthropods and the evolution of the development of these animals. Funded by: CNPq Brazil

Program Abstract #192
Investigating sex-specific expression of five doublesex genes in the common house spider, P. tepidariorum
Jessica Petko
Penn State York, USA
There is an astonishing variety of methods by which animals govern sex. Sex determination mechanisms generate differences in gonad development and various other dimorphisms exhibited by males and females. In arthropods, sex is determined genetically, and for many species this relies on the dosage of the X chromosome. In Drosophila, a double dose of the X chromosome results in early accumulation of a special group of RNA splicing factors. These regulators determine a female-specific cascade of splicing events that culminates in differential activation a transcription factor called doublesex (DSX). Alternative splicing of DSX serves to activate sex-specific genes. Since diverging from their last common ancestor with insects, spiders have incurred a whole genome duplication. It is unknown what effect this genome duplication has had on the evolution of sex determination mechanisms in this lineage. A search of the common house spider (P. tepidariorum) transcriptome revealed five DSX paralogs, several of which display alternative splicing. The purpose of this study was to analyze the evolution sex specific expression/splicing of spider DSX transcripts. RT-PCR was used to demonstrate that all of the DSX paralogs are expressed in male and female adult spiders at approximately equal levels. There is, however, expression of alternative splice variants during particular stages of embryonic and postembryonic development, that is not seen in the adults. The sex of juvenile spiders cannot be determined until after several molts when male pedipalps form, and therefore, it has been difficult to discern whether the embryo-specific splice variants are also sex-specific. Future studies aim at identifying sex earlier in the development process to determine whether the alternative splice variants are specific to one sex. This project was funded by a Research Development Grant awarded by Penn State York.

Program Abstract #193
FET-1 in avian sex determination
Chiron Loubser, Natalya Nikitina
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The mass culling of male chicks of egg-laying breeds is ethically and financially questionable. This practice is estimated to cost close to $1 billion worldwide. Understanding sex determination in birds is essential to the
development of an all-female chicken breed, which would eliminate the need for mass culling. In birds, where females are ZW and males are ZZ, sex determination is hypothesised to be the result of either a W determinant or Z dosage. One of the few candidate W determinants is the Female Expressed Transcript -1 (FET1), a seemingly attractive candidate, previously localized to the W chromosome with female specific expression. The aim of this research was to further investigate the function of FET1 and its potential role in avian sex determination. RT PCR on extracted RNA from E4.5 gonads confirmed FET1 expression. Unexpectedly, expression was significantly higher in females than in males. RT PCR and a nucleotide blast of FET1 against the chicken genome suggested multiple FET1 integrations on the Z chromosomes and/or autosomes, consistent with retroviral elements such as FET1. Using RACE (Rapid amplification of cDNA ends) two lincRNA populations were identified that overlapped along the FET1 transcript. RNA expression was observed for both of the lincRNA populations as well as the FET1 protein coding sequence at E4.5 and E6.5 embryos; stronger gonadal and epidermal staining observed in females compared to males, suggesting a sex specific role. Whole mount IHC using a rabbit polyclonal anti-FET1 antibody showed the presence of FET1 protein in the epidermis but not the gonads. Collectively our data suggests that FET1 is not likely a W determinant; however predominant expression in female suggests a role in the development of sex specific characteristics. Ethical clearance provided by Wits Animal Ethics Committee (permit number 2017/01/01/0) and funding provided by the National Research Foundation of South Africa (grant number 105821 to N Nikitina).

Program Abstract #194

Developmental evolution of mammalian pregnancy
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While changes characterizing the female menstrual cycle and establishment of pregnancy are not generally thought of as a classical developmental system, they share multiple processes with basic development. Decidualization, for example, is a differentiation of endometrial stromal fibroblasts (ESF) into decidual stromal cells (DSCs). DSCs evolved in the Eutherian lineage and are essential for the initial establishment of pregnancy along with subsequent reprogramming of the endometrium, fetal immunotolerance and invasive placentation. To identify and characterize the genetic, cellular and molecular changes that underlie the evolution of decidualization, I use a cell culture-based model. TERT-immortalized human ESFs in culture decidualize into DSCs upon treatment with medroxyprogesterone acetate and cAMP, recapitulating the process occurring during normal initiation of pregnancy. Using siRNA knockdown in ESFs followed by RNAseq analysis, I screened multiple transcription factors (TFs), whose uterine expression was gained in Eutherians. The results show that a significant proportion of downstream affected genes are involved in cell cycle, senescence and stress response pathways. I am extending this research by studying the roles of screened TFs in more detail using CRISPR-Cas9 stable knockouts and investigating the secretory profile and the cell cycle status of DSCs. These experiments will be informative about the potential of decidualization to produce an inflammatory-like environment and are relevant in the broader context of preterm birth in humans. I am also in the process of establishing a culture model of 3D uterine organoids from human primary tissue and perturbing gene expression in them. These studies will shed additional light on the changes taking place in uterine tissue, expanding our knowledge on developmental and evolutionary specificities of human pregnancy. Funding source: March of Dimes Prematurity Research Center Grant

Program Abstract #195

Convergence and divergence: The story of placenta evolution as told by Poeciliopsis fishes
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The placenta is an essential organ that connects mother and fetus during pregnancy, allowing for proper embryonic development. Gene expression studies of mammal placentas reveal convergence at a molecular level,
but we understand little about how this extends to other vertebrate placentas. Here we present some of the first work exploring this in the live-bearing fish genus, *Poeciliopsis*. This genus of fish has three independent origins of placentation, a stark contrast from mammals where the placenta evolved once. This provides a unique opportunity to study how similar the molecular basis of placenta establishment is in a system where placentation evolved convergently, not only from mammals but also from closely related species. *Poeciliopsis* placentas have a maternal follicle and a fetal epithelium where we find expression of mammalian placenta genes in their cognate tissue types, suggesting molecular convergence. Most notably we find expression of prolactin in the maternal follicle of placental *Poeciliopsis* species. This pattern mirrors prolactin expression in mammal placentas, suggesting convergence in placenta Prl regulation. Though we observe convergence in gene expression, placentas display stunning amounts of morphological diversity, but understanding how this is coordinated at the molecular level is difficult because this divergence exists between different species. To overcome this barrier, we are studying natural populations within a single species, *Poeciliopsis retropinna*, that has colonized many different Costa Rican rivers. Preliminary studies indicate that these populations vary in their placentation. We sequenced placenta transcriptomes of these populations to identify genes that change expression to allow for rapid changes in placentation. We believe these differences within the same species will provide new insight into the rapid divergence of placenta morphology between species. NSF GRFP and EDEN research exchange grants support this work.

**Program Abstract #196**

**The cholesterol synthesis pathway regulates erythropoiesis by discrete molecular mechanisms**

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Erythropoiesis is the conserved process by which new red blood cells (RBCs) are formed. Erythropoiesis is tightly regulated as defects in this process can lead to human disease. The formation of new RBCs is regulated in part by the gata family of transcription factors, each of which modulate the step wise differentiation into a mature RBC. However, the upstream mechanisms that regulate the expression of specific gata genes are not completely characterized. Cholesterol is known to be an integral part of the RBC membrane and is one potential mediator of gene expression because it forms lipid rafts and is a precursor to steroid hormones. In addition, defects in cholesterol synthesis cause defects in RBC differentiation. Thus, we hypothesized that mutations in *hmgcs1*, the gene that encodes the first enzyme in the cholesterol synthesis pathway interfere with RBC maturation. To test this hypothesis we analyzed the number and differentiation of RBCs in a zebrafish harboring mutations in the *hmgcs1* gene. We show that inhibition of cholesterol synthesis pathway results in decreased numbers of RBCs. Moreover, the targeted inhibition of isoprenoids and cholesterol, the two major products of the pathway, via pharmaceutical inhibition, suggests that each branch of lipids regulates RBC differentiation, but through distinct molecular mechanisms. Most notably, we demonstrate that the synthesis of isoprenoids is essential for the expression of *gata1*, a key modulator of RBC specification, whereas, cholesterol regulates RBC development by a *gata1* independent mechanism. Collectively, our results reveal two novel upstream regulators, cholesterol and isoprenoids, of erythropoiesis. This work was supported by NIMHD Grant no. 2G12MD007592 and NINDS Grant no. NS099153-01A.

**Program Abstract #197**

**Investigating the relationship between cell cycle arrest and development in anoxia-tolerant zebrafish**

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Zebrafish embryos can tolerate anoxic conditions (absence of oxygen) by arresting development and the cell cycle. Survival in this arrested state is remarkably long, lasting up to 50 hours for sphere-stage embryos. Furthermore, this arrest has been shown to be reversible, with embryos resuming development and the cell cycle upon the return of oxygen. The arrest of development and cell cycle promote the induction of a hypometabolic state, that preserves ATP. The signaling mechanisms that mediate oxygen-sensing and that orchestrate arrest in
anoxia-tolerant organisms are for the most part unknown. Understanding the molecular underpinning of anoxia-induced arrest will be paramount in developing treatments and therapies to combat hypoxia-related injuries such as acute ischemic stroke (AIS), emphysema, and chronic kidney disease. Here we aim to characterize the process of anoxia-induced developmental arrest. Preliminary data suggest that cell cycle arrest is a key event that significantly delays the morphological progression of development. Furthermore, we have demonstrated that blocking translation arrests the cell cycle and delays development. Interestingly, AMP-activated Protein Kinase (AMPK), a molecule implicated in the metabolic adaptation to low oxygen, is rapidly activated in zebrafish embryos exposed to anoxia and has been shown to block cell division (Mendelsohn et al., 2008). We hypothesize that AMPK functions via mTOR inhibition to block initiation of protein translation and prevent cell cycle progression under low oxygen. Data supporting this hypothesis will be presented.

Funding sources: UMBC Department of Biological Sciences, Department of Defense Grant #RT150086

Program Abstract #198
Developmental plasticity, thyroid hormone signaling and the evo-devo of bite mechanics in the zebrafish and other minnows
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This project seeks to understand how both thyroid hormone levels and diet affect variation in the development of fish bite mechanics. The minnows (Family Cyprinidae) comprise a lineage of fishes with highly kinetic linkages between the bones of their skulls. Work with the zebrafish suggests that: 1) the most dynamic movements of minnow skulls do not arise until post-larval development; and 2) a spike in thyroid hormone production during the larva-to-juvenile transition appears necessary for the development of their adult skull mechanics. Thyroid hormone signaling has large effects on skeletal remodeling and the bite mechanics of many fishes can be profoundly altered if different mechanical forces are exerted on their developing skulls. We sought to determine if thyroid hormone levels affect the plasticity of post-larval skull remodeling in the zebrafish. Jaw linkages that are efficient at producing either force or speed lie at opposite ends of a mechanical spectrum. Starting at 30 days post-fertilization we exposed fish with high, low and normal hormone levels to nutritionally identical treatments that required them to use either forceful or rapid bites throughout their post-larval development. We collected a developmental series from each treatment and quantified both skull shape (using geometric morphometrics) and bite kinematics (using high-speed video) for each series through at least 6 months of treatment. We then compared the variation in bite mechanics induced by these treatments to variation in feeding kinematics that has evolved during the diversification of the minnow lineage to which the zebrafish belongs (Subfamily Danioninae).

Program Abstract #199
Airway cartilage patterning and the development of the avian vocal organ
Evan Kingsley, Clifford Tabin
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Despite the presence of vocal communication in all major tetrapod clades, surprisingly little is known about the evolution and development of vocal structures. Nowhere is this gap in our understanding more evident than in the case of the avian vocal organ, the syrinx. Present only in birds, the syrinx is the site of all avian vocal production, but its development is critically understudied. The syrinx is located at the junction of the trachea and bronchi and it is not homologous to the larynx, which is the organ of nearly all non-avian tetrapod vocalization. The lack of larynx-syrinx homology and the unique anatomical location of the syrinx raise several questions regarding the evolution of novel structures and the evolution of developmental patterning mechanisms. Here, we investigate the development of the syrinx in the context of airway cartilage patterning. The periodic pattern of cartilage in the trachea and bronchi is broken up by the larger modified cartilage elements of the syrinx, which provides an opportunity to identify the molecules that generate and orient the pattern by studying how the ancestral pattern is disrupted. We demonstrate two novel roles for Hedgehog signaling in the patterning of airway cartilage: first, we show how temporally dynamic gene expression allows formation of the modified syrinx
cartilages, and second, how a gradient of Hedgehog activity orients airway cartilage elements. In addition to defining new roles for Hedgehog signaling in the developing tetrapod airway, this work represents early steps toward understanding the developmental changes that underlie the evolution of the avian syrinx. This work is funded by Gordon and Betty Moore Foundation.

Program Abstract #200
Comparative transcriptomics reveals conserved loci of GRN interaction underling the development of skeletal cells
Patsy Gómez Picos, Katie Ovens, Amir Ashique, Ian McQuillan, Brian Eames
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Comparative transcriptomics can help to reveal gene regulatory networks (GRNs) underlying a given biological process, but potential interactions between distinct GRNs can complicate understanding of their evolution and organization. In order to provide insight into GRN interaction, here we focus on transcriptional control of skeletal cell differentiation. Skeletal cells are a good model to test GRN interaction since they are distinct, but they also share many features. Here, we hypothesize that evolutionary conserved mechanisms of GRN interaction between immature chondrocytes (IMM) and osteoblasts (OST) GRNs direct the differentiation of mature chondrocytes (MAT). To analyze GRN interaction globally in an unbiased manner LCM-RNA-seq was used. RNA was isolated from specific skeletal cells from the mouse and the chick embryos, and then data was analyzed bioinformatically. Several findings supported the hypothesis. In both species, MAT expressed fewer differentially expressed genes than the other cell types, consistent with the idea that overlapping actions of the IMM and OST GRNs regulate MAT. With the goal of understanding how changes in GRNs underlie skeletal cell evolution, gene co-expression network (GCN) analysis was used to estimate skeletal cell GRNs. This analysis showed that in both mouse and chick, two independent GRNs driving IMM and OST overlap partially in MAT. Moreover, MAT contributed several genes to both IMM and OST GRNs, whereas genes enriched in IMM and OST were located in opposite networks. Finally, model-based cluster analyses revealed that GRNs present in IMM and OST interact via synergism and averaging in MAT, leading to specific gene expression patterns in this cell type, and these mechanisms of GRN interaction are conserved in distinct vertebrate clades. These results outline a novel experimental system through which to understand GRN interaction and organization in order to elucidate evolutionary origins of cartilage and bone.

Program Abstract #201
The muscle-less jerboa foot as a novel system to understand muscle degeneration
Mai Tran, Rio Tsutsumi, Joel Erberich, Michelle Flores, Kevin Chen, Hannah Grunwald, Kimberly Cooper
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Since the first terrestrial vertebrates crawled out of water 350 million years ago, natural selection has shaped limbs in extraordinary ways for diverse functions. For example, mouse-like quadrupedal rodents have muscles in their hands and feet that enable grasping and climbing. In contrast, the three-toed jerboa (Jaculus jaculus), a close relative of the laboratory mouse, can no longer climb and instead has dramatically elongated lever-like hindlimbs as an adaptation to leap bipedally through open desert habitats. The jerboa has all of the muscles in the thigh and calf that facilitate jumping but entirely lacks muscles distal to the ankle. Surprisingly, newborn jerboas have fully differentiated muscles, suggesting that muscle migration and patterning are normal in early development. Muscle fiber loss is initiated and completed within a few days after birth, but we are unable to detect any evidence of apoptotic or necrotic cell death by a variety of methods. How are muscles specifically lost in the jerboa foot while more proximal muscles are maintained? Our research indicates that the immature contractile apparatus is disassembled in a stereotyped manner that correlates with increased expression of the Murf1 and Atrogin-1 E3 ubiquitin ligases, similar to muscle atrophy. However, the short-term effect of atrophy in adult mice and in humans is to reduce myofiber size, but not number. We are currently performing lineage-tracing experiments to determine if differentiated myofibers are transformed to another cell type, or if their loss is due to an unusual mechanism of cell death. Since natural selection serves to modify existing developmental mechanisms, muscle
loss in the jerboa foot may serve as a model to better understand the cellular processes of muscle maturation and degeneration in a variety of contexts. Cell & Molecular Genetics training grant (T32GM724038).

**Program Abstract #202**

**A comparative study of eye development in Branchiopoda (Anostraca, Notostraca, Spinicaudata, Cladocera)**

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We propose that evolutionary genetic changes to Branchiopoda led to a variety of eye forms, and members of the class with two eyes exhibit ancestral morphology while those with a single eye exhibit more recent morphology. We have established profiles of staged eye development for *D.magna*, *T. longicaudatus*, and *A. franciscana*. Further evidence supporting our proposal is found in the current phylogenetic trees and in trees which we have assembled using a gene involved in eye development. Building on this understanding of the relationship between phylogeny and eye form, we will investigate the evolutionary genetic process that led to cyclopia in Branchiopoda. Our current focus is to determine what differences in the hedgehog gene exist between the cyclopic *D. magna* and the other orders within the class that show imperfect or no fusion of the eyes. In this way, we are taking advantage of natural evolutionary relationships to discern the origin of cyclopia. We have studied *Daphnia magna*, a member of Cladocera and a full cyclops; a spinicaudatan clam shrimp with eyes “on the way” to cyclopia; *Triops longicaudatus*, two eyes; and *Artemia franciscana*; two eyes widely separated on lateral eye stalks. We propose two possible mechanisms for hedgehog involvement in *D. magna* cyclopia. First, a partial loss of hedgehog function in *D. magna* compared to closely related two eyed branchiopods by a mutation causing a reduced function of the hedgehog protein. A second proposed mechanism is lowered expression levels of the protein. Furthermore, both mechanisms may conspire to cause cyclopia. To investigate our first hypothesis, we have identified and analyzed key amino acid residue changes unique to the *D. magna* hedgehog protein that could cause reduced function. We are modeling how these amino acid changes may affect protein function. To address the second proposed mechanism, we are measuring hedgehog gene expression levels throughout *D.magna* development using RT-qPCR. Funding: URGO

**Program Abstract #203**

**Cis-regulatory control of Onecut1 in a fate-restricted retinal progenitor cell population**

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Many diurnal vertebrate animals have a greater number of cone photoreceptors in the retina, to allow for sufficient visual acuity in the daytime. In contrast, nocturnal vertebrates often have fewer cone photoreceptors and more rods. Variation in the compositions of diurnal and nocturnal vertebrate photoreceptor layers may have been produced by modifications at the cis-regulatory elements of retinal development genes. The gene Onecut1 (OC1) is expressed in a population of retinal progenitor cells (RPCs) that are restricted to either the cone photoreceptor fate or horizontal cell fate. This TF binds to an enhancer called ThrbCRM1, which reliably marks this fate-restricted progenitor population (Emerson et al., 2013). However, it is unknown which upstream factors are required for the expression of OC1 in cone/HC restricted progenitors. Gain or loss of expression of OC1 in the retina results in an increase in the number of cone cells or rod cells, respectively. This suggests that the expression of OC1 during development could act as a molecular switch to control the ratio of rod and cone photoreceptors that are generated. Here, we conduct a screen of non-coding DNA elements flanking the Onecut1 gene to identify a cis-regulatory element active in Onecut1(+) restricted RPCs in the early chick retina. We use reporter assays to determine enhancer activity, EdU as a progenitor marker, flow cytometry for quantification and lineage-tracing to determine the outcome of the enhancer’s activity. Identifying an enhancer active in Onecut1(+) RPCs will allow us to understand what upstream molecular events restrict the fate potential of ThrbCRM1(+) cells to generate cones and horizontal cells. Additionally, variation in one or more regulatory elements upstream of Onecut1 may
contribute to the varying cone-to-rod ratios in nocturnal and diurnal vertebrates. Funding: NSF CAREER 1453044 and Sloan Foundation/CUNY award to ME; NIH 8G12MD7603 to CCNY.

Program Abstract #204
Patterning of cone photoreceptors in mammalian eyes
Sarah Hadyniak, Kiara Eldred, Kasia Hussey, Cameron Avelis, Elijah Roberts, Robert Johnston
Johns Hopkins University, USA
Cone photoreceptors are responsible for daytime, color, and high acuity vision in mammals. Long- or middle-wavelength (L/M) photoreceptors comprise the majority of cone cells in the mammalian retina, with short-wavelength (S) photoreceptors in the minority. Cone cell subtype and pattern vary throughout the animal kingdom, and these differences have been shown to correlate to eye position, open/closed terrain, or 24-hour cycle. We present a comparative study using whole retina immunohistochemical analysis from diverse species of mammals. Human retinas show concentric patterning of photoreceptor subtypes, with random distributions of L/M- and S-wavelength photoreceptors, while Rhesus Macaque retinas show similar concentric patterning with a grid-like organization of the three cone subtypes. Mouse and Guinea Pig retinas show a dorsal-ventral gradient of M- and S-wavelength photoreceptors, while retinas from the suborder Ruminant contain random distributions. Using custom software, we are mapping the patterns of cone cells in the whole retina to understand the evolution of photoreceptor patterning in mammalian retinas. Funding sources: Pew Scholars Program, NIH NEI R01EY025598-01, Johns Hopkins University Catalyst Award.

Program Abstract #205
The dorso-ventral patterning of Musca domestica embryos: insights into BMP/Dpp evolution from the base of the lower cyclorraphan flies
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In the last years, accumulated information has indicated that the evolution of extra-embryonic membranes in dipterans was accompanied by changes in the gene regulatory network controlled by the BMP/Dpp pathway, which is responsible for dorsal patterning in these insects. However, only comparative analysis of gene expression levels between distant species with two extra-embryonic membranes, like A. gambiae or C. albipunctata, and D. melanogaster, has been conducted. Analysis of gene expression in ancestral species, which evolved closer to the amnioserosa origin, could provide new insights into the evolution of dorsoventral patterning in dipterans. Here we describe the spatial expression of several members of the Dpp pathway and show the compared patterns of expression between Musca domestica and D. melanogaster embryos, both dipterans with amnioserosa. Mostly of the analyzed gene showed a high degree of expression conservation, however, we found differences in the expression pattern of M. domestica orthologs for sog and tolloid. Bioinformatics analysis of the promoters of both genes indicated that the variations could be related to the gain of several binding sites for the transcriptional factor Dorsal in the Md.tld promoter and Snail in the Md.sog enhancer. These altered expressions could explain the unclear formation of the pMad gradient in the M. domestica embryo, compared to the formation of the gradient in D. melanogaster. We believe that this data can contribute to understanding the evolution of the BMP/Dpp pathway, the regulation of BMP ligands, and the formation of a Dpp gradient in higher cyclorraphan flies. This work was supported by Fondecyt 11130231 to C.Hodar and Fondecyt 1120254 to V. Cambiazo.

Program Abstract #206
Functional divergence between eRpL22 paralogues in Drosophila melanogaster interommatidial bristle morphogenesis
Brett Gershman
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The Drosophila melanogaster eRpL22 ribosomal protein (Rp) family contains two structurally diverse members: eRpL22-like and eRpL22. eRpL22-like has a tissue-specific expression pattern in the testis and eye, while eRpL22 is
expressed ubiquitously. The developmental significance of differential eRpL22 paralogue expression is poorly understood and will be addressed through characterization of paralogue localization and phenotypic consequences of paralogue manipulation. In the midpupal retina, immunohistochemistry (IHC) analysis revealed that eRpL22-like is restricted to one cell type. Co-localization with tissue-structure-specific F-actin expression patterns revealed that eRpL22-like is localized to the growing interommatidial hair cell. Both eRpL22 paralogues are co-expressed within the developing hair cell but are present in temporally dependent, shifting asymmetry. Paralogue exclusion in early bristle development shifts to overlapping patterns of localization at later time points. eRpL22 paralogue asymmetry within the developing hair cell suggests paralogue-specific roles in cell-type-specific processes. IHC of eye-specific paralogue manipulation shows that eRpL22 paralogue depletion yields similar interommatidial bristle defects; eRpL22 depletion resulting in more severe phenotype. These data reinforce the hypothesis that eRpL22-like and eRpL22 have redundant but distinct roles in hair growth. Studies assessing co-localization of eRpL22 family paralogues with the core ribosomal component RpL23a support a ribosomal role for eRpL22 and an extra-ribosomal role for eRpL22-like in the developing eye. Ongoing investigations involving heat-shock-mediated eRpL22-like knock-out in the developing eye aim to examine tissue-specific requirements of eRpL22-like with greater temporal resolution than is possible with Gal4/UAS-mediated RNAi. Funding provided by Sigma Xi GIAR and Lehigh University faculty research grant to VCW.

Program Abstract #207
Tumor suppressor Scribble acts on multiple partners to orient the mitotic spindle
Nicole Paterson, Kenneth Prehoda
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Spindle orientation is a cellular mechanism for creating spatially organized tissues of diverse cell types within the context of development. Mis-regulation of spindle orientation can result in morphological defects and tumorigenesis. Using a minimal Drosophila S2 cell-based system we have found evidence regarding the molecular mechanisms of one of the major tumor suppressors involved in the process of spindle orientation, which we believe scaffolds the activities of at least two proteins, one that passively couples to the cell cortex and another that acts as a downstream effector, both of which are necessary for orienting the spindle.

Program Abstract #208
Crumbs complex dynamics controls apical membrane disassembly during epithelial cell ingression
Sergio Simoes, Thomas Lam, Paraskevi Giannatou, Rodrigo Fernandez-Gonzalez, Ulrich Tepass
University of Toronto, CA
Epithelial to mesenchyme transitions (EMTs) are frequently observed in development and during epithelial tumor progression. EMT involves the loss of apical-basal polarity and cell-cell adhesion and the acquisition of migratory and/or proliferative ability. However, the mechanisms of how cells leave the epithelium remain poorly understood. Using a novel in vivo model, the ingressing neuroblasts (NB) of the Drosophila embryo, and applying quantitative live imaging, we address the cellular and molecular mechanisms involved in the loss of the apical domain and E-cadherin-based cell junctions, which is not driven by transcriptional downregulation of E-cadherin in NBs. We focused on three cellular machineries: the contractile actomyosin cytoskeleton, endocytosis and recycling, and apical-basal polarity. Apical cell contacts between NBs and neighbors are disassembled sequentially driven by periodic pulses of junctional and medial myosin II, and progressively stronger apical contractions and differential coupling of actomyosin to junctions (Simoes et al., JCB, 2107). Moreover, we found that endocytosis, degradation and recycling of the apical determinant Crumbs regulates ingestion kinetics. Crumbs loss accelerates ingestion whereas Crumbs overexpression slows it down. Crumbs is shuttled from the apical cortex of NBs to endosomes for degradation to promote ingestion, whereas Crumbs recycling counteracts ingestion. We show that internalization of Crumbs relies on ubiquitination of its cytoplasmic tail. Notably, completion of ingestion also depends on interactions between the Crumbs binding partner Stardust and the E3 Ubiquitin ligase Neuralized, a component of the Notch pathway that specifies NBs. Our results suggest that temporally regulated
ubiquitination of the Crumbs complex mediates its internalization to precipitate the loss of the apical domain during ingression. (Funding: Canadian Cancer Society)

Program Abstract #209
Tumbling nuclei, polarizing cytoplasm: cellular mechanisms of early oocyte differentiation by a novel centrosome organizing center
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Differentiating cells acquire multiple functional traits consecutively or simultaneously, but how they coordinate the acquisition of distinct traits is unknown. We discovered a novel centrosome-based cellular organizer in the zebrafish early oocyte that integrates multiple facets of differentiation. Universal to differentiating oocytes is formation of the Balbiani body (Bb), a large prion-like aggregate of specific mRNP granules. The Bb establishes the oocyte animal-vegetal polarity and is crucial for oocyte and embryonic development, but how it forms and cellularly positioned was unknown. We traced the oocyte symmetry breaking to a nuclear asymmetry at the onset of meiosis called the chromosomal bouquet. The bouquet is a universal meiotic feature, where telomeres associate with Sun/KASH proteins on the nuclear envelope (NE), connecting to cytoplasmic centrosome-based microtubules. Microtubule-associated telomeres rotate to facilitate chromosomal pairing and recombination, and cluster to the NE pole that faces the centrosome. Bb granules first localized to the bouquet centrosome, and centrosome-based microtubules mechanistically coupled their localization with nuclear bouquet formation. Granule nucleation around the centrosome assembled the mature Bb and required the prion-like protein Bucky ball, demonstrating prion-like mechanisms in oocyte polarization. Thus, the bouquet centrosome functions as a cellular organizer that we termed the Centrosome Organizing Center (COC), coupling meiosis and oocyte patterning. Furthermore, the COC connects to a novel stage-specific primary cilium that likely mechanically regulates bouquet chromosomal pairing. Earlier, the last mitotic division of oocyte precursor-cells positioned the COC towards differentiation, aligning oocyte polarity perpendicular to this division plane. We uncovered a cellular organizer that integrates mitosis, meiosis, cell polarity, cilia biology and prion-like mechanisms in early oocyte differentiation.

Program Abstract #210
Non-canonical Wnt/PCP signaling is required for endoderm morphogenesis
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The endoderm, the deepest germ layer, contributes to development of the gut and associated organs. During embryogenesis, the endoderm undergoes convergence and extension (C&E) and is transformed from a broad endodermal sheet into the narrow gut tube. Non-canonical Wnt/planar cell polarity (Wnt/PCP) signaling has been implicated in endoderm morphogenesis, but the underlying cellular and molecular mechanisms are not clear. We found that Glypican 4 (Gpc4, a protein that promotes Wnt/PCP signaling) and VanGogh-like 2 (Vangl2, a core Wnt/PCP protein) are required for endodermal C&E during zebrafish segmentation. In these mutants, the endodermal sheet is significantly widened, the gut tube is enlarged, and the digestive organs are malformed. We discovered that during the early and middle stages (1-12 somite) of segmentation, endodermal cells become increasingly elongated and polarized mediolaterally in the plane of endodermal sheet. Such endoderm cell polarity is lost in either gpc4 or vangl2 mutants. Notably, mosaic experiments reveal that the anterior edge of the most elongated endodermal cells is enriched for GFP-Vangl2. These findings suggest that during segmentation endodermal cells acquire planar cell polarity and that this requires Wnt/PCP signaling. Furthermore, confocal time-lapse imaging revealed that in addition to becoming elongated, the endoderm cells undergo intercalation mediolaterally, and that this process is driven by both shrinkage of cell junctions and polarized cellular protrusive activity. In both gpc4 and Vangl2-deficient endodermal cells, this protrusive activity is lost and the intercalation of the cells is impaired. Additionally, Wnt5b and Receptor-like tyrosine kinase (RyK) are also required for normal endoderm C&E, and our genetic analyses have shown that Gpc4 interacts with Wnt5b and RyK to regulate
endodermal C&E. Currently, we are investigating whether the control of endoderm morphogenesis by Gpc4 involves the Wnt5b-RyK signaling axis.

Program Abstract #211
Gpr125 Adhesion GPCR/Adgra3 plays a role during zebrafish early development by regulating Wnt canonical and non-canonical pathways
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The establishment of the vertebrate body plan is achieved by coordinated gastrulation movements that narrow the embryonic tissues mediolaterally while extending them in the anteroposterior direction. Planar cell polarity (PCP) signaling has been shown to be essential for during this process. Zebrafish mutants carrying mutations in core Wnt/PCP proteins present impaired mediolateral cell polarization and intercalation movements leading to phenotypically shorter and mediolaterally broader embryos. We have identified the G-protein coupled receptor protein Gpr125/Adgra3 as a modulator of the Wnt/PCP pathway. By RNA overexpression experiments in blastula stage embryos, we showed that Gpr125/Adgra3 protein is able to recruit a subset of core Wnt/PCP components: Dishevelled, Frizzled7 and Glypican 4 into specific membrane subdomains while does not influence Vangl2 subdomains localization. Gpr125 is a seven-pass transmembrane protein. While the functional domains of Gpr125 are largely uncharacterized, molecular analyses demonstrated a direct interaction of Gpr125 intracellular domain with Dishevelled (Li et al., 2013, PMID: 23821037). Using TALEN genome editing, we generated gpr125 mutants. Whereas zygotic gpr125 mutants do not exhibit any visible gastrulation defects, mutants lacking both maternal and zygotic gpr125 function (MZ) show convergence and extension defects and consequently shorter and wider embryonic bodies. gpr125 mutants show a strong genetic interaction with vangl2 and scribble1 mutants. In addition to its role in gastrulation via Wnt non-canonical/PCP signaling, we found evidence supporting a role of gpr125 in the Wnt/β-catenin canonical signaling. We are pursuing analyses of the MZgpr125 mutants, the protein’s functional domains possibly linked to particular morphogenetic behaviors and its downstream signaling partners. (This work is supported by MIRA NIH Grant # 1R35GM118179-01).

Program Abstract #212
An interaction between Drg1 and Dsh modulates ciliogenesis during vertebrate development
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Cilia are critical structures for proper embryonic development and maintaining homeostasis. Although ciliogenesis has been extensively studied, there are still significant gaps in our understanding of all the proteins involved in regulating and directing ciliogenesis. We identify Drg1 (Developmentally regulated GTP binding protein) as a novel binding partner of Dishevelled (Dsh), a known regulator of ciliogenesis. Using the Xenopus laevis embryo as a model system, we show that morpholino-mediated knockdown of Drg1 causes a reduction in the length and number of cilia in the gastrocoel roof plate (GRP) and multiciliated cells (MCCs). While expression of wild type Drg1 rescues these ciliogenesis defects in the morphant, a Drg1 mutant with a reduced ability to interact with Dsh fails to rescue the phenotype. In addition, Drg1 morphants display defective basal body migration and docking to the apical surface of MCCs, as well as abnormal rotational polarity of basal bodies. Moreover, apical actin and RhoA activity are also decreased in Drg1 morphants, suggesting a possible cause for the deficient basal body behaviors. Lastly, Drg1 knockdown results in decreased protein complex formation between Dsh and Daam1, and between Daam1 and RhoA. These results further support the concept that the Drg1/Dsh interaction regulates the nucleation and stability of MCC apical actin. Thus, Drg1 is a newly identified partner of Dsh in regulating ciliogenesis. Funding Sources: Intramural Research Program of the NIH
Program Abstract #213
Patterns of Cellular Addition to the Developing Tendon
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Tendons are an essential tissue that join together and transmit force between muscle and bone. While much is known about muscle and skeletal growth mechanisms, remarkably little is known about the regulation and tendon growth. Our lab aims to identify key signals and processes that direct tendon growth during development. We began our analysis by quantifying normal tendon growth. By examining cross-sections of mouse forelimbs, we have evaluated tendon tissue growth and cellular addition through development. Our data show cell addition continues from embryonic stages through to adulthood primarily along the longitudinal axis of the tissue. Our data also show a reduction of actively proliferating cells from P10 captured even as large numbers of cells continue to be added to the tissue, suggesting the possibility of stem-like progenitors or recruitment of external cells. To further probe this we used reporter mouse models to label tendon cells and their progeny to directly observe the pattern of cell clones generated by proliferation in the tendons. Our data show that in postnatal stages, most proliferation arises from a large group of cells dividing only once or twice, as seen by the presence of clones only containing only 2-3 cells. Surprisingly, while some clones are arranged along the length of the longitudinal cell arrays in tendons many of these divisions occur laterally, resulting in non-contiguous clonal clusters. These studies are also extended to mutant models in which tendon growth is altered. One such model is a mouse model of dwarfism, a condition in which limb and tendon elongation is arrested at an early stage of development by targeting deletion of the Indian Hedgehog gene. We will used the methods established in the first study to investigate how tendon growth parameters are altered in the mutant model. This work was supported by the grant from Shriners Hospital for Children (SHC 85410-POR-14).

Program Abstract #214
A non-canonical role for Wnt16 in the Wnt signaling network involved in specifying and patterning the early anterior-posterior axis of sea urchin embryos
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In early development of the deuterostome sea urchin embryo, anterior-posterior specification and patterning depends on integrated information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways, forming an interconnected Wnt signaling network. We have previously shown that a non-canonical signaling pathway involving the Wnt receptor, Fz1/2/7, antagonizes the progressive posterior-to-anterior downregulation of the anterior neuroectoderm (ANE) gene regulatory network (GRN) by canonical Wnt/β-catenin and non-canonical Wnt1/Wnt8-Fz5/8-JNK signaling. This interaction is critical to establish the spatial expression of the early GRNs along the AP axis. Yet, the exact mechanism by which Fz1/2/7 signaling antagonizes the other Wnt signaling branches is unclear. Here, we show that maternal wnt16 is expressed ubiquitously during cleavage stages and that zygotic wnt16 expression is concentrated in the endomesoderm as early as the mid-blastula stage. We used morpholino and dominant negative interference approaches to analyze the function of Wnt16 during early AP specification and patterning. Our results indicate that Wnt16 antagonizes ANE restriction mediated by Wnt/β-catenin and Wnt1/Wnt8-Fz5/8-JNK signaling and that this activity depends on a functional Fz1/2/7 receptor. Our results also indicate that zygotic wnt16 expression in posterior endomesoderm cells during gastrulation depends on both the Fz5/8 and Wnt/β-catenin signaling. In addition, we demonstrate that while Wnt16 is unnecessary for the activation and/or maintenance of many genes in the endomesoderm GRN in posterior blastomeres at the beginning of gastrulation, it is essential for gastrulation. Together, our data suggest that Wnt16 activates the Fz1/2/7 pathway that antagonizes the ANE restriction mechanism mediated by Wnt/β-catenin signaling and Wnt1/Wnt8-Fz5/8-JNK signaling as well as has a role in the morphogenetic movements of gastrulation.
Program Abstract #215

Development of Hofstenia miamia and the embryonic origin of neoblasts

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Many animals are capable of “whole-body” regeneration, i.e., they can replace any missing cell type. Regeneration in these species is often mediated by proliferation and differentiation of populations of cells that are effectively pluripotent, e.g., the i-cells of Hydractinia and the neoblasts of planarians. The mechanisms of how these populations of pluripotent cells are first specified are unknown. Identifying the embryonic origins of these adult stem cell populations in regenerative species is a crucial first step in deciphering the molecular genetic control of stem cell specification. We are focusing our study of this question in Hofstenia miamia, a highly regenerative acocel species that produces abundant and accessible embryos in laboratory culture conditions. Hofstenia have a large population of parenchymal stem cells, also called neoblasts, that are required for regeneration and express homologs of piwi and other markers of planarian neoblasts. At 43-55 hours into development (“Dimple” stage), we found that embryos begin to internalize cells on the animal pole. At this time-point we also detected a significant upregulation of neoblast genes in RNAseq data and the presence of internal cells with high piwi-1 expression through in situ hybridization. Taken together, this suggests that the earliest neoblasts may be specified at this time. We are combining in situ hybridization, lineage tracing, and single cell sequencing to validate our hypothesis that these piwi+ cells at the Dimple stage are bonafide neoblast precursors. These experiments will then enable a functional investigation of neoblast specification.

Program Abstract #216

Cas9 ribonucleoprotein complex allows direct and rapid analysis of target genes in Pleurodeles waltl development and regeneration

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Newts have remarkable ability to regenerate their organs and have been used in research for centuries. However, the laborious work of breeding has hampered reverse genetics strategies in newt. Here, we present simple and efficient gene knockout using Cas9 ribonucleoprotein complex (RNP) in Pleurodeles waltl, a species suitable for regenerative biology studies using reverse genetics. Most founders exhibited severe phenotypes against each target gene (tyrosinase, pax6, tbx5); notably, all tyrosinase Cas9 RNP-injected embryos showed complete albinism. Moreover, amplicon sequencing analysis of Cas9 RNP-injected embryos revealed virtually complete homozygous disruption at target loci in founders, allowing direct phenotype analysis in the F0 generation. In addition, we demonstrated the generation of tyrosinase null F1 offspring within a year. Finally, we expanded this approach to the analysis of noncoding regulatory elements by targeting limb-specific enhancer of sonic hedgehog, known as the zone of polarizing activity regulatory sequence (ZRS). Disruption of ZRS led to digit deformation in limb regeneration. From these results, we are confident that this highly efficient gene knockout method will accelerate gene functional analysis in the post-genome era of salamanders. This work was supported by JSPS KAKENHI Grant Numbers JP15K06802, JP16K08467, JP16H01254, JP22124002, JP16H04794, JP16H06376 and JP17J04796.

Program Abstract #217

The Marine Pinfish, Lagodon rhomboides, as a Model for Developmental Neurogenesis and Retinal Development

Patience Moseley

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In contrast to mammals, the fish CNS produces new neurons throughout life and can fully regenerate neurons after injury. Understanding mechanisms that govern persistent neurogenesis in fish may be critical for developing the ability to treat CNS injury and disease in humans. The freshwater zebrafish is a powerful genetic model but,
unlike many marine fishes, does not have a prolonged larval stage followed by metamorphosis. During metamorphosis, the marine fish CNS rapidly generates new neurons, and during this time the retina rapidly adds rod photoreceptors. Information about the mechanisms that regulate metamorphic neurogenesis is critically lacking but could be key for understanding the potential for the vertebrate retina/CNS to produce new neurons. To understand these mechanisms, our objective is to develop the pinfish, *Lagodon rhomboides*, as the first marine fish developmental model species that can be reliably reared in a laboratory. To accomplish this, we have established systems and protocols to successfully breed pinfish within a small laboratory setting. Pinfish naturally breed in winter, but by controlling lighting and temperature, we can induce spawning throughout the year. We have determined that metamorphosis begins earlier at lower salinity (9 days at 15 ppt) compared with higher salinities (17+ days at 27 ppt) but, regardless of salinity, rod genesis begins around 10 dpf. This suggests that, in contrast to the generally accepted paradigm, rod genesis is not necessarily linked to metamorphosis and is regulated by different mechanisms. To understand these mechanisms and establish pinfish as a developmental model, we have sequenced and are assembling the pinfish genome, have cloned rhodopsin and other genes, and have created mRNA probes to label several retina specific molecules using in-situ hybridization. Funding sources: UWF Hal Marcus College of Science and Engineering -New Faculty Award (Dr. Scott Taylor); Graduate Research Grant (Patience Moseley)

**Program Abstract #218**

*From random walk to directed cell migration: a quantitative study of killifish embryogenesis*

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The proper patterning and coordination of cell movements is critical for embryogenesis. Our current understanding of teleost morphogenesis during gastrulation is that patterning dictates cell behavior. This consensus view is based on the idea of prepatterning, whereby maternally provided mRNAs determine the dorsal side of the embryo. In annual killifish, prior to gastrulation during diapause I, individual blastomeres exhibit a random walk, which has led us to hypothesize that prepatterning is an unlikely mechanism. This view is supported by the expression of tissue specific markers throughout development. Here we track the nuclei of approximately 2,000 cells of an H2A:dendra line of *Nothobranchius furzeri* during cell aggregation, gastrulation, and axis formation. Taking advantage of the relatively low cell number, we quantify the change in cell behavior during the transition from a random walk to a directed migration towards the site of aggregation. Using a pharmacological approach we are currently investigating which signaling pathways are involved in the proper formation of the aggregate. We propose that specification and migration occur concurrently during the initial formation of the aggregate and that prepatterning is dispensable for annual killifish development. This work is supported by the NIH and the Damon Runyon Cancer Research Foundation.

**Program Abstract #219**

*No oxygen, no water: the unique physiology of annual killifish embryos across development*

*Daniel Zajic, Jason Podrabsky*

*Portland State University, USA*

In most organisms, even brief episodes of low oxygen supply can cause irreparable damages to vital organs, such as the brain and heart. The annual killifish (*Austrofundulus limnaeus*) survives in ephemeral ponds and their embryos have the remarkable ability to tolerate anoxic and dehydrating conditions for months. Survival of dehydrating conditions is achieved through reduction of evaporative water loss and thus is likely to highly limit gas exchange. The mechanisms that support survival and buffer these embryos from damage during environmental stress are unknown. This study examines four developmentally distinct stages of *A. limnaeus* embryos that differ in their anoxia and dehydration tolerance. When exposed to anoxia, embryos of *A. limnaeus* respond by producing significant amounts of γ-aminobutyric acid (GABA) and lactate. However, when exposed to desiccation, embryos of *A. limnaeus* do not produce significant amounts of GABA or lactate, suggesting different metabolic pathways to combat desiccation stress. This study aims to 1) understand the role of GABA and lactate in
supporting the metabolic response to anoxia and 2) uncover the molecular mechanisms that allow survival during desiccation across development. Our findings show no glutamate decarboxylase (GAD) activity, the primary enzyme responsible for producing GABA in most vertebrates, suggesting a different GABA synthesis route in response to anoxia. GABA has been found to provide excitatory actions in the developing vertebrate nervous system, but conversely, typically functions as an inhibitory neurotransmitter in adults. The high levels of GABA accumulated during anoxia in A. limnaeus embryos suggests GABA may serve a purpose other than as a neurotransmitter, and raises questions about role of GABA in neural development in this species. Metabolite profiling of desiccated embryos provides insight into the unique molecular physiology behind embryological survival with no water. (Funding: NSF IOS 1354549 JEP).

Program Abstract #220
Vitamin D Pathway Enzyme Inhibition Promotes Alternative Developmental Trajectory in Annual Killifish Embryos
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Deciphering the mechanisms by which organisms sense and react to environmental cues is an essential part of understanding embryonic development where stress can have both detrimental and adaptive effects on individual organisms, populations, and perhaps entire species. Embryos of the annual killifish, Austrofundulus limnaeus, enter a state of diapause to endure environmental extremes typical of their environment. Entrance into diapause is regulated by vitamin D signaling in a temperature-dependent manner. This pathway is homologous to DAF-12 signaling in C. elegans and ecdysone signaling in Drosophila, both of which regulate metabolic dormancy. Vitamin D signaling is highly conserved across all eukaryotes and is potentially an important unexplored mechanism for integration of environmental information into developmental programs across a diversity of species. Using selective endocrine disrupting chemicals, we provide physiological evidence for the role of the vitamin D pathway in regulating developmental dormancy in A. limnaeus. When exposed to Dafadine A, a known inhibitor of DAF-9 in C. elegans and CYP27A1 in vertebrates, A. limnaeus embryos enter into diapause even under laboratory conditions known to support active development. CYP27A1 is a mitochondrial enzyme that synthesizes 25-hydroxyvitamin D$_3$, a critical intermediate in the synthesis of hormonally active 1,25-dihydroxyvitamin D$_3$. Further, Dafadine A exposure caused developmental arrest in zebrafish embryos at a stage similar to diapause II in A. limnaeus. This project explores the potential role of vitamin D signaling as a conserved mechanism among fishes to regulate developmental progression and integrate environmental cues into major life history decisions. Future study may provide evidence for this pathway as an evolutionarily ancient mechanism for sensing and responding to environmental changes in vertebrates. Funding: NSF IOS-1354549 to JEP

Program Abstract #221
Annotation and characterization of candidate germline development genes in a tardigrade genome
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A key event during animal embryogenesis is the segregation of germ cells from somatic cells. Two general mechanisms for this segregation have been described in animals. In some species, including the model organisms Caenorhabditis elegans and Drosophila melanogaster, germ cells are specified early in development by restriction of maternally-provided specialized cytoplasm, or “germ plasm”, to a single lineage. In other species, including mammals, germ cell fate is determined later in development through signaling between potential germ cells and neighboring cells. However, the signaling mechanisms that specify a few germ cell precursors from a pool of competent cells are incompletely characterized. To address this question, my lab has focused on the emerging model tardigrade Hypsibius dujardini, which has been proposed to rely on cell-signaling mechanisms to specify germ cells. Tardigrades, (“water bears”), comprise a phylum of small, segmented invertebrates that are part of the Ecdysozoa clade. The genome has been sequenced, but remains largely unannotated. To begin to characterize the specification of germ cells in tardigrades, my lab is targeting candidate genes with highly-conserved role in germ
cell specification and maintenance. I describe here preliminary efforts to annotate and characterize several key candidates genes in the tardigrade genome, carried out by undergraduates in my Principles of Development course and in my laboratory at Seattle Pacific University. Given tardigrades' key evolutionary position, this research provides a starting point to better understand the evolution of germline development. This research was supported by a Murdock College Research Program for Natural Sciences grant and a Seattle Pacific University start-up research grant to J. Tenlen.

Program Abstract #222
Transdifferentiation in Turritopsis dohrnii (Cnidaria, Hydrozoa): Model system for regeneration, cellular plasticity and aging
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The medusae (jellyfish) of Turritopsis dohrnii (Cnidaria, Hydrozoa) undergoes reverse development to avoid death caused by physical damage, adverse environmental conditions, or aging. Weakened or damaged jellyfish will undergo a whole-body transformation into a cluster of uncharacterized tissue, referred to as the cyst stage, which then will metamorphose back into an earlier lifecycle stage, the juvenile polyp. This unique ability has granted the species the name, the "Immortal Jellyfish". The underlying cellular mechanism that permits its reverse development is called transdifferentiation or cell reprogramming. Cell transdifferentiation allows fully mature and differentiated cells to reprogram themselves into a new cell type of any lineage. Thus, transdifferentiation is highly regarded in the biomedical sector as a potential mechanism to transform mature cells into any needed cell type after tissue damage. The polyp, jellyfish and cyst stage of T. dohrnii were sequenced through RNA-sequencing and the transcriptomes were assembled de novo. The transcriptomes were then annotated to create the gene expression profile of each stage. Comparative functional gene enrichment analyses with the cyst as the central stage of comparison reported significant Gene Ontology GO categories that were over-expressed, such as telomere maintenance and DNA repair, in the cyst as compared to other stages. The enrichment analyses also reported significantly under-expressed categories, such as mitotic cell division, cellular differentiation and development, in the cyst as compared to the other stages. Ultimately, our work produced a foundation to develop an alternative model system to further investigate and understand regeneration, cellular plasticity and aging in metazoans.

Program Abstract #223
Determining the effects of a coding mutation in a pigmentation patterning gene
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Color patterns in birds and other vertebrates are incredibly diverse and can impact fitness by affecting communication, mate choice, and crypsis. The genetic and developmental basis of pattern diversity is not well understood. To investigate this diversity, we use rock pigeons (Columba livia), which have variation in many traits. All rock pigeons are one of four major wing patterns: T-check, checker, bar, and barless (in decreasing order of melanism and dominance). Barless is characterized by a lack of the ancestral bars and an incompletely penetrant vision defect called “foggy vision”. Previously, we used genome wide comparisons to show that variation in Ndp (Norrie disease protein) is associated with the four major wing pattern phenotypes. In humans, Norrie disease is characterized by an array of symptoms, including vision defects. Ndp encodes a secreted protein that binds to its receptor to initiate Wnt signaling. Barless pigeons are fixed for a start codon mutation in Ndp that truncates the protein’s signal peptide sequence. The start codon is also mutated in two human families with Norrie disease. We are working to assess whether these mutations alter secretion of NDP by expressing different versions of EGFP-tagged pigeon and human proteins: truncated mutants, wild type, and a version with no signal peptide sequence. The latter two will serve as positive and negative controls, respectively. We will express our tagged proteins in HeLa cells and visualize cellular localization using fluorescence microscopy. We will also separate the extracellular fluid and cells of a liquid culture and use western blots to evaluate secretion of the tagged proteins. With these
experiments, we will understand how a truncated signal sequence of NDP functionally affects secretion and gain more insight into the barless phenotype. We thank the SDB’s Choose Development! program, the NSF (Career DEB1149160 to M.D.S., GRFP 1256065 to A.I.V.), and NIH (ROI6M115996 to M.D.S.) for funding.

Program Abstract #224
EMC1 a Candidate Gene for a Diverse Array of Congenital Diseases is Important for Neural Crest Cell Development
Jonathan Marquez, June Criscione, Emily Mis, Mustafa Khokha
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The endoplasmic reticulum (ER) membrane protein complex (EMC) is a conglomerate of 10 subunits in Homo sapiens (EMC1-10). This complex is essential for the synthesis, folding, and localization of multi-pass transmembrane proteins. Genetic studies have provided evidence for the role of mutations in EMC1 in a variety of childhood diseases. Investigating mechanisms of dysfunction will help us understand disease in the context of EMC1 mutations. Xenopus tropicalis provides a model that has allowed us to observe the severity of Emc1 loss of function in the neural crest cell (NCC) lineage and has provided insight into how mutations in EMC1 may contribute to disease. To investigate developmental phenotypes in an Emc1 loss of function model, we used morpholino oligos (MOs) to deplete Emc1 in whole embryos by injecting at the one cell stage or in one-half of the embryo by injecting one cell at the two-cell stage to maintain the contralateral side as an internal control. This loss of function model results in various developmental anomalies. Pigment cells, cardiac outflow tracts, and craniofacial cartilage display morphological abnormalities. Given the importance of neural crest cells (NCCs) in the development of these tissues, we investigated this population of cells. Markers of neural crest specification and migration are abnormally distributed or lost in the injected half of embryos. Given the importance of the EMC for multi-pass transmembrane proteins, we assessed possible connections between this function and NCC development. A prominent class of multi-pass transmembrane proteins important for NCC development is the Frizzled proteins that serve as part of the WNT signaling pathway. WNT signaling is one of the drivers of the NCC GRN. Indeed, Fzd7 decreases over time in Emc1 depleted embryos. These findings point to a potential mechanism for the dysfunction seen in NCC derived tissues of patients with mutations in EMC1. Support: Yale MSTP T32GM007205 and CMB T32GM007223

Program Abstract #225
RDH10 and retinoic acid signaling mediate ECM-composition and neural crest cell migration during early colonization of the gut and in the pathogenesis of Hirschsprung Disease
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Vagal neural crest cells (vNCC) are a migratory cell population that give rise to the neurons and glia of the enteric nervous system (ENS). Defects in vNCC may lead to incomplete formation of the ENS in the gut which is the principal cause of Hirschsprung Disease (HSCR). HSCR occurs with an incidence of 1/5000 live births and is treated via surgical resection of the aganglionic bowel. A number of genes have been identified in the etiology and pathogenesis of HSCR, however a significant proportion of affected individuals have an unknown genetic diagnosis. Therefore additional genes or modifiers must participate in the etiology of HSCR. Our lab identified Rdh10 as a novel critical regulator of vitamin A metabolism and retinoic acid (RA) synthesis during embryogenesis (Sandell et al. 2007). Interestingly, Rdh10 is highly expressed in the mesenchyme around the foregut through which vNCC migrate, and we have shown that retinoid signaling is required early from E7.5-E9.5 for proper neural crest cell colonization. Furthermore Rdh10 loss-of-function mouse embryos (Rdh10<sup>trex/trex</sup>) exhibit total intestinal aganglionosis, thus providing an excellent model of HSCR. vNCC form in Rdh10<sup>trex/trex</sup> embryos, but fail to enter the foregut. To mechanistically understand the basis for this phenotype, we performed comparative RNA-sequencing of wild-type and mutant embryos. KEGG pathway analyses identified (i) changes in ECM composition with an up-regulation in ECM-receptor interactions and focal adhesion pathways; and (ii) down-regulation of Ret signaling pathway genes, Ret, Gfra1 and Gdnf. Therefore we hypothesize that Rdh10-mediated RA formation and signaling
is essential for vNCC migration into the gut through extrinsic regulation of the ECM composition, and the intrinsic regulation of Ret pathway genes which are associated with the etiology and pathogenesis of HSCR. Funding: Stowers Institute for Medical Research; Madison and Lila Self Graduate Fellowship, University of Kansas

Program Abstract #226

A small intronic deletion in Snrpb models Cerebro-costomandibular Syndrome (CCMS)

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Cerebro-costomandibular Syndrome (CCMS) is a very rare autosomal dominant disorder where patients mostly have craniofacial abnormalities and rib defects. SNRPB, which codes for a common core component of the spliceosomal small nuclear ribonucleoproteins (snRNPs) is mutated in patients with CCMS. As patients have abnormalities in specific tissues due to mutation in SNRPB, it is possible that a subset of transcripts in those tissues is sensitive to the level of SNRPB during development. Exome sequencing of patients reveals mutations occur mostly in the highly conserved alternative exon 2 (AE2) of the gene, where it plays an instrumental role in autoregulating SNRPB. Using Crispr/Cas9 system, we generated a mutant mouse line with a 61 base-pair (bp) intronic deletion upstream of the AE2. A subset of heterozygous and homozygous mice and embryos for this deletion show phenotypic abnormalities seen in CCMS patients such as reduced size of face and head, rib abnormalities, and limb defects. Intriguingly, heterozygous and homozygous embryos show reduced SNRPB protein levels, suggesting that this 61 bp sequence plays a critical role in regulating SNRPB levels. Our preliminary data suggests the deletion of the intronic sequence changes splicing of the gene, where the mutants preferentially include AE2 in the transcript. Future studies will elucidate the mechanism by which this small intronic sequence regulates SNRPB. Funding Source: Supported by CIHR, Queen Elizabeth Scholarship

Program Abstract #227

Mutation of Eftud2, the gene responsible for mandibulofacial dysostosis with microcephaly (MFDM), leads to pre-implantation arrest in mouse

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Haploinsufficiency of EFTUD2 is associated with MFDM, but how mutations of EFTUD2 cause abnormalities associated with this synrdrom remains unknown. In the present study, we first aimed to characterize the expression of Eftud2 during mouse development and to generate a mutant mouse line carrying Eftud2 mutation. In situ hybridization revealed expression of Eftud2 throughout embryonic development, with tissue-specific expression in ectodermal and mesodermal components of the developing head and craniofacial region. The CRISPR/Cas9 system was used to delete exon 2 of Eftud2. Eftud2 heterozygous animals were viable and fertile despite a 32% and a 30% reduction of Eftud2 mRNA and protein expression, respectively. Furthermore, heterozygous embryos showed delayed development prior to the onset of organogenesis. Noticeably, RNA sequencing revealed that Eftud2 was the only transcript affected in heterozygous mice. In addition, collection of embryos from embryonic day (E)3.5 to E18.5 from matings between Eftud2 heterozygous males and heterozygous females, indicated pre-implantation arrest of homozygous mutant embryos after E3.5. Further ex vivo analysis of E3.5 embryos from Eftud2⁻/⁻ inter se crosses showed a 32% embryonic death compared to embryos collected from wild-type crosses. Arrested embryos failed to grow more beyond the blastocysts stage and were unable to hatch. Our observations indicate that reduced levels of Eftud2 is associated with developmental delay and does not model MFDM in mouse, and reveal a role for Eftud2 during implantation.

Program Abstract #228

Ranbp1 models cleft palate and craniofacial patterning defects in 22q11.2 Deletion Syndrome

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22q11.2 Deletion Syndrome (22q11 DS) is a neurodevelopmental disorder that impacts 1 in 4,000 live births. Craniofacial anomalies, including cleft palate and other structural defects of the palate, as well as anomalies in bone morphology, are apparent in most individuals with 22q11.2 deletions. We have found that Ranbp1, a 22q11.2 DS candidate gene, is a key regulator of multiple aspects of craniofacial development, and accounts for a subset of craniofacial defects in 22q11.2 DS. Mice with homozygous null mutations of Ranbp1 have a robust cleft palate phenotype, and also have significant disruptions in cranial bone formation. Conditional neural-crest specific knockout of Ranbp1 yields a highly-penetrant but less-severe phenotype, which impacts palate structure and cranial bone formation. The neural crest CKO model shows asymmetries and anomalies in palatal structure that mirror “submucosal cleft palate” – a condition seen in the majority of children with 22q11.2 deletions. Intriguingly, heterozygous Ranbp1 mutants display a subtle and variable phenotype: heterozygotes show a delay in palatal shelf elevation/extension, and subtle anomalies in craniofacial bone formation that mirror those observed in the model of the full 22q11.2 deletion. This suggests that Ranbp1 is the primary gene responsible for at least a subset of craniofacial defects in 22q11.2 DS. Loss of Ranbp1 appears to disrupt BMP signaling by impairing nuclear export of signaling intermediates, thus this mutation likely disrupts key craniofacial signals that pattern the cranial neural crest. The series of Ranbp1 mutants we have identified are models of common craniofacial defects observed in many neurodevelopmental disorders.

Program Abstract #229
Correction of Craniofacial Defects in Pax9 Deficient Mice by Small Molecule and Antibody Treatments
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PAX9 is known to be associated with human craniofacial abnormalities such as cleft palate and tooth agenesis. Mice lacking Pax9 gene die at birth with cleft palate and tooth agenesis. The objective of this study is to use this mouse model to explore the in-utero correction of craniofacial defects by therapeutic treatments. This study will also provide new molecular mechanism of Pax9 in regulating palate formation and candidates for the therapeutic treatment of the patients with craniofacial defects. To compensate the disrupted signaling activities in Pax9-/− palate, the Wnt agonists WAY-262611 and IIIc3a, Eda/Edar agonist Anti-EDAR antibody (mAbEDAR) and Bmp agonist Isoliquiritigenin were injected through the tail vein into pregnant Pax9+/− mice which had been mated with Pax9+/− males. Time course, which covered the early developmental stages of palate and tooth formation, and dose dependent analysis were performed to achieve higher correction ratio. Results: WAY-262611 and IIIc3a treatments rescued cleft palate defects in Pax9−/− embryos with a range of ratio from 37.5% to 100%. mAbEDAR antibody treatment corrected cleft palate defects in Pax9−/− embryos with a range of ration from 71% to 90%. And Isoliquiritigenin treatment resulted in 50% to 67% palate closure in Pax9−/− embryos. However, correction of the palatal defects did not prevent postnatal death of Pax9−/− pups. None of these treatments had negative effects on the mother or control littermates. Conclusions: The small molecules and antibody treatments could rescue the craniofacial defects in Pax9−/− embryos without any overt associated toxicities, suggesting that they have the potential to be used as safe therapeutic drugs for treating developmental abnormalities related to Pax9 deficiency. The Wnt, Bmp and Eda/Edar signaling pathways shared interaction networks downstream of Pax9 during palate formation. (Supported by NIH/NIDCR Grant DE019471 and and DE027255 to RDS)

Program Abstract #230
Altered progenitor dynamics prefigure craniosynostosis in a zebrafish model of Saethre-Chotzen syndrome
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Sutures separate the skull bones and house stem cells for growth and repair. In Saethre-Chotzen syndrome, mutations in TCF12 or TWIST1 ablate a specific suture, the coronal. Despite this suture forming at a neural-crest/mesoderm interface in mammals and a mesoderm/mesoderm interface in zebrafish, we find that zebrafish lacking TCF12 and TWIST1 homologs display similarly specific coronal suture loss. Sequential live bone staining reveals an initial acceleration of bone production across the skull, which correlates with increased...
osteoprogenitor proliferation in both zebrafish and mouse mutants. Later, selective stalling of bone growth occurs at the future coronal suture, with the severity prefiguring suture loss in individual mutants. Conditional mouse genetics also reveals Twist1 requirements in both the frontal and parietal bones for suture patency. These findings reveal remarkable conservation of coronal suture formation despite evolutionary shifts in its embryonic origins, and demonstrate that early progenitor exhaustion prefigures later suture loss. This work was supported by NIH grant R01DE026339 to R.E.M. and G.C, and NIH training fellowships F31DE024031 and T90DE021982 to C.S.T.

Program Abstract #231

Requirement for Bmp signaling in endoderm and jaw development identified from a gene-ethanol screen in zebrafish

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Most birth defects are likely due to poorly understood interactions between genetic and environmental factors. To elucidate these interactions, we performed genetic screens for mutations that enhance ethanol teratogenicity. Ethanol induces similar jaw defects in bmp4 and au15 mutants, neither of which has facial defects when untreated. The jaw defects in ethanol-treated bmp4 mutants resemble those in some endoderm mutants, suggesting a role for Bmp signaling in endoderm development. We analyzed Bmp activity using a Bmp response transgenic line. We found that the endoderm receives Bmp signaling from 10-18 hpf, the same window when bmp4 mutants are ethanol sensitive. Using genetic and chemical inhibitor approaches, we show that endoderm morphogenesis requires Bmp signaling during this time window. Genetic chimeras show that Bmp signals directly to the endoderm and that cells lacking Bmp signaling are excluded from the endodermal pouches. Time-lapse imaging shows that loss of Bmp signaling disrupts pouch out-pocketing, similar to Fgf signaling mutants. Using an Fgf response transgenic line and loss-of-function analyses, we show that a Bmp-dependent Fgf response in the pouches is necessary for craniofacial development. Our current work centers on how ethanol interacts with multiple members of the Bmp pathway by creating allelic combinations of Bmp mutants to identify those that recapitulate the effects of bmp loss. While phenotypically similar to ethanol-treated bmp4 mutants, the ethanol-sensitive time window of au15 mutants is from 24-48 hpf, after the widow for bmp4 mutants. We are currently genetically mapping au15 and characterizing the relationship of ethanol-induced jaw loss in au15 and Bmp pathway mutants. Overall, these data suggest that au15 may function downstream of Bmp as part of an ethanol-sensitive genetic pathway regulating jaw development. This research was supported by K99AA023560 to CBL and R01AA023426, R01DE020884 and U24AA014811 (Riley, PI) to JKE.

Program Abstract #232

Exploring the developmental mechanisms underlying Wolf-Hirschhorn Syndrome: Uncovering evidence for defects in neural crest migration using Xenopus laevis as a model system

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Wolf-Hirschhorn Syndrome (WHS) is a neurodevelopmental disorder characterized by craniofacial malformations, heart defects, skeletal defects, and intellectual disabilities. Previous genetic studies have determined that WHS likely arises from a microdeletion on chromosome 4, which includes the four genes WHSC1, WHSC2, LETM1 and TACC3. However, the mechanism by which their heterozygous loss results in WHS is not understood, and their embryonic functions have not been well-characterized. Given that these genes have all been directly or indirectly linked to cell migration in various contexts, we hypothesize that WHS results from a defect in neural crest cell migration. Recently, we have determined that WHSC1, WHSC2, TACC3 and LETM1 are all expressed along the developing neural tube, neural folds, and are enriched in the pharyngeal arches, during Xenopus laevis embryonic development. Knockdown of these WHS-related genes leads to variable defects in both craniofacial and cartilage morphology. Moreover, the loss of WHS gene expression causes specific defects in forebrain and midbrain development. This implicates these four genes in the WHS phenotype. Further analysis of WHSC1 function shows
that its knockdown causes defective neural crest cell migration, supporting the notion that the WHS phenotype is a result of erroneous neural crest cell motility. We have also identified other defects in both neural crest cell motility and proliferation after knockdown of other WHS genes. Thus, our analysis shows that the WHS-associated genes all may play contributing roles in the WHS phenotype of craniofacial malformation, skeletal abnormality, and microcephaly. Future studies will determine the combinatorial effects of their knockdown on neural crest cell migration and will further elucidate the mechanism by which WHS develops. Funding: March of Dimes 1-FY16-220, NIH R03 DE025824

Program Abstract #234
Mutations in Bcl9 and Pygo genes cause congenital heart defects by tissue-specific perturbation of Wnt/β-catenin signaling
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Bcl9 and Bcl9l (Bcl9/9l) are developmentally expressed genes that encode Wnt/β-catenin transcriptional co-factors. Mechanistically, BCL9/9L mediate the interaction between β-catenin and the chromatin code-reader PYGO via two evolutionarily conserved domains, HD1 and HD2, respectively. Genetic alterations in human BCL9 gene have been repeatedly found in congenital heart disease (CHD) with as-of-yet unclear causality. Nonetheless, their contributions to vertebrate heart development remain uncharted. Here, combining zebrafish and mouse genetics, we document tissue-specific functions in canonical Wnt signaling for BCL9 and PYGO proteins during heart development. In a CRISPR-Cas9-based genotype-phenotype association screen, we uncovered that zebrafish mutants for bcl9 and pygo genes largely retain β-catenin activity yet develop cardiac malformations. In the mouse, both systemic and lineage-specific loss of the β-catenin-BCL9-PYGO complex caused heart defects with outflow tract malformations, aberrant cardiac septation and valve formation, and compact myocardium hypoplasia. Mechanistically, these phenotypes coincide with transcriptional deregulation during heart patterning, and PYGO2 associates with β-catenin at cis-regulatory regions of cardiac genes. Taken together, our results establish BCL9 and PYGO as tissue-specific β-catenin co-factors during vertebrate heart development. Our results, importantly, implicate alterations in BCL9 and BCL9L in human CHDs as possibly causative.

Program Abstract #235
A Novel Developmental Disorder Linked to UBA2: Implications for SUMOylation
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UBA2 is an important player in SUMOylation, a post-translational modification involved in nuclear transport and protein degradation. We present a novel human disorder due to UBA2 haploinsufficiency, a potential cause of 19q13.11 microdeletion syndrome. Phenotypes include variable expression of developmental delays, dysmorphic facial features, microcephaly, limb anomalies, cardiac defects, genitourinary malformations, and cutis aplasia. During zebrafish development, expression is dynamic. From 12 hours to 7 days post-fertilization (dpf), generalized expression becomes restricted to the developing nervous system, heart, genitourinary system, gastrointestinal tract, and fins. We hypothesized that loss of uba2 function would result in developmental defects homologous to the human disorder. To test this hypothesis, we generated a 14bp deletion in exon 1 by CRISPR-Cas9 targeting. Distribution of genotypes in live young was consistent with Mendelian inheritance. Lethality was observed in uba2del14/ubad14 fish by 12 dpf. Homozygous null fry exhibited microcephaly and an array of incompletely penetrant phenotypes, including craniofacial defects, shortened pectoral fins, and notched tail fins. Immunohistochemical examination of the embryonic brain demonstrated abnormal neurogenesis. Microscopic
computerized tomography (CT) was used to characterize craniofacial and fin development. These results support our hypothesis that UBA2 loss of function leads to abnormal development of multiple tissues. Ongoing work will clarify whether hypomorphic traits are present in adult heterozygous fish. Funding for this work includes intramural funds from the National Eye Institute.

Program Abstract #236

Knockdown of the zebrafish orthologue of GABRA1 results in absence seizures.

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The Gamma-Aminobutyric Acid receptor A (GABA\(_A\)R) is a multi-subunit receptor for the major inhibitory neurotransmitter (GABA) of the central nervous system. Mutations in the genes that encode the major sub-units of the GABA\(_A\)R have been associated with syndromic forms of epilepsy. We used trio based whole exome sequencing (WES) to identify the genetic etiology of a patient diagnosed with a multiple congenital anomaly syndrome characterized by a severe seizure disorder. WES identified a heterozygous de novo missense mutation in the GABRA1 gene (c. 875C>T), which resulted in a single amino acid substitution in the transmembrane domain (p. Thr292Ile). GABRA1 encodes the alpha subunit of the GABA\(_A\)R and previous studies have associated mutations in this gene with epileptic phenotypes. Thus, we hypothesized that mutations in GABRA1 disrupt GABA\(_A\)R function causing seizure like disorders. To begin to test this hypothesis, we developed a zebrafish model of GABRA1 deficiency using antisense morpholinos. Our preliminary analysis demonstrates that gabra1 expression in zebrafish is nervous system restricted. Moreover, we performed behavioral analysis of larval morphants and their control injected siblings using the Zebrabox automated tracking system. Our data suggests that knockdown of gabra1 results in hypoactivity with phenotypes reminiscent of an absence seizure. Collectively, our data suggests that mutations in GABRA1 are essential for appropriate GABA\(_A\)R function and likely result in various seizure phenotypes. This work was supported by the Bridges to the Baccalaureate Program, Grant no. 2R25GM049011-16, the RISE program Grant no. R25GM069621-11, and the Border Biomedical Research Center Grant no. 2G12MD007592 from National Institute on Minority Health and Disparities, and the University of Texas El Paso New Investigator Laboratory Start-up Funds.

Program Abstract #237

Mutations in hcfc1a disrupt neural proliferation by way of deficiencies in asxl1 expression

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HCFC1 is an X-linked gene that is highly conserved throughout vertebrates and encodes for a transcriptional co-factor which mediates cell proliferation and cell-cycle division in vitro. Mutation of HCFC1 causes cblX, a multiple congenital anomaly syndrome characterized by intellectual disability, craniofacial abnormalities, and epilepsy. The neurological phenotypes associated with cblX are quite profound, however treatment options are limited because very little is known about the function of HCFC1 in vertebrate brain development. Studies attempting to characterize the function of HCFC1 in murine models have provided some insight into HCFC1’s developmental function, but these studies have been plagued by compensatory mechanisms that limit developmental studies. Therefore, novel model systems with which to understand HCFC1 function are warranted. Zebrafish represent one alternative model system because they do not have sex chromosomes and therefore avoid the impediments associated with mouse models. Therefore, we developed a zebrafish harboring germline mutations in the hcfc1a ortholog using CRISPR/Cas9 genome editing technology. Our preliminary data demonstrates that mutation of hcfc1a results in abnormal numbers of neural precursors caused by disruption of the G1/S transition. Moreover, RNA-sequencing revealed an upregulation of a novel gene asxl1 in Hcfc1a mutant fish. ASXL1 encodes a transcription factor which is known to regulate the G1/S transition. Collectively, our data provides a putative mechanism by which mutations in HCFC1 interfere with neural precursor proliferation and differentiation causing severe neurological phenotypes.
Program Abstract #238

Going with the Flow: A novel kinesin and its Role in Cerebrospinal Fluid Flow

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The delicate balance of cerebrospinal fluid (CSF) production and flow dictates brain structure and function. Disrupting this balance through loss of flow causes hydrocephaly, characterized by dilation of ventricles in the brain. The flow of CSF is in part orchestrated by ependymal cell cilia, making them an integral part in brain structure and function. Here, we used analysis in both mouse and zebrafish models, as well as human genetics studies, to identify an evolutionarily conserved function for Kinesin family member 6 (Kif6) in ependymal cell cilia. We report a homozygous nonsense mutation in Kif6 in a child displaying macrocephaly, cognitive defects, and seizures. We generated a 'humanized' Kif6 mutant mouse strain to test the pathogenicity of this novel human patient mutant and observe progressive expansion of the skull coinciding with dilation of the ventricles, characteristic of hydrocephaly. Using light and electron microscopy, we observe a reduction of ependymal cell cilia. However, the specification and apical/basal polarization of ependymal cells were unaffected in Kif6 mutant mice. Furthermore, we observe similar phenotypes in kif6 mutant zebrafish. Interestingly, we did not observe expression of Kif6 or defects in other multiciliated cells or tissues, in either zebrafish or mouse Kif6 mutants. For these reasons, we propose that Kif6 has an ependymal cell specific role in building the axoneme of ependymal cell cilia. Future experiments will determine the subcellular localization of this novel kinesin and begin to address how this protein functions for establishment and maintenance of ependymal cell cilia. Funding for this project was supplied by the University of Texas at Austin Provost Graduate Excellence Fellowship.

Program Abstract #239

PI3K pathway overactivation induces cortical gyrification in mouse

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Evolution of the human brain is characterized by cortical expansion and gyrification complexity; yet mechanisms driving cortical gyrification remain poorly understood. From a clinical perspective, several genetic mutations that cause gyral abnormalities have been identified. A major outstanding question is how disease-causing gene function relates to cortical folding. Over-activating mutations of the phosphoinositide-3 kinase (PI3K) signaling pathway genes, especially of PIK3CA gene, encoding the catalytic subunit of the PI3K enzyme, result in severe human brain overgrowth and cortical dysplasia including dysplastic gyral pattern. To investigate mechanisms, we generated mouse models of human–related Pik3ca alleles. We previously published that these models recapitulate the key human pathological features including large brain, cortical dysplasia and epilepsy. Underlying developmental mechanisms included increased proliferation, enlarged cell-size and altered cell migration that correlated with defects in neural scaffold. Phenotypic severity was dependent on the mutant allele and its time of activation. We now demonstrate that these mutations also induced gyrencephaly in mice, a species which is normally lissencephalic. The most pronounced gyrification phenotype was observed in Pik3ca H1047R mutants in the dorsomedial neocortex and hippocampal CA1 zones. Timed induction experiments revealed the folding phenotype to have a critical period of PI3K overactivation from embryonic day (E)13.5 to E15.5. Our highly stereotypical and finely regulatable model of cortical folding represents the first of its kind in the field and has enabled us to define the cellular and molecular events that initiate and propagate cortical gyrification. This work was supported by NIH awards R01 NS050375 to WBD and R01 NS099027 to KJM.

Program Abstract #240

Targeted deletion of Crb1 and Crb2 in the optic vesicle induces crucial features of Leber congenital amaurosis 8

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The Crb1 and 2 (Crumbs homolog 1 & 2) genes encode large, single-pass transmembrane proteins essential for
apicobasal polarity of epithelial cells and cell-cell adhesion. Crb1 and 2 form apically localizing adhesion belts between retinal progenitor cells (RPCs) in the embryonic retina and between photoreceptors and Muller glial cells in the mature retina. Crb1 mutations cause inherited retinal degenerative diseases in humans: Leber congenital amaurosis type 8 (LCA8) and retinitis pigmentosa type 12 (RP12). LCA, which is characterized by severe vision loss at birth, affects 20% of children attending schools for the blind. In LCA impaired photoreceptor development and/or survival is thought to lead to blindness during early infancy, whereas, in RP12, progressive photoreceptor degeneration leads to peripheral vision impairment later in life. To generate a mouse model of LCA8 in which to investigate functions of Crb1/2 in developing ocular tissues, we used a mRx-Cre driver to generate allelic combinations that enabled conditional gene ablation from the optic vesicle stage. In this series, only Crb1/2 double knock-out (dKO) mice exhibited characteristics of human LCA8 pathologies, including; thickened retina at P21, potential hyper-reflective spots from P0, aberrant retinal cells, and severely disrupted retinal lamination reminiscent of the defective irregular interkinetic nuclear migration of RPCs. We also detected depigmented RPE, which may mimic pigmentary changes observed in human LCA8. The onset of these retinal defects was before E12.5, far earlier than the stage at which photoreceptor cells differentiate. These results suggest that human LCA8 can be modeled in the mouse by simultaneously ablating Crb1/2 from the beginning of eye development. Importantly, they also indicate that LCA8 is caused by malfunction of RPCs during ocular development rather than by defective photoreceptor-Muller glial interaction, which is a proposed mechanism for RP12.

Program Abstract #241
**Notch1 receptor alleviates cognitive dysfunction of diabetic mice via cross-talk with Wnt/beta-catenin and ERK1/2 signaling**
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Cognitive dysfunction is high-incidence in diabetes mellitus (DM). However, the detail mechanism is still unclear. Notch1 signaling pathway is considered as an important molecular of modulating metabolism. Until now, the function of Notch1 receptor (NR1) is poorly understood in the hippocampus of diabetic mice. In this study, adult male wild-type C57BL/6J mice and NR1 knock-down (Notch1+/-) mice were randomly divided into control group (WT CON), DM group (WT DM), Notch1+/- control group (Notch1+/- CON) and Notch1+/- DM group (Notch1+/- DM). Streptozocin was intraperitoneally injected in WT DM and Notch1+/- DM group for five days. After 8 weeks, the spatial learning and memory was examined by Morris water maze, the long-term potentiation (LTP) was recorded by electrophysiology, and then hippocampus were detected by Western blot assay. Data showed that knock-down NR1 exacerbated the spatial learning and memory impairment and the damage of LTP compared to those of WT DM group. The NR1 and Jagged-1 expression were markedly increased in WT DM group. However, knock-down NR1 led to the further decrease of NMDAR2A, NMDAR2B and PSD95 in Notch1+/- DM group compared to those of WT DM group. Interestingly, the beta-catenin expression and phosphorylation of ERK1/2 were decreased, meanwhile the p-beta-catenin was increased in Notch1+/- DM group. Our results suggest that diabetes induced cognitive dysfunction is at least partly via disturbing the cross-talk between Notch1 signaling and Wnt/beta-catenin and ERK1/2 signaling.

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Program Abstract #242
**Modulating the WNT pathway in Drosophila models of Cornelia de Lange Syndrome**
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Cornelia de Lange Syndrome (CdLS) is a rare genetic disorder affecting neurodevelopment and the gastrointestinal and musculoskeletal systems. CdLS is caused by mutations within NIPBL, SMC1A, SMC3, RAD21, HDAC8 and BRD4 genes. These genes codify for the cohesin complex (or associated proteins), a multiprotein structure playing a role in chromatid adhesion, DNA repair and gene expression regulation. Our laboratory has shown that a strong correlation exists between cohesin complex function and WNT signalling, an intracellular pathway involved in
regulation of expression of several genes controlling cell division and embryonic development. Recently, it has been observed that in nipblb- and smc1a-loss-of-function zebrafish embryos, in NIPBL- and SMC1A- mutated patient fibroblasts and in CdLS murine neural stem cells (NSCs) similar patterns of canonical WNT pathway alterations and cyclinD1 downregulation are present. Indeed, zebrafish embryos adverse phenotype was rescued by chemical activation of the WNT pathway. Drosophila melanogaster is an inexpensive model to study CdLS and to screen in vivo for therapeutic compounds. Therefore, we have used flies’ strains mutated in nipped-B and hdac3 genes (respectively NIPBL and HDAC8 in humans) for assessing the existing correlation between cohesin complex and WNT pathway. Moreover, we have selected D. melanogaster mutants to screen for chemicals that revert the CdLS associated-phenotypes efficiently. In particular, we have tested WNT activators in order to investigate which effects they have on the mutated flies, assessing body weight and changes in brain structures (i.e. mushroom bodies) and possibly select compounds to test on vertebrate models. Funding: This work has been supported by Fondazione Cariplo, grant 2015-0783 to Valentina Massa.

Program Abstract #243
The impact of human mutations in Dishevelled1 on WNT signaling
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Here we used the chicken embryo to understand the functional impact of nonsense mutations in the penultimate exon of DVL1 that cause Robinow Syndrome. The mutations delete most of the C terminal and replace it with a highly basic peptide. Avian retroviruses containing the wild-type gene or DVL1 with a single bp deletion (1529ΔG) were injected into the face and limb of chicken embryos. At E12, DVL11529ΔG mutation caused upper beak shortening (13/14) with many ectopic cartilages, whereas wtDVL1 resulted in mild deviations (10/25). Long bones were shorter both in DVL11529ΔG (14/18) and wtDVL1 (11/15) infected limb buds. In control limbs, chondrocytes assume a flattened morphology where the long axis of the cell is perpendicular to the long axis of the bone. Planar cell polarity (PCP) protein, Prickle, is typically concentrated at each end of the flattened chondrocytes. In limbs infected with DVL11529ΔG, chondrocytes are round and have lost restricted Prickle expression. RNAseq analysis revealed that the DVL11529ΔG significantly downregulated WNT5A and FZD2 which could explain why similar phenotypes are caused by mutations in different genes. We next investigated signaling defects caused by the mutation using the SuperTopflash luciferase reporter. Wild-type DVL1 induced luciferase activity 60-fold, whereas mutant DVL1 (1529ΔG) was significantly less effective (2-fold). When Wnt3a protein with wtDVL1 was added, luciferase activity was induced more than 100-fold above empty plasmid. In contrast, Wnt3a+DVL11529ΔG significantly suppressed activity (20-fold). When WNT ligand binds to its receptor, DVL1 dimerizes to create a signalosome. Thus, autosomal dominant RS can be partially explained by the mutant DVL1 dimerizing with wtDVL1 causing dominant negative effects on canonical WNT signaling. The dominant effect of DVL11529ΔG may cause randomized distribution of PCP molecules in the cells including Prickle. Funded by CIHR grant #MOP-123536 to JMR and a UBC fellowship to SG.

Program Abstract #244
Scoliosis Mutations in Juvenile/Adult Zebrafish
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Adolescent idiopathic scoliosis (AIS) causes twisting and bending of the spine and affects about 3% of the population with an onset during adolescence. Although common, the causes of AIS are not understood. We are currently performing a forward genetic screen of ENU-generated mutations leading to scoliosis in juvenile/adult zebrafish. Because of intensive fish husbandry, we can grow fish with adult phenotypes that may not thrive elsewhere. Here we present a few of the mutations recovered. They fall into 2 classes: fish that are strongly runted and develop scoliosis, and fish that develop into fertile adults with scoliosis. An example of the later category is mutation in BLOC-1s4. This gene is a subunit of the Biosynthesis of Lysosomal Organelles Complex,
effecting lysosomes and lysosomal related organelles in melanocytes, platelets, and the notochord. Adult zebrafish homozygous for a \textit{BLOC-1s4} mutation lack melanin pigmentation, have defective swim bladders, and frequently have multiple bends in the spinal column. Other pigment cells are present in adults. \textit{BLOC-1s4} mutants initially develop pigmentation but by 4 dpf (days post fertilization) have mostly lost melanin. Conditions that cause the notochord to kink, (loss of \textit{col8a1}, Gray, et.al. 2014 PMID: 24333517, low copper levels, Mendelsohn BA, et.al.,2006, PMID: 16890543) can cause the development of fused or malformed vertebrae. We find that notochord cells inflate on time at 24 hpf, but by 48 hpf, appear to be filled with small ball-like structures. We also report on timecourse of development of bends of the spine from juvenile to adult stages. We hope that analysis of these mutants will lead to a genetic animal model of scoliosis and an understanding of the mechanisms underlying the disease. Funding source: NIH (No. P01 HD084387)

\textbf{Program Abstract #245}

\textbf{Investigating the role for motile ciliated cell lineages in spine morphogenesis}

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The goal of this project is to further understand the connection between motile cilia loss and idiopathic scoliosis (IS). IS is a highly prevalent disease characterized by an abnormal three-dimensional curvature of the spine. Unlike congenital or neuromuscular scoliosis it has no known underlying cause, arising in the absence of obvious congenital or physiological defects. Genetic heterogeneity in patients plus a historical lack of suitable animal models have confounded our understanding of the disease. Surprisingly, teleost fish have been observed to develop spinal curves naturally. The Ciruna lab defined the first genetic model of idiopathic scoliosis in zygotic protein tyrosine kinase (\textit{ptk7}) mutant zebrafish (Hayes et. al., 2013; 2014). Ptk7 is a key regulator of the canonical and non-canonical Wnt pathways. Analysis of these mutants revealed defective ependymal cell cilia and disrupted cerebrospinal fluid (CSF) flow within the brain ventricles (Grimes et. al., 2017). The mechanisms however by which cilia and CSF flow defects cause scoliosis still remain unclear. To gain better insight, I am investigating the causes of IS in zebrafish \textit{katanin} p80 subunit (\textit{katnb1}) mutants, which also develop spinal curvatures during adolescence. Katnb1 is a microtubule severing protein that has roles at the cilia and basal body in single celled organisms, and is important in vertebrate neurogenesis, early embryonic patterning and ciliary signaling. We have demonstrated that zebrafish \textit{Katnb1} is not required for primary or nodal cilia formation, though strikingly ependymal cell cilia are completely lost in \textit{katnb1} mutants. By comparing distinct differences between \textit{katnb1} and \textit{ptk7} scoliosis phenotypes, I aim to determine when, where and how motile cilia function in spine morphogenesis, as well as investigate downstream mechanisms that sense CSF flow within the spine. Funding Sources Ontario Trillium Scholarship to A.M-M, Canadian Institutes of Health Research operating grant to B.C

\textbf{Program Abstract #246}

\textbf{Investigating the role of the subcommissural organ, Reissner’s fiber and SCO-spondin in spine morphogenesis and idiopathic scoliosis pathogenesis}

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Scoliosis is characterized by an abnormal lateral curvature and rotation of the spine. Idiopathic scoliosis (IS), which accounts for 80% of spinal curvatures, has no known cause. Adolescent IS is the most prevalent form, affecting approximately 4% of children between the ages of 11-18 worldwide. The genetic and biological mechanisms underlying IS are poorly understood and, as a result, treatment options remain limited to bracing and surgery. Our lab characterized the first genetically defined developmental model of IS in protein tyrosine kinase 7 (\textit{ptk7}) mutant zebrafish and linked defects in ependymal cell cilia and cerebrospinal fluid (CSF) flow to IS pathogenesis. However, how CSF homeostasis controls spine morphogenesis and whether CSF flow defects are linked to human IS remain unknown. To gain further insights, we are investigating the biological mechanisms of spinal curve formation in
SCO-spondin (sspo) mutants, a new zebrafish model of idiopathic-like scoliosis. SSPO is a high molecular weight glycoprotein secreted by the subcommissural organ (SCO), a highly conserved gland located in the back of the third ventricle of the brain. SSPO is the main component of Reissner’s fiber (RF), a threadlike structure which extends from the SCO down the central canal of the spine in most vertebrates. The SCO, RF and SSPO have been implicated in hydrocephalus, neurogenesis and CSF homeostasis; however, their roles in spine development have not yet been investigated. We hypothesize the SCO, RF and SSPO regulate adolescent spine morphogenesis and that defects in these structures can cause scoliosis in both zebrafish models and humans. We have identified a mutation in sspo that causes a dominant scoliosis phenotype in zebrafish and here, I will present work characterizing the role of the SCO, RF and SSPO in spine morphogenesis and idiopathic-like scoliosis pathogenesis. This work is funded by an operating grant from the Canadian Institutes of Health Research (CIHR).

Program Abstract #247
Focal neuroinflammatory signals drive spinal curve formation in zebrafish models of idiopathic scoliosis
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Idiopathic scoliosis (IS) is a debilitating disease characterized by three-dimensional curvatures of the spine that arise in the absence of observable physiological or anatomical defects. Although there has been investigation into genetic components of IS, the underlying cell biological and molecular mechanism for disease progression remains unknown. Consequently, treatment options are limited to bracing or surgery, which act more as preventative measures rather than a cure. Our lab has previously described the first genetically defined developmental model of IS in ptk7 mutant zebrafish. Homozygous ptk7 mutant zebrafish display hallmark phenotypes of the human disease, including 3-dimensional rotational spinal deformities, and sexual dimorphic bias in phenotype severity (Hayes et al., 2014). It was found that ptk7 mutants display a late-onset motile cilia defect that was directly responsible for the scoliosis phenotype (Grimes et al., 2016). To identify underlying mechanisms of disease, we employed next generation sequencing, lineage tracing and conditional genetic methodologies to define the spatial and biological origins of spinal curve formation in ptk7 mutant zebrafish. RNA-sequencing revealed an unexpected upregulation of immune factors. This, in conjunction with previous work, suggests that scoliosis may be caused by a neuroinflammatory response due to defective CSF flow. We demonstrate that focal activation of proinflammatory signals within the spinal cord is sufficient to initiate idiopathic-like spinal curvature, and that administration of anti-inflammatory molecules to juvenile ptk7 mutant animals significantly impacts the onset of scoliosis. Together, our data suggests that neuroinflammatory signals downstream of disrupted CSF homeostasis can initiate spinal deformity, and that modulation of these inflammatory signals could provide a treatment for this disease.

Program Abstract #248
Osteoarthritis induced by Has2 gene knockout of cartilage
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Osteoarthritis (OA), also known as degenerative joint disease, is the most common form of arthritis affecting over 26 million people in the US. It is characterized by the degradation of articular cartilage cells leading to joint dysfunction usually in the hips, hands, knees and spine. This debilitating disease has limited palliative care options and there are no effective treatments to date, because its genetic causes are unknown. The goal of our study is to determine one of the primary genetic causes of OA. The central dogma of molecular biology is an explanation of the flow of genetic information within a biological system. The Has2 gene codes for a major enzyme that produces hyaluronic acid, which is a glycosaminoglycan in the articular cartilage matrix. Using Has2 gene knockout in joint cartilage prevents the production of hyaluronic acid. Therefore, creating a hyaluronic acid deficient environment in joint cartilage. We used an inducible cartilage cell specific Cre-LoxP conditional transgenic mouse model to look into the genetic role of Has2 for the development of OA. To generate this system, the following mouse models were used: AgcCreERT2, tdTomato, and Has2. The correlation of Has2 to OA would be the first proven link.
Program Abstract #249

Adgrg6 is critical for homeostasis of the intervertebral disc in mouse
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Degenerative spine disorders are associated with significant morbidity and health care expenditures worldwide. A common tissue affected in spine disorders are the intervertebral discs (IVD) of the spine. Though age and genetic risk factors are contributors to spine disease, we currently understand little about the molecular signaling promoting homeostatic mechanisms of the IVD. Here, we have used conditional mouse genetics and chondrogenic cell culture approaches to identify a cellular role for the Adhesion G-protein coupled receptor G6 (Adgrg6) for normal spine alignment and homeostasis of the disc. We find no evidence for Adgrg6 function in bone forming lineages of the spine, however its deletion in chondrogenic lineages resulted in a low penetrance scoliosis in juvenile mutant mice, with progressive disc degeneration in aged mice. The loss of Adgrg6 in cartilaginous tissues resulted in the misregulation of genes important for cartilage development and extracellular matrix synthesis, but more interestingly, we observed upregulation of pro-inflammatory IL6/STAT3 signaling in young Adgrg6 mutant mice which we hypothesize underlies the accumulation of degenerative changes of the disc during skeletal maturation and aging in these mutant mice. We propose that ADGRG6 acts through a cAMP-mediated pathway to promote normal anabolic gene expression as well as dampen catabolic, pro-inflammatory pathways of the cartilaginous components of the IVD.

Program Abstract #250

Role Of Brachyury In Maintenance Of Neonatal Mouse Intervertebral Disc
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Intervertebral disc degeneration and associated lower back pain affect almost 70% people. Despite its high incidence, current treatments are palliative, which is mainly due to our limited understanding of normal disc growth, maintenance, and aging or degeneration. Each disc has three main components; a central core of notochord derived nucleus pulposus (NP), surrounded by collagenous annulus fibrosus, and adjacent cartilaginous endplates. We have previously shown that the postnatal NP expresses notochordal markers like Shh, Brachyury (Bra/T) and Krt19. We also showed that Shh signaling, which is essential for postnatal disc growth and maintenance, even regulates BRA expression in the mouse discs. The expression of SHH and BRA decreases with age. However, we do not know the role of BRA is postnatal NP cells. As the current Bra mutants have a severe skeletal deformity, we generated a floxed allele of Bra to understand its role in the postnatal NP cells. First, we characterized the Bra floxed allele which did not display any defects in development, skeleton, or viability. Next, we conditionally targeted Bra in the newborn mice using NP-specific driver line and observed serve pathological changes in disc structure including clumping of NP cells and loss of organization of the AF layers ten days later. We also found a decline in cell proliferation and extracellular matrix markers that are essential for the maintenance of disc structure and function. Loss of BRA also resulted in increased cell death in the NP cells, suggesting it is crucial for the viability of the postnatal NP cells. We did not observe any changes in the SHH expression, suggesting BRA acts downstream of SHH. However, we do not know whether BRA continues to regulate NP cells in the adult stages, and will require further studies. Acknowledgments: NIAMS/NIH R01AR065530 and Gerstner Family Foundation.
Program Abstract #251
Altered glucose metabolism in TRIM32 deficient muscle
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The E3 ubiquitin ligase TRIM32 is a member of tripartite motif (TRIM) family of proteins involved in various processes including differentiation, cell growth, muscle regeneration and cancer. The N-terminus of this protein is characterized by a RING domain, B-box domain, and Coiled-Coil region, while the C-terminus contains six NHL repeats. In humans, mutations that cluster in the NHL domains of TRIM32 result in the muscle disorders Limb-Girdle Muscular Dystrophy type 2H and Sarcotubular Myopathy. The model organism Drosophila melanogaster possesses a TRIM32 homolog, encoded by the thin (tn) gene, that is highly expressed in muscle tissue. We previously showed that TRIM32 is localized to Z-discs of the sarcomere and is required for myofibril stability. Muscles form correctly in tn mutants, but exhibit a degenerative muscle phenotype once contraction ensues. It is predicted that mutations in the NHL domain either affect protein structure or are involved in protein-protein interactions. However, the molecular mechanism by which these mutations affect the interaction properties of TRIM32 is not understood. We utilized biochemical pulldown assays using TRIM32-NHL as bait to identify TRIM32 binding proteins in larval muscle tissue. Many key glycolytic enzymes were present in the TRIM32 pulldowns and not in control experiments. Strikingly, many glycolytic proteins are also found at the Z-disk, consistent with TRIM32 localization. Our biochemical binding assays show a direct interaction between TRIM32 and the glycolytic proteins Aldolase and Phosphoglycerate mutase. TRIM32 also regulates glycolytic enzyme levels and protein localization at their sites of action. Moreover, metabolomic studies revealed metabolites involved in glycolysis and amino acid metabolism were altered. These data together suggest a role for TRIM32 in coordinating glycolytic enzyme function, possibly for localized ATP production or to maintain muscle mass via glycolytic intermediates.

Funding - NIAMS.

Program Abstract #252
Protein dynamics of the intraflagellar transport (IFT) complex
Jaime Hibbard, John Wallingford
The University of Texas at Austin, USA

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

Program Abstract #253
A novel protein complex in ciliogenesis and development
Janelle Leggere, Elle Roberson, Edward Marcotte, John Wallingford
The University of Texas at Austin, USA

Cilia are microtubule-based organelles that protrude from the majority of cells in the human body and play essential functions in cell-cell communication. Defects in the formation or function of cilia cause a broad class of
common developmental disorders, termed ciliopathies, that result in debilitating or lethal congenital birth defects. In collaboration with clinical geneticists, recent work by our labs has revealed that multiple ciliopathies are caused by mutations in a novel protein complex necessary for ciliogenesis, CPLANE (Cilia and PLANar cell polarity Effectors). However, the mechanism of CPLANE action in mammalian primary cilia remains unclear. We are therefore combining mouse genetics, cell biological studies of CPLANE mutant cells and novel proteomic methods to elucidate CPLANE’s molecular mechanism in mammalian primary cilia. Current work focuses on genetic interactions between the CPLANE genes and on the role of CPLANE in assembly of the ciliary transition zone. This work is funded by the NIHCD.

Program Abstract #254
A phase separated organelle for axonemal dynein assembly in ciliated cells
Chanjae Lee, Ryan Huizar, Alex Boulgakov, Edward Marcotte, John Wallingford
University of Texas at Austin, USA

Motile cilia are microtubule based cellular projections and are involved in fluid flows that are critical for development and homeostasis. The motile behavior is generated by multi-protein dynein motors attached to axonemal microtubules. Dynein motors are pre-assembled in the cytoplasm before being deployed to cilia. Importantly, mutations in an array of dyneins or cytoplasmic Dynein Arm Assembly Factors (DNAAFs) result in primary ciliary dyskinesia (PCD) manifesting in chronic respiratory infections, situs inversus and male and female infertility. Here, we show that dyneins and their assembly factors concentrate all together in cytosolic foci named Dynein Assembly Particles (DynAPs). DynAPs also contain core Hsp70/90 chaperones and specific co-chaperones. Interestingly, DynAPs are multiciliated cell specific and are induced by ectopic expression of motile ciliogenic transcription factors, such as Mcidas and Foxj1, which are sufficient to convert nonciliated cell to ciliated cells. In addition, DynAPs display hallmarks of biological phase separation including rapid fission and fusion between particles and rapid exchange of material with cytosol. These data suggest that DynAPs are novel cell type specific liquid-like organelles and that one such organelle is defective in a human genetic disease. This work was supported by NIH grants (R01 HL117164, R21 GM119021 and R01 HD085901).

Program Abstract #255
Regulation of Inorganic Phosphate at the Maternal-Fetal Interface
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Phosphorus is an essential nutrient required for cellular energetics, growth, bone ossification, and other critical processes. In adults phosphorus is obtained by dietary consumption and circulates through the blood stream as phosphoric acid, referred to as inorganic phosphate (Pi). Dysregulated Pi levels can lead to significant health challenges, including Pi toxicity, refeeding syndrome, skeletal phenotypes, and ectopic calcification. The mechanism(s) that regulate mammalian maternal-fetal Pi transport during embryogenesis are poorly understood. In the study presented herein we examined Pi transport mechanisms by defining symport kinetics, comparing expression levels of sodium-dependent Pi transporters, and establishing protein localization patterns for the most abundant transporters, Slc20a1 and Slc20a2. We then tested the hypothesis that Slc20a2 is required for maternal-fetal Pi transport. We observed decreased amniotic fluid Pi levels and impaired fetal bone mineral deposition as well as ectopic calcification in the placenta. Together, the data support that Slc20a2 is required for maternal-fetal Pi transport, effectively promoting embryonic Pi accretion and protecting the placenta from ectopic calcification. We further observed atypical vascular morphology prior to pathological mineral deposition in Slc20a2 null placentas. A morphogenetic phenotyping approach was used to evaluate molecular mechanisms by which Slc20a2 may promote normal vascular development of the placenta. In conclusion, we now propose that Slc20a2 promotes placental development by protecting placental cells from high extracellular Pi, and subsequent cell death, inflammation, and osteoblastic differentiation. The findings presented herein provide the first
comparative study of maternal-fetal Pi transport biology and support that Slc20a2 plays a critical role in both placental sufficiency and mammalian embryonic development. Funding: NICHD K99HD090198, PI: Wallingford, MC.

Program Abstract #256
Manipulation of the Redox Environment Alters the Oncogenic Activity of Ras and Src in Drosophila
Leslie J. Saucedo, Bridget N. Alexander, Rosalie E. Triolo, Kate E. Segar
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Oxidative stress helps establish cancers by mutating genes that regulate cell growth and by modulating Src and Ras: two oncoproteins that have been shown to be activated by reactive oxygen species (ROS). Once the empowering genetic mutations are obtained, cancerous cells benefit from an increase in antioxidants in order to protect themselves from oxidative stress-induced apoptosis. Established cancers can aberrantly express enzymes that control the redox environment and the administration of antioxidants has been demonstrated to accelerate the progression of some cancers. Because cancers are genetically heterogeneous in humans and mammalian models, we use the UAS-GAL4 system in Drosophila to tightly control gene expression and quantify subsequent phenotypes within a few days. In this study, we genetically manipulated the levels of NRF2 and Keap1; two master regulators of the cellular redox environment while simultaneously overexpressing Src or Ras. We found that either increasing or decreasing antioxidant activity suppresses the oncogenic activity of Ras in multiple cell types. In contrast, we found that decreased antioxidant activity promotes a Src-induced tumorigenic phenotype. In spite of the differences between mammals and Drosophila, flies have successfully informed numerous mechanisms that contribute to human cancer biology. Because inhibiting antioxidants as a therapeutic approach to cancer is gaining momentum, we hope that our findings can provide a more complete understanding of how the redox state can either promote or suppress oncogene activity. Funded provided by the University Enrichment Committee of the University of Puget Sound.

Program Abstract #257
Are You What Your Mother Ate?: Embryo Environment Shapes Adult Phenotype
Caralina Marin de Evsikova
University of South Florida, USA
Common daily environmental exposures, such as heavy metals, airborne pollutants, and pesticides, can shape embryonic development and alter adult phenotype. Long-term consequences of sub-lethal environmental exposures are often unknown. The round worm C. elegans is an excellent model organism to investigate how embryo environment shapes the adult phenotype. To determine developmental windows when environmental exposures act to alter adult phenotype, we exposed C. elegans to sub-lethal doses (0.1-100 µM) during specific larval stages or at adulthood and measured changes in growth, fertility, and energy balance. Environmental exposures include: estrogen mimics Bisphenol A (BPA) and diethylstilbestrol (DES), metals and metalloids (cadmium Cd, arsenic As), biocides (triclosan TRI, tributyltin TBT, fenthion FEN) and airborne pollutants (cigarette smoke CSE, nicotine NIC, benzo-α-pyrene BAP). Some environmental exposures stunted growth (Cd, BAP, TBT), impaired sexual maturity and fertility (Cd, As), while others increased egg-laying (NIC, BPA). Notably, reproductive fitness and longevity were intertwined because hatching and embryo viability were greater in eggs laid early, albeit these parents died sooner than late layers. Most environmental exposures shifted energy balance and altered lipid storage, but only in offspring, while BPA also affected adults. In mammals, environmental exposures are suggested to affect embryogenesis and alter adult phenotype via nuclear hormone receptor (NHR) signaling, specifically PPARy. C. elegans and other nematodes have an exceptionally expanded NHR gene family (~300 vs. ~40 in mammals) but identification of PPARγ homolog proves challenging. Consequently, we created a combined, “double bait” domain homology identification program to detect candidate PPARs in nematode genomes and found strong candidate homologs of PPARγ in worms, with a candidate signaling akin to PPARα, and candidates were validated using RNAi. Funding: Impact Assets.
Program Abstract #258
Trehalose improves social and repetitive behaviors in a Valproic Acid-Induced rat model of Autism
Jingxuan Fu, Tao Zhang
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Autism is a neurodevelopmental disorder characterized by the impairments of social interaction and repetitive behavior. It reports that the valproic acid (VPA) exposure in utero may downregulate autophagy. Furthermore, trehalose has gained attention for its ability to induce cellular autophagy. In the present study, we investigated if social and repetitive behaviors were improved in a rat model of autism through regulating autophagy. Male Wistar rats, which were offspring of pregnant rats exposed to a single injection of VPA, were used. The animals were randomly divided into 3 groups: CON group (n=6), VPA group (n=6) and VPA+TRE group (n=6). Trehalose at 2% concentrations in drinking water was given from postnatal day 21(P21) to P60 in the VPA+TRE group. Sociability was assessed by the three chamber test. The duration in the stranger chamber is shorter in the VPA group than that in the CON group (p<0.05), however, it is longer in the VPA+TRE group than that in the VPA group (p<0.05). Moreover, the duration in the empty chamber is longer in the VPA group than that in the CON group (p<0.001), while it is shorter in the VPA+TRE group than that in the VPA group (p<0.05). There are no statistical differences of the duration in the center area among these three groups. Moreover, the marble burying test is used to measure repetitive behavior. The rats in the VPA group baried more marbles compared to that in the CON group (p<0.01), however, the animals in the VPA+TRE group buried less marbles compared to that in the VPA group (p<0.01). It suggests that trehalose improves social and repetitive behaviors in VPA rats, and the up-regulation of autophagy is possibly an underlying mechanism which needs to be explored in future. The work was supported by NSFC (31771148).

Program Abstract #259
Zebrafish Models of Pediatric Brain Tumors
Mattie Casey, Katarzyna Modzelewska, Rodney Stewart
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Zebrafish models of cancer are a powerful system to identify cell of origin, oncogenic drivers and drug targets in human cancers. In addition, the unique imaging properties of transparent zebrafish and scalability allow tumor growth and dissemination to be easily measured in real time for testing tumor response to new therapeutics. Here we use comparative genomics between human, mice and fish, together with genomic editing and transgenic approaches, to identify oncogenic drivers in pediatric brain tumors and model these changes in zebrafish brain tumors. Using these techniques, we generated zebrafish with specific pediatric brain tumors, including medulloblastoma and primitive neuroectodermal tumors (CNS-PNETs). We show that amplification of NRAS, observed in a subset of human PNET tumors, is sufficient to drive malignant tumors when targeted to embryonic oligodendrocyte precursor cells. Comparative genomics and immunohistochemical analysis show the NRAS-driven tumors represent a distinct subgroup of human CNS-PNET tumors that occur along the entire CNS axis. To complement these studies, we developed novel orthotopic embryonic brain tumor transplantation assays for high-throughput drug screening and show MEK activity is essential for the oligodendrocyte-derived CNS-PNET tumor growth in vivo. Thus, MEK inhibitors may represent the first targeted therapy option for children with CNS-PNET or other embryonal tumors with RAS/MEK pathway activation. As many pediatric brain tumor entities have no cell- or animal-based models available for drug development, the zebrafish system offers a unique platform to rapidly identify cellular origins, oncogenic drivers and therapeutics for childhood brain tumors.

Program Abstract #260
The Tumor Suppressor Hypermethylated in Cancer 1 (Hic1) Interacts with Wnt Signaling Pathways During Neural Crest Migration
Heather Ray
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Many members of the POZ-ZF transcription factor (TF) family are known regulators of both developmental processes and cancer. The POZ-ZF TF Hypermethylated in Cancer 1 (HIC1) is an epithelial tumor suppressor that is recognized as a valuable prognostic marker. Additionally, loss-of-function mouse alleles show embryonic lethality accompanied with developmental defects that are reminiscent of human Miller-Dieker syndrome, a complex developmental disorder resulting from a large genomic deletion that includes the HIC1 gene. To date, little is known as to the mechanisms of HIC1 function in cancer or embryogenesis, prompting us to use the power of the *Xenopus laevis* model system to further investigate this TF. We find that *hic1* is expressed throughout early development, including within the branchial arches. Targeted micro-injection of either *hic1* mRNA (overexpression) or a translation-blocking morpholino (*hic1* MO, knockdown) to the neural-neural crest domain results in craniofacial defects including malformation of the craniofacial cartilages. In situ hybridization (ISH) reveals that early patterning of neural and cranial neural crest (CNC) domains is mostly unaffected, but migration of the CNC population is altered. Transplant and explant studies using CNC cells from *hic1* morphants further demonstrate that overall CNC migration is decreased and morphant cells exhibit defects in lamellipodial formation and persistence. Mechanistic studies using ISH, RT-PCR and secondary axis formation assays reveal that *hic1* interacts with Wnt signaling pathways. Our data help to shed light on the mechanisms of *hic1* function. Ongoing studies are aimed at elucidating the molecular nature of *hic1*-wnt interaction during CNC migration, with implications for our understanding of both developmental disorders and cancer. Funding sources: National Science Foundation grant ISO-1558067 UAB IRACDA-MERIT Program K12 GM088010

**Program Abstract #261**

**LvLOX is required for midline PMC positioning and skeletal patterning in sea urchin embryos**

Daniel Zuch, Kristin Dionne, Michael Piacentino, Sviatlana Rose, Kanwal Aziz, James Huth, Cynthia Bradham

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Our understanding of developmental pattern formation remains superficial, especially for complex, 3D structures. Sea urchin skeletal patterning offers a comparatively simple model to study the molecular basis for embryonic pattern formation. The larval endoskeleton is secreted by migratory primary mesenchyme cells (PMCs). PMCs integrate local positional cues from the overlying ectoderm, then establish spatially-delimited expression profiles while migrating into a stereotypical 3D arrangement, along which they secrete calcium carbonate biomineral. From an RNA-Seq-based screen for skeletal patterning genes, we identified Lv-Lipoxygenase (Lv-LOX) as a potent regulator of PMC migration to the embryonic bilateral midline. Lv-LOX expression is initially restricted to the apical plate, then transitions to the ventrolateral ectoderm during skeletogenesis. Knockdown or inhibition of Lv-LOX results in embryos with truncated midline skeletal elements and elements that are abnormally rotated with respect to the midline. Accordingly, PMCs in perturbed embryos fail to migrate to the midline and form abnormal filopodial connections between non-adjacent PMCs, which may underlie observed skeletal rotation defects. Using a combination of RNA *in situ* hybridization and immunofluorescent labeling of PMCs, we mapped the 3D spatial expression pattern of several PMC subset-specific genes throughout skeletogenesis. Perturbation of LOX function causes a dramatic increase in the expression of the subset-specific gene *pks2*, particularly within PMCs occupying ventral and midline positions. Further, PMCs in *pks2* morphants exhibit perturbed ventral midline migration, indicating that *pks2* is required for territory-specific PMC migration. These results demonstrate that Lv-LOX is a key regulator of sea urchin skeletal patterning, specifically with respect to midline elements and bilateral alignment of the skeletal halves. This project is funded by an award from the National Science Foundation to CAB

**Program Abstract #262**

**Expression and function of sfrp1/2/5c, a secreted Wnt modulator, in the leech embryo**

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The evolutionarily conserved Wnt signaling pathway is important in segmentation, anterior-posterior differentiation and regulation of stem cells and has been studied primarily in vertebrate, insect and nematode embryos, representing two of the three super-phyla of bilaterally symmetric animals (Deuterostomia and
Ecdysozoa). Thus, understanding how Wnt signaling functions in representatives of the third super-phylum (Lophotrochozoa) should contribute to elucidating the range of developmental mechanisms within the evo-devo space. The leech Helobdella austinensis (phylum Annelida) is a segmented worm that is amenable to lab culture and reproduces via large, experimentally tractable embryos that develop through stereotyped cell lineages. This species thus represents a useful model for studying how annelid embryos use the Wnt pathway. Previous in situ hybridization surveys indicate that Wnt ligands and modulators are expressed in the posterior growth zone (PGZ) of the embryo (Cho et al., 2010). Specifically, transcripts encoding Secreted Frizzled-Related Protein 1/2/5c (SFRP1/2/5c; Bastin et al. 2015), an extracellular Wnt modulator, are expressed in ventral and dorsal ectodermal cell lineages (N and Q, respectively), suggesting a role in cell fate determination and PGZ function. We aim to further characterize wild-type sfrp1/2/5c expression in the Q lineage of H. austinensis embryos, and to analyze the function of SFRP1/2/5c in the PGZ using a CRISPR/Cas9 approach. Mutant embryos developed significant germinal band malformations, suggesting that SFRP1/2/5c affects germinal band migration in the leech embryo, presumably by its effects on Wnt signaling. This work was funded by NIH.

Program Abstract #263
Probing Hox-related developmental mechanisms in the leech Helobdella austinensis
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Hox genes encode transcription factors that are well known for their roles in establishing segment identities in arthropod and vertebrate embryos. Within Annelida (segmented worms), Hox functionality is not as well understood; however, Hox expression in various taxa is at least partially consistent with presumed ancestral patterns, such as spatiotemporal co-linearity and complementary expression at tagma boundaries. For clitellate annelids (oligochaetes and leeches), genome-level analyses have revealed expanded Hox-gene complements and an atomized Hox cluster in the leech Helobdella, suggesting that Hox cluster evolution was an ongoing process during clitellate diversification, and that clitellate Hox genes may be under relaxed regulatory constraint. We are examining the roles that Hox genes play during embryonic development of the leech H. austiinesis by examining their expression patterns and CRISPR knockout phenotypes. We are focusing primarily on lox4a, lox4b, post2a, post2b, and post2c—Hox genes that are duplicated, unclustered, and associated with the development of novel posterior features in the leech, such as the caudal sucker, dorsal rectum and anus, and the large posterior ceca of the midgut. We find that, in contrast to their single-copy homologs in polychaete annelids, expression of most of these leech genes is initiated after segment identities are specified, and their transcripts accumulate in non-overlapping tissues, indicating significant regulatory differences within annelids. Our analyses of post2a knockouts show that this gene functions in morphogenesis of the caudal sucker, rectum, and anus, and that it does so, and least in part, by repressing lox4b expression in caudal mesoderm. Finally, we are also investigating whether Hox expression in the caudal tagma of Helobdella is activated by wnt11c signaling. We acknowledge the NIH for funding our research.

Program Abstract #264
The maternally expressed Hox gene Ax6a is required for gastrulation and the formation of bilateral symmetry in the cnidarian Nematostella vectensis.
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Hox genes are homeobox transcription factors that are responsible for patterning along the primary axis and are found in all bilaterians, a group that makes up 99% of metazoan life. Cnidarians, such as anenomes and corals, represent the only phylum outside of the Bilateria to have Hox genes, making them an important sister group for studying Hox gene evolution. The anthozoan gene Ax6a is a cnidarian specific Hox gene that has maternal expression and is expressed at the site of gastrulation, suggesting it has an important role in early development. Furthermore, Ax6a is asymmetrically expressed along the future site of gastrulation when visualized with in situ hybridization. When Ax6a is prevented from being expressed through the injection of eggs with an Ax6a
antisense morpholino, treated embryos fail to gastrulate and oral, aboral, and directive axis is disrupted, suggesting that it has a large effect on the embryos development outside of its own asymmetrical oral domain. Furthermore, when Ax6a mRNA is injected ectopically into random blastomeres at the 8-32 cell stage, a second site of invagination, and later, gastrulation is induced, with expression of some markers for oral development. This implies that Ax6a plays a very important role in axial patterning and suggests that anterior Hox genes may have had fundamental roles in establishing the primary body axis in the bilateral lineage. Funded by the Society for Developmental Biology Choose Development! program.

Program Abstract #265
Identification of Hox and GATA as context factors for the Wnt effectors TCF and β-catenin regulating early lineage patterning in C. elegans embryos
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The Wnt signaling pathway activates many target genes in the developing C. elegans embryo through its effectors TCF/POP-1 and β-catenin/SYS-1. To understand how Wnt activates distinct target genes in different signaled cells, we investigated the regulation of two Wnt targets: Hox1/ceh-13 and Hox9/nob-1. Unlike most animals, expression of these Hox genes are early, broad, and overlapping, with Hox1/ceh-13 expressed one cell cycle before Hox9/nob-1 in several Wnt-signalized posterior daughter lineages at the 24-cell stage. We identified enhancers that control this early embryonic expression of Hox1/ceh-13 and Hox9/nob-1, including several putative shadow enhancers with overlapping expression. Hox1/ceh-13 and the Hox co-factor Meis/unc-62 activate Hox9/nob-1 expression in one particular sublineage through two enhancers, but a third Hox9/nob-1 enhancer active in this sublineage does not require Hox1/ceh-13 or Meis/unc-62, suggesting the use of multiple regulatory strategies in these overlapping enhancers. We identified GATA/elt-1 as a regulator of Hox9/nob-1 expression in a distinct sublineage. Each Hox9/nob-1 enhancer has multiple predicted binding sites for HOX1 or GATA and for TCF. This suggests that Hox1/ceh-13 or GATA/elt-1 provide context information to define which lineages express Hox9/nob-1, while TCF and β-catenin further limit Hox9/nob-1 expression to Wnt-signalized daughter cells. These results demonstrate that Hox9/nob-1 is regulated by different combinations of context transcription factors in different sublineages. Our observation of an anterior Hox gene positively regulating expression of a posterior Hox gene to specify extreme posterior structures is novel. Furthermore, it appears that, in C. elegans, Hox genes have acquired unique early lineage specification functions alongside their normal role in later positional identity, suggesting more diverse functions for Hox genes may exist in other simple or unsegmented organisms. Support: NIH GM11825, GM105676.

Program Abstract #266
The Ryanodine Receptor intracellular calcium release channels are required for Left/Right patterning in the zebrafish embryo
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Although humans appear highly symmetric from the outside, on the inside many of our organ systems develop predictable L/R asymmetries. Our goal is to identify the events that initiate asymmetric gene expression and consequently pattern left-right (L/R) differences. In zebrafish, at the base of the notochord, Kupffer’s Vesicle (KV) generates a signaling cascade that begins with fluid flow and leads to asymmetric patterning of gene expression and subsequent asymmetric organ morphogenesis. Elevated intracellular calcium in neighboring tissue on the left side of the KV is associated with fluid flow and has been proposed to be a relay signal functioning to affect the initiation of asymmetric gene expression. First we demonstrate that asymmetric calcium release is dependent on fluid flow within the KV. As ryanodine receptors (RyR) mediate the release of calcium from intracellular stores into the cytoplasm, and as these channels are maternally supplied and broadly expressed in the embryo, we tested the
hypothesis that RyR function may be required for proper L/R patterning. We generated protein null mutations in zebrafish ryr genes. Although loss of zygotic ryr expression does not affect heart looping, a downstream consequence of normal L/R patterning, loss of maternally supplied RyR channels disrupts heart looping. Using loss-of-function ryr mutations, morpholino knockdown of ryr expression, and pharmacologic inhibition of RyR channel function, we demonstrate RyR-dependent Ca^{2+} fluxes are required for the asymmetric expression pattern of southpaw, which encodes the signaling growth factor responsible for propagating left-side-specific gene expression. Loss of RyR activity also disrupts the right-sided bias of the earliest asymmetrically expressed gene, charon, which is a local inhibitor of southpaw gene expression. Thus our work suggests RyR activity is required for early asymmetric gene expression which is necessary for proper L/R patterning of the developing embryo.

Program Abstract #267
Characterization of narigoma, a regulator of anterior gut left-right asymmetry in Drosophila melanogaster
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Left-right (LR) asymmetry is a common feature which is genetically determined to obtain specific LR directions in many organisms. Comparing to the well-studied asymmetry developing systems in vertebrates, studies in invertebrates are poorly done. Thus, we use Drosophila melanogaster as an invertebrate model for studying LR asymmetry. In Drosophila, the first organ that shows LR asymmetry is the gut. The embryonic gut can be divided into three main parts: foregut, midgut, and hindgut, all of which show directional asymmetry. By studying the embryonic gut, we can decipher the first symmetry-breaking cues in flies, and understand the origin of LR asymmetry more comprehensively. Previously, we conducted a set of genetic screens to identify mutations that affect LR asymmetry of the embryonic gut. The narigoma (nag) mutant shows a high frequency of LR inversed anterior gut (foregut and anterior midgut) during embryogenesis. Additionally, nag mutant flies are homozygous viable and fertile, suggesting that its function is primarily involved in determining laterality, and is not crucial for survival. The locus of nag has been narrowed down by complementation test and whole genome sequencing analysis to a ~50kb region on the 3R chromosome. Among all the genes within this region, CG6475 is the most compelling candidate. CG6475 is an unannotated gene predicted to encode a UDP-glucuronosyl/UDP-glucosyltransferase (UGT), which catalyzes the addition of a glycosyl group from a nucleotide sugar to other small lipophilic molecules. Past research in our lab has shown that dally-like (dlp), a glypican encoding gene, functions to determine LR asymmetry in the anterior gut by regulating the Wnt signaling pathway. Therefore, our present results suggest that CG6475 is involved in modifying the heparan sulfate chains on Dlp, which changes its ability to regulate Wnt signaling properly, and eventually leads to laterality defects in the anterior gut.

Program Abstract #268
Nodal patterning without Lefty inhibitory feedback is functional but fragile
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Developmental signaling pathways often activate their own inhibitors. Such inhibitory feedback has been suggested to restrict the spatial and temporal extent of signaling or mitigate signaling fluctuations, but these models are difficult to rigorously test. Here, we determine whether the ability of the mesendoderm inducer Nodal to activate its inhibitor Lefty is required for development. We find that zebrafish lefty mutants exhibit excess Nodal signaling and increased specification of mesendoderm, resulting in embryonic lethality. Strikingly, development can be fully restored without feedback: Lethal patterning defects in lefty mutants can be rescued by ectopic expression of lefty far from its normal expression domain or by spatially and temporally uniform exposure to a Nodal inhibitor drug. While drug-treated mutants are less tolerant of mild perturbations to Nodal signaling
levels than wild type embryos, they can develop into healthy adults. These results indicate that patterning without inhibitory feedback is functional but fragile.

Program Abstract #269
Regulation of Head Development by DNA Polymerase δ
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The head development during mouse early-stage embryogenesis requires accurate spatial-temporal regulation. Recently we identified an ENU-induced mutation that disrupts head development featuring a small head with abnormal anterior patterning, which resembles the phenotype in Otx2 or Lhx1 null mutants. Surprisingly, whole exome sequencing revealed a missense mutation (D939Y) in Pold1 gene. Pold1 encodes the catalytic subunit of DNA polymerase δ, which is required for DNA synthesis and DNA repair. We find that a knockout-first allele of Pold1 fails to complement the Pold1D939Y allele: Pold1D939Y/− arrests development at E6.5, confirming that the Pold1D939Y partially disrupts the activity of POLD1. Pold1 mutation has been reported in human genetic diseases such as mandibular hypoplasia, deafness, progeroid and lipodystrophy (MDPL). Patients with MDPL harbor the same pS605del mutation in POLD1, indicating tissue-specific function of POLD1 in development. In contrast to the Pold1 null mouse mutant (Uchimura et.al., 2009), which has a severe cell proliferation defect causing peri-implantation lethality, the Pold1D939Y/D939Y embryos do not have a global cell proliferation defect. At E8.0, the overall morphology of the mutant is abnormal: the mutant head is located at the distal tip of the embryo and body extends along distal-proximal axis of the embryo. In situ hybridization for Otx2 shows that expression of this anterior marker is restricted to the distal tip in mutant embryos. At E8.0, SOX2 is expressed in the anterior epiblast but is only expressed at the distal tip of the mutants, consistent with the in situ data. Given the unique phenotype, the Pold1D939Y hypomorphic allele suggests that DNA polymerase δ may have complex roles in early embryonic development. We will generate Pold1 conditional knockout mice to determine which lineage requires POLD1 activity to promote head development. This work is supported by the NIH grant (NIH R37HD03455 to K. Anderson).

Program Abstract #270
Uncovering novel regulators of neural fate commitment in embryonic stem cells
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How do pluripotent embryonic stem (ES) cells reliably select a neural fate during differentiation? When ES cells are exposed to a homogenous signalling environment differentiation is variable and unpredictable. Whilst the key extrinsic signalling pathways that inhibit neural lineage commitment have long been established, less progress has been made in identifying downstream effectors of these pathways. The finding that BMP signalling blocks entry into the neural lineage via transcriptional upregulation of Inhibitor of Differentiation (Id) proteins provides strong evidence that neural induction proceeds via derepression of an unknown positive-acting regulator. We have previously demonstrated that the major binding partners of Id proteins in ES cells are E-protein members of the bHLH family of transcription factors, the activity of which is dependent on the formation of functional homo- or heterodimers. We hypothesise that this interaction represents an important first step towards neural lineage commitment. Using a CRISPR/Cas9 targeting approach to generate endogenously-tagged Id1 and E-protein reporter cell lines, we are able to follow and quantify changes in protein expression at the onset of differentiation. We have also demonstrated using a doxycycline-inducible system that overexpression of a specific forced E-protein homodimer, but not monomer, is sufficient to induce neural commitment in pluripotent ES cells, even under self-renewal conditions. In combination with ongoing loss-of-function approaches we aim to use this information to gain greater mechanistic insight into the earliest stages of the multi-step process of neural commitment.
Program Abstract #271
Measuring genome-wide transcription rates in the *Caenorhabditis elegans* embryo
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Rapid and timely transcription is critical in cells that have to respond quickly to environmental and developmental cues. In the dividing embryo, developmental factors need to accumulate to threshold levels to specify cell fate, despite the constraints of short cell cycles. Early zygotic genes in many species have short primary transcripts, suggesting that they have evolved to sustain rapid transcription. High rates of PolII loading and elongation influence patterning in *Drosophila* embryos, indicating the importance of rapid mRNA accumulation during development. Yet, genome-wide mRNA accumulation rates have not been measured in individual embryonic cell types. The *C. elegans* embryo is an ideal system to examine temporal transcription at the single cell level, as it develops with an invariant lineage and shows rapid changes in zygotic transcripts. For example, transcripts of *end-3*, an endoderm specification factor, accumulate from 0 to ~600 mRNAs in 15 minutes in a single cell. We hypothesize that rapid accumulation is a common feature of early zygotic regulators, is controlled by multiple mechanisms, and is required for robust cell fate specification. We have performed single cell RNA sequencing (scRNA-seq) from ~15,000 embryonic cells and identified distinct transcription profiles in early and late progenitors, including many genes with rapid changes. We are currently measuring transcript accumulation rates of all zygotic genes by combining 5-ethynyluridine (EU) labeling of RNA with scRNA-seq using the 10X genomics platform. The pooled data from labeled and unlabeled RNA, and the knowledge of expression patterns of zygotic factors will allow us to estimate the transcription rates and mRNA abundances in each embryonic cell. This strategy for identifying recently transcribed RNAs can also be used to refine worm embryonic lineage maps and extended to other systems to find conserved properties of rapidly transcribed genes. Funded by the NIH GM105767 and HD085201.

Program Abstract #272
Indexing tissue development specific transcription start sites in mouse embryogenesis
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Cell fate determination of a metazoan involves both common and tissue-specific genes, whose dysregulation can cause developmental disorders. As existing tissue specificity indices of genes were designed for *developed* tissues, based on static gene expression, they are not readily extendable to *developing* tissues whose transcriptome is highly dynamic. To characterize the tissue development specificity of a gene, we employ a model-free statistical method called functional chi-squares (FunChisq) to test the mathematical functional dependency of tissue type on gene expression. A FunChisq-derived conditional functional index ($\xi_f$) measures the effect of a functional relationship, ranging from 0 (no or constant) to 1 (perfect) functionality. We use $\xi_f$ to contrast the expression profile of a gene in tissue type 'x' versus others and call it conditional tissue specificity index (xTSI). A gene is tissue specific if its expression range in tissue type 'x' is distinct from other tissue types. Next, we characterize the dynamic of a gene as a function of developmental time point by development dynamics index (xDDI) also based on $\xi_f$. We demonstrate the advantage of xTSI on transcriptome data for five tissue specific Gene Ontology terms in contrast to other methods of assessing tissue specificity. Using both xTSI and xDDI, our analysis of 159K transcription start sites (TSSs) in four developing mouse tissues yields 1884/183/365/6892 genes specific to heart/kidney/testis/visual cortex development. We also captured 101 common genes that show strong dynamics in all four developing tissue types and in whole-body samples. The threshold of xTSI was selected at adjusted P-value <= 0.05 and the threshold of xDDI was at >=90% of known developmental genes. Our analysis contributes novel putative coding and noncoding genes that are either tissue-specific or common to development. This reported work is supported by National Science Foundation grant 1661331.
Program Abstract #273
Rewired second-order differential gene interactions in developing mammalian tissues
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Phenotypic differences across tissue types can be attributed to gene network rewiring during embryonic development. Previous statistical methods for detecting network rewiring are not specific to intrinsic differences between molecular interactions but rather to extrinsic molecular changes due to varied network input. This inadequacy prevents one from understanding fundamental differences in molecular interactions between tissue types that are derived from the same genome. We designed a novel statistical method to characterize the second-order differences in gene interaction patterns across multiple tissue types. By second order, we mean the relationship between two genes irrespective of their individual marginal distributions. We applied the new method to highlight second-order differences in miRNA-gene interactions across developing liver and lung tissues of the mouse. Specifically, we examined the interactions between pre-miRNAs and transcription start sites (TSSs) on transcriptome data from the FANTOM5 project. As miRNAs act as a post-transcriptional gene silencer, we revealed dramatical changes in miRNA-TSS interactions between developing liver and lung in mouse. Some miRNA-TSS interactions demonstrated non-monotonic increase in lung versus non-monotonic decrease in liver, providing evidence for what distinguishes the development of the two tissue types. Our work is applicable to studying intrinsically rewired interactions across many tissues to understand the molecular mechanisms underlying mammalian cell differentiation. This work is supported by National Science Foundation grant 1661331.

Program Abstract #274
Scanning fluorescence correlation spectroscopy reveals that Dorsal/NF-κB exhibits a dorsal-to-ventral mobility gradient in the Drosophila embryo
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In a developing organism, tissue patterning by a concentration gradient in a signaling protein, or morphogen, has been the predominant theory for how tissues are patterned. To more fully understand tissue patterning, real-time, quantitative measurements of biophysical parameters associated with morphogen gradients, including diffusivities, are needed. In this study, we report measurements of the mobility of Dorsal (Dl), a Drosophila homolog of NF-κB, by using scanning fluorescence correlation spectroscopy methods. Raster image correlation spectroscopy (RICS) and pair-correlation analysis reveal the local diffusivity and nuclear export, respectively, of Dl-GFP both vary along the (Dorsal-Ventral) DV axis, as well as in mutants that mimic the Dl gradient at different DV positions. Cross-correlation RICS (ccRICS) suggests this DV variation is the result of a higher fraction of Dl binding to the DNA on the ventral side than on the dorsal side. These results are consistent with our modeling work that suggests some of the Dl-GFP fluorescence in the nuclei stems from Dl/Cact complex. We conclude that most of the Dl-GFP fluorescence on the dorsal and lateral portions of the embryo emanates from Dl/Cact complex, which cannot bind the DNA. These observations could be explained by a significant pool of DNA-bound Dorsal on the ventral side of the embryo, which is confirmed by cross-correlation between Dl-GFP and a Histone-RFP. We propose that Cactus binding to Dorsal explains the DV asymmetry in these two biophysical processes. (Funding: NC State's "Research and Innovation Seed Funding" program).

Program Abstract #275
The FGF Signaling Pathway Plays an Important Role during Xenopus Pharyngeal Development
Emily Shifley, Breanna Bond, Sarah Kunkler, Leigh Ann VanDyke
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The Fibroblast Growth Factor (FGF) signaling pathway plays an important role during the development of the pharynx. The embryonic pharynx gives rise to a number of important organs including craniofacial structures of the head and neck as well as the thymus and parathyroid glands. When the genetic signals that guide pharyngeal development are disrupted, this can cause birth defects such as 22q11.2 deletion syndrome or 1st/2nd pharyngeal
arch syndromes. Using *Xenopus laevis* as our model organism, we hypothesized that the FGF signaling pathway controls the expression of important downstream targets during the embryonic patterning of the pharynx. Using microarray and *in situ* hybridization, we discovered genes such as the *Iroquois* transcription factors are downregulated when FGF signaling is inhibited in the developing pharynx. Knocking down *Iroquois* transcription factors by morpholino caused reductions in gene expression in the pharynx and some craniofacial malformations. Therefore FGF targets such as the *Iroquois* factors may play distinct roles in helping to pattern the embryonic pharynx. In the future, we would like to further characterize the role FGF signaling and *Iroquois* factors play during pharyngeal development, for instance, in signaling between the endoderm and the mesoderm. This research will aid in building a model of how genetic signaling cascades control pharyngeal development and help us understand how these cascades might be disrupted causing birth defects. Funding sources: Kentucky Biomedical Research Infrastructure Network NIGMS grant #8P20GM103436-14, NKU CINSAM, NKU Faculty Senate.

**Program Abstract #276**

**Effects of polycyclic aromatic hydrocarbons (PAHs) on pharyngeal system development**

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Polycyclic aromatic hydrocarbons (PAH’s) such as Naphthalene (naph), are hazardous compounds which could be found at high concentrations in fossil fuel pollutants and everyday consumables alike. To study how environmental teratogens such as PAHs can intersect and interfere with embryonic development, we have taken advantage of the extrauterine development of the utilized zebrafish model system. We aim to identify the cellular and molecular processes that mediate PAH teratogenesis of the craniofacial system. The craniofacial skeleton is derived in part from cranial neural crest cells (NCCs) which migrate through transient structures known as the pharyngeal pouches and arches. We postulated that naph could be targeting its effect on three possible cell types: the cranial NCCs, cells of the developing pharyngeal pouches, or epibranchial placodal cells that are known to interact with pouch and arch growth. Using antibody and transgenic reporters for both arches and pouches, we demonstrate that naph causes specific malformations in the posterior most region of the pharyngeal system. Our data supports a model in which PAH mediated teratogenesis first targets the posterior pharyngeal pouches that then indirectly impacts cranial neural crest cell migration. Placodal cells do not appear to be involved. This model is supported by changes in the early expression of the pouch marker *hoxa2b* among others. We are currently assessing the involvement of the Aryl hydrocarbon receptor pathway as mediating these teratogenic events. This project is sponsored by Smith College.

**Program Abstract #277**

**Distinguishing between function-dependent and function-independent modules of atrioventricular canal patterning**

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Differentiation of the cardiac atrioventricular canal (AVC), a specialized region at the junction between the atrial and ventricular chambers, is critical for cardiac function. Cells at the AVC orchestrate a complex morphogenetic program to create cardiac valves and also adjust their electrical properties to generate a conduction delay that results in sequential chamber contraction. AVC differentiation is under the spatiotemporal control of a patterning network whose exact mechanism remains unclear, although several of its components have been identified. Notably, it is thought that canonical Wnt signaling initiates a signaling cascade that drives AVC differentiation, and recent studies suggest that Wnt ligands are expressed in response to retrograde blood flow within the AVC. These results support a model in which the spatial distribution of biomechanical forces resulting from cardiac function control region-specific differentiation within the heart. However, certain elements of AVC differentiation do not seem to depend upon biomechanical inputs: for example, in embryos lacking cardiac function, the atrioventricular myocardium still creates a characteristic morphological constriction and exhibits a conduction delay. These data
suggest that there are function-independent spatial cues that drive certain aspects of AVC patterning. Here, we will present a systematic analysis of the effects of cardiac function on AVC patterning. Our studies will reveal which characteristics of the AVC depend on cardiac function for their establishment and/or maintenance, and our experiments will determine which patterning cues require cardiac function to exert their effects. Together, our results will distinguish function-dependent and function-independent AVC patterning modules, which will clarify the role of biomechanical forces in AVC development and provide a framework for the interpretation of phenotypes in embryos with altered cardiac function.

Program Abstract #278

Multiplexed in situ Hybridization Chain Reaction and Hyperspectral Phasor analysis reveals novel patterns of hand2, gata4 and gata5 gene expression

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The lateral plate mesoderm (LPM) is an embryonic tissue that gives rise to multiple adult structures, including the heart, blood, smooth muscle, limbs and spleen. Following gastrulation, the zebrafish LPM becomes morphologically identifiable and molecularly defined by the expression of a number of transcription factors (TFs), including hand2, gata4 and gata5. Overlapping expression of these TFs is thought to provide a molecular code that defines the LPM along the anterior-posterior and medial-lateral axes, which is critical for patterning of the LPM. Thus, the ability to spatially resolve overlapping gene expression at single cell resolution in the context of the same embryo is critical for improving patterning dissection in the LPM. However, spatially resolving multiple overlapping gene expression remains a significant challenge. Here, we overcome these limitations and describe multiplexed imaging of hand2, gata4 and gata5 parallel expression by combining in situ Hybridization Chain Reaction (HCR) with Hyperspectral Imaging and Phasor Analysis (HySP). We demonstrate subcellular resolution of hand2, gata4 and gata5 gene expression simultaneously in embryos during the stages of cardiac fate specification. Our analysis reveals previously undescribed non-overlapping expression patterns of these TFs that mark sub-regions of the LPM. We explore the longitudinal dimension by identifying nascent and cytoplasmic transcripts of hand2, gata4 and gata5 at different stages in development, opening a window into the dynamics of gene regulation in LPM patterning. We show here that by capturing the subcellular gene expression patterns of multiple genes simultaneously, we gain unique insights into the molecular mechanisms that pattern the LPM. This project is funded by the Eli and Edythe Broad Foundation Innovative Pilot Stem Cell Research Project and NIH 2P30DK048522.

Program Abstract #279

Mitochondrial energetics define cardiac patterning and early signaling in vertebrate development

Alexandra MacColl Garfinkel, Emily Mis, Mustafa Khokha

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Congenital heart disease is the leading cause of birth defects and infant mortality in the USA, affecting 9 of every 1000 children born each year. One especially severe form of CHD is heterotaxy, or the rearrangement of internal organs along the left-right axis. Through recent genetic analysis of affected patients, novel candidate genes for CHD have been identified and analysis of their underlying mechanisms is ongoing. One such gene is LRPPRC, which encodes a mitochondrially-localized mRNA binding protein that regulates mitochondrial gene expression and OXPHOS, critical for aerobic metabolism. LRPPRC, although implicated in human developmental disease, has not yet been studied in a developmental context. The molecular mechanism of LRPPRC’s involvement in CHD and the specific tempero-spatial requirements of aerobic metabolism in organ patterning are unknown. Through the use of Xenopus tropicalis, a powerful genetic model for human cardiac disease, and the use of modern gene editing tools such as CRISPR/Cas9 we have begun to elucidate the role of LRPPRC in embryonic patterning. We have identified a potential role for LRPPRC and aerobic metabolism in the regulation of Wnt signaling during...
gastrulation, required for proper patterning of the left-right axis and organ morphogenesis. This work was supported by NIH training grant ST32GM007223-42 to AMG.

Program Abstract #280
Characterizing the Role of MINK1 in Congenital Heart Disease
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Congenital Heart Disease (CHD) is the most common birth defect, affecting approximately 1% of all live births and is a of the leading causes of infant mortality globally. A recent genetic analysis of CHD patients identified a novel candidate gene, mink1. Mink1 encodes a serine-threonine germinal-center kinase with known functions in the JNK and PCP/Wnt signaling pathways. However, it has no known role in LR patterning or cardiac development. CRISPR knockout strategies to deplete mink1 in the high-throughput human disease model, Xenopus, resulted in malformation of the cardiac outflow tract, recapitulating the patient phenotype, and LR patterning defects. Additionally, novel defects in formation of multiciliated cells were observed. The overall goal of this proposal is to investigate the molecular mechanism by which mink1 affects LR patterning, heart development, and multiciliated cell formation in the Xenopus (frog) model system. Loss of function experiments were employed to determine the earliest time point of mink1 activity during the LR patterning cascade by testing molecular markers pitx2 and coco. Observation of the structure of the left-right organizer tissue supports the hypothesis that early patterning defects during gastrulation of the embryo cause downstream defects in left-right axis patterning and organogenesis. Mechanistic hypotheses will be guided by an analysis of temporal and spatial expression of mink1 in the whole embryo and the left-right organizer. On the multiciliated epidermal surface, loss of function experiments have confirmed a regulatory role for mink1 during formation of multi-ciliated cells of the Xenopus epidermis. Focused hypotheses will for formed based on the role of mink1 in the Notch signaling pathway, which will be tested by rescue of the multiciliated cell phenotype. Finally, the role of each mink1 domain, as well as the nature of patient mutations, will be determined using multiple functional assays in Xenopus. This work was in part supported by the National Institute of Health (T32 GM007499).

Program Abstract #281
The Congenital Heart Disease Candidate Gene Myelin Regulatory Factor Plays an Unexpected Role in Left Right Patterning
Sarah Amalraj, Emily Mis, Mustafa Khokha
Yale University School of Medicine, USA
Congenital heart disease (CHD) is the most common major birth defect and is the leading cause of infant mortality. Although the causes of CHD are not well understood, abnormalities in left-right (LR) patterning, known as Heterotaxy (Htx), are associated with severe forms of CHD. In a recent analysis of CHD/Htx patients, the transcription factor Myelin Regulatory Factor (MYRF) emerged as a candidate gene in three patients, who all carried mutations in highly conserved regions of the DNA binding domain. This is intriguing, as MYRF has an identified role in myelination, but no known function in cardiac development or LR patterning. Here, we show that depletion of myrf using CRISPR based gene modification in Xenopus tropicalis causes midline heart looping defects, phenocopying our patients (p<.001). We then analyzed global LR patterning markers and found abnormal bilateral expression of pitx2 (p<.001), but normal coco expression in myrf depleted embryos. We also examined nodal expression and found that although nodal was properly expressed on the left side of myrf depleted embryos, nodal expression intensity was increased in myrf depleted embryos (p<.001). Additionally, we depleted myrf in one cell of a two-cell embryo and found that left-sided depleted embryos resulted in heart looping defects (p<.001), abnormal bilateral pitx2 expression (p<.01), and normal coco expression. Conversely, right-sided depleted embryos had no LR patterning defects. Together, our data suggests MYRF plays a role in LR patterning, possibly by acting as a midline protein regulating transcription of nodal. Loss of myrf may allow nodal protein to diffuse into the right side of the embryo, leading to bilateral pitx2 expression and heart looping defects. We conclude that patient driven gene discovery can provide new insights into the molecular mechanisms that drive
cardiac patterning and LR axis formation. This project is supported by the HHMI Medical Research Fellows Program.

Program Abstract #282
FACS-Seq analysis of Pax3-derived cells identifies non-myogenic lineages in the embryonic forelimb
Chrissa Kioussi
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Skeletal muscle in the forelimb develops during embryonic and fetal development and perinatally. While much is known regarding the molecules involved in forelimb myogenesis, little is known about the specific mechanisms and interactions. Migrating skeletal muscle precursor cells express Pax3 as they migrate into the forelimb from the dermomyotome. To compare gene expression profiles of the same cell population over time, we isolated lineage-traced Pax3+ cells (Pax3EGFP) from forelimbs at different embryonic days. We performed whole transcriptome profiling via RNA-Seq of Pax3+ cells to construct gene networks involved in different stages of embryonic and fetal development. With this, we identified genes involved in the skeletal, muscular, vascular, nervous and immune systems. Expression of genes related to the immune, skeletal and vascular systems showed prominent increases over time, suggesting a non-skeletal myogenic context of Pax3-derived cells. Using co-expression analysis, we observed an immune-related gene subnetwork active during fetal myogenesis, further implying that Pax3-derived cells are not a strictly myogenic lineage, and are involved in patterning and three-dimensional formation of the forelimb through multiple systems.

Program Abstract #283
Role of paracrine signaling and cell movements in the spatial patterning of stem cells in an in vitro model of human gastrulation
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Gastrulation is a crucial stage in embryonic development when a homogeneous population of stem cells transforms into a trilaminar disk comprising the three germ layers of the embryo proper: endoderm, mesoderm, and ectoderm. In mammals, gastrulation occurs post implantation, which makes it very challenging to study in vivo. As a result, the mechanisms underlying this developmental stage are largely unknown. In a previous study, we showed that spatially confined human embryonic stem cells (hESCs) treated with Bone Morphogenic Protein (BMP4) ligand, self-organize to form robust spatial patterns comprising consecutive radial rings of distinct germ layers, thus recapitulating gastrulation-like events in vitro. In the current study, we quantitatively examined the role of cell communication via paracrine signals through the Wnt and Nodal pathways, and cell movements in this self-organized spatial patterning. Using live-cell imaging, we determined the spatial and temporal dynamics of paracrine signals and the movement trajectories of cells during spatial patterning. Our experimental results suggest that waves of paracrine signals and not cells themselves, move through the stem cell colonies to initiate spatial patterning. Based on these results, we propose a reaction-diffusion based mathematical model that recapitulates the signaling wave and correctly predicts gastrulation fate patterns in spatially confined stem cell colonies. This work was supported by Cancer Prevention Research Institute of Texas (CPRIT) grant RR140073 and NSF grant MCB-1553228

Program Abstract #284
NOing the Heart: Endoderm Nitric Oxide Focally Elevated “Hotspots” at the Heart Fields Signal in Early Cardiogenesis in Chicken Embryos
Devan Shah
San Francisco State University, USA

Nitric oxide (NO) signals in a diverse array of higher vertebrate physiological and pathological processes to regulate cell proliferation, growth, division, and apoptosis. The NO/sGC/cGMP canonical pathway has been shown to induce cardiomyocyte differentiation in mouse embryonic stem cell cultures. However, the spatiotemporal
nature and tissue source of NO signaling at the earliest stages of cardiac tissue formation in developing embryos has not been well-studied. Therefore, we investigated NO signaling to coincide with cardiac morphogenesis during gastrulation that produce the heart fields and cardiac crescent for formation of the heart tube. To investigate NO signaling in cardiogenesis, gastrula staged chick embryos (HH3–HH9) were labeled by DAF-2DA, fluorescent NO indicator, and embedded in agarose for imaging by confocal microscopy (n=23). We show that NO is produced as dynamic focal signals in “hotspots” in the endoderm layer that correlate with mesoderm heart field formation, the cardiogenic mesoderm, nascent cardiomyocyte differentiation, and formation of the early embryo heart tube. Furthermore, HH4-5 stage embryos were cultured in NOS inhibitor, L-NAME, or NO donor, DETA-NONOate, in ovo and developed to heart tube formation (HH 8 -10) stages to observe effects of the manipulation of NO production on cardiac morphology and muscle formation. L-NAME treated heart tubes exhibited noticeably reduced myosin heavy chain compared to controls, while the converse was true for DETA-NONOATE treated embryos. These are the first studies to show spatiotemporal NO signaling in correlation with early heart tube formation, and demonstrate that NO is a biomolecular regulator functioning in chicken embryo cardiogenesis. Funding: (1) NSF STC CCC: 1548297 (2) SFSU IRA award

**Program Abstract #285**

**Regulation of β-dystroglycan by miR-206 in early muscle development in Xenopus laevis**

Coohleen Coombes, Julio Ramirez
San Francisco State University, USA

During embryonic development in *Xenopus laevis*, cells within the presomitic mesoderm are compartmentalized into somites, which eventually give rise to adult muscle, bone, and cartilage. Complex cellular and morphological changes lead to the formation of 45 pairs of somites, with each somite consisting primarily of aligned myotome fibers. These morphological events correlate with the deposition of the extracellular matrix (ECM), which drives formation of the intersomitic boundaries. Muscle cells will rotate and adhere to nascent intersomitic boundaries to form elongated myotome fibers within each somite. A key complex involved in this process is the β-dystroglycan complex, which links the ECM to the cytoskeleton. We find that knockdown of a muscle-specific microRNA, miR-206, disrupts the formation of the β-dystroglycan complex, which leads to abnormal muscle cell adhesion and a failure to form elongated and aligned muscle fibers. In the absence of β-dystroglycan, we find that lamin in, the ECM molecule that is crosslinked by β-dystroglycan is significantly reduced as well as utrophin, an intracellular protein that binds to β-dystroglycan. Importantly, since utrophin directly binds F-actin, in the absence of utrophin, we also observe a significant reduction in F-actin expression. Thus, the knockdown of miR-206 leads to the inability of muscle cells to form β-dystroglycan complex and likely focal adhesions that are necessary to achieve and maintain a muscle fate. Understanding this process during de novo formation of muscle, will further our understanding of the role of β-dystroglycan in various muscular diseases.

**Program Abstract #286**

**An orderly interplay of Bmp, Wnt and Fgf8 specify the otic fate**

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At vertebrate gastrula, the neural border (NB) includes the pre-placodal ectoderm (PPE) that will give rise to a number of neurogenic placodes such as the otic placode (OP). Bmp/Wnt signaling is known to help establish the NB and Fgf signaling induces individual placode. We sought to test whether Fgf is involved in NB/PPE development by implanting Fgf protein-soaked beads into the presumptive PPE and epidermal ectoderm (EE) at the shield stage of zebrafish. Judged by ectopic otic vesicle (OV) induction, the shield-staged PPE appears in a differentiated state, exerting a rostra-caudal gradient of Wnt-Foxi1 activity (low to high). The caudal PPE is highly potent in obtaining an otic fate, in response to Fgf8-bead implantation. Unlike PPE implants, however, the EE implants are able to induce not only ectopic OVs, but also ectopic hindbrain and PPE (caudal) in wild-type background. Since ectopic OVs are usually in pairs and zebrafish *fgf8a* is transiently expressed in dorsal margin cells at late blastula to determine future hindbrain/spinal cord, we speculate that ectopic hindbrain and PPE are induced by Fgf8 beads
before ectopic OVs form. Indeed, blocking Foxi1 function prevents ectopic PPE formation, and knocking down factors that affect OP/OV formation does not affect ectopic PPE. Therefore, Bmp/Wnt and Fgf8 allow the PPE cells to obtain their caudal identity that favors the otic fate.

**Program Abstract #287**

**FGF signaling induces neuromesodermal progenitors in vivo**

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The discovery of FGF signaling as an embryonic inducer of mesoderm helped usher in the molecular age of developmental biology. FGF signaling was later discovered to also induce neural tissue, yet how it is capable of inducing tissues from two different germ-layers remains unknown. We re-evaluated the role of FGF signaling in the context of the more recently discovered neuromesodermal progenitors (NMPs), which are an essential cell-type of vertebrate embryos that continuously make a germ-layer decision to become spinal cord or posterior mesoderm, even after gastrulation, until the entire anterior-posterior axis is established. These cells give rise to majority of the tissues in the posterior body. Using zebrafish heat-shock inducible transgenic lines, paired with cell-transplantation, we show that FGF signaling induces NMPs during gastrulation. Individual cells lacking FGF signaling that are transplanted into a wild-type host are restricted to the epiblast. Although these FGF deficient cells can contribute to the posterior body, they give rise only to epidermal tissue and not NMP derived neural or mesodermal populations. NMPs are defined by the co-expression of *brachyury* and *soxb1* transcription factors, which FGF signaling activates during gastrulation. Surprisingly, restoring only Soxb1 function in transplanted cells lacking FGF signaling is sufficient to rescue both neural and mesodermal contribution, showing that *soxb1* transcriptional activation is the critical cell-autonomous role of FGF signaling during NMP induction. Our results show how FGF signaling is capable of inducing both neural and posterior mesodermal tissues through induction of the precursor NMP population, which later gives rise to spinal cord or mesoderm depending on local signaling cues.

**Program Abstract #288**

**Expression pattern of atg4d in *Xenopus laevis* embryos**

Hui Wang, Hui Zhao  
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Autophagy as a self-cleaning system is essential in homeostasis. It occurs at a basal level but can be further induced during embryonic development. The autophagy-related gene 4d (*atg4d*) is a cysteine protease, which regulates the processing of Atg8 in the phagophore expansion stage. Here, we have examined the expression pattern of *atg4d* during embryogenesis of *Xenopus laevis*. At two-cell stage embryos, the weak signals can be observed in the animal pole. It maintained almost a background level through cleavage, gastrulation and early neurulation. At late neurula stage, the strong signal appeared in the otic placode, which gives rise to spherically shaped ear vesicle. *Atg4d* is highly expressed in the otic vesicle through tadpole stages. In addition, signals can be observed in the somites. The high expression of *atg4d* in the otic vesicle suggests it may have other functions except being involved in autophagy. This data provides a basis for further functional analysis of autophagy-related genes in *Xenopus* embryos. This work is supported by the Research Grants Council of Hong Kong (CUHK24100414, CUHK14167017)

**Program Abstract #289**

**Zinc deficiency disrupts neural tube closure through attenuation of p53 ubiquitination**

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Micronutrition is essential for neural tube closure and zinc deficiency is associated with human neural tube defects, but the mechanism(s) underlying zinc deficiency remains unclear. Here we modeled zinc deficiency in
mouse embryos and used live imaging and molecular studies to determine how zinc deficiency affects neural tube closure. Embryos cultured with the zinc chelator TPEN failed to close the neural tube and showed excess apoptosis. TPEN induced p53 protein stabilization \textit{in vivo} and in neuroepithelial cell cultures and TPEN-associated apoptosis was dependent on p53. Mechanistically, zinc deficiency in embryos and primary neuroepithelial cells resulted in disrupted interaction between p53 and the zinc-dependent E3 ubiquitin ligase Mdm2 and greatly reduced p53 ubiquitination, both of which were rescued by zinc supplementation. Overexpression of Chip, a zinc-independent E3 ubiquitin ligase that targets p53, relieved TPEN-induced p53 stabilization and reduced apoptosis in neuroepithelial cells. Expression of p53 pro-apoptotic target genes was upregulated by zinc deficiency. Correspondingly, embryos cultured with p53 transcriptional activity inhibitor Pifithrin-α, but not the inhibitor of p53 mitochondrial function Pifithrin-μ, could overcome TPEN-induced apoptosis and failure of neural tube closure. Our studies indicate that zinc deficiency impacts neural tube closure through decreased p53 ubiquitination, increased p53 stabilization, and excess apoptosis.

Program Abstract #290
Making the best of bad genes: how zebrafish overcome a deleterious mutation
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Identical deleterious mutations often only penetrate into a phenotype in a subset of mutant individuals, known as incomplete penetrance. Mutations in the zebrafish transcription factor encoding gene \textit{mef2ca} produce various craniofacial phenotypes with incomplete penetrance. We discovered that selectively breeding zebrafish carrying a deleterious mutant \textit{mef2ca} allele can shift mutant penetrance both downward and upward. Excitingly, through our ongoing selective breeding program the once fully recessive-lethal \textit{mef2ca} mutation is now homozygous viable in our low-penetrance strain. These low- and high-penetrance strains provide a model for understanding how some individuals can overcome a deleterious mutation, while in other individuals the same mutation has disastrous developmental consequences. To learn how animals from the low- and high-penetrance strains might differentially respond to the \textit{mef2ca} mutation, we profiled craniofacial progenitor cell transcriptomes from genetic wild types and mutants from both strains. These experiments reveal strain-specific activity of signaling axes like BMP and Notch in response to \textit{mef2ca} loss of function, along with alterations in \textit{mef2ca} paralog expression between strains. Our studies motivate a model in which activation of a compensatory signaling network downstream of the \textit{mef2ca} mutation, and heritable changes in paralogous gene expression allow genetic mutants to develop the wild-type phenotype. Funding support: NIH R00DE024190

Program Abstract #291
Asymmetric deposition of the cardiac jelly establishes initial left-right asymmetry in the developing heart
Evan Brooks, Mike Dush, Martha Alonzo-Johnsen, Nanette Nascone-Yoder
North Carolina State University, USA
The heart is a left-right (LR) asymmetric organ. It develops from a straight embryonic heart tube that undergoes rightward looping to form a four-chambered structure. The importance of looping for normal heart function is indicated by the fact that many congenital heart defects are a direct effect of disrupted LR asymmetric development. While it is known that the TGF-β family member Nodal and its downstream transcriptional target Pitx2c are required for asymmetric morphogenesis, very little is known about the molecular and cellular mechanisms that underlie looping itself. We hypothesized that the development of LR asymmetry in the straight heart tube is mediated by LR asymmetric changes in the cardiac jelly, an extracellular matrix-rich region located between the endocardial and myocardial layers of the early heart tube. Indeed, morphometric measurements of the endocardial-myocardial space through the developing heart tube of \textit{Xenopus laevis} embryos reveal that the cardiac jelly has greater volume on the left side than the right at stages just prior to the rightward looping event. To ascertain whether Nodal-Pitx2c signaling was required for this difference, we dosed embryos with the TGF-β inhibitor SB50S124 or ectopically expressed \textit{Pitx2c}, and measured the endocardial-myocardial space in the developing heart tube. Both experiments resulted in a loss of LR asymmetry in the volume of the cardiac jelly,
accompanied by abnormal heart looping. These results demonstrate that the cardiac jelly is LR asymmetric, and that this asymmetry is dependent on conserved LR patterning events. Our results suggest that abnormal extracellular matrix deposition in early heart development may underlie many congenital heart defects. This work was supported by an SDB “Choose Development!” fellowship awarded to EB, the NC State University Initiative for Maximizing Student Diversity Intensive Research Training Program (NIH R25 GM083242), and NIH R21 OD01796 awarded to NNY and MAJ.

Program Abstract #292
The left-right asymmetry of liver lobation is generated by Pitx2c-mediated asymmetries in the hepatic diverticulum
Mandy Womble, Nirav Amin, Nanette Nascone-Yoder
North Carolina State University, USA
Internal organs exhibit left-right asymmetric sizes, shapes and anatomical positions, but how these different lateralities develop is poorly understood. Here we use the experimentally tractable Xenopus model to uncover the morphogenetic events that drive the left-right asymmetrical lobation of the liver. On the right side of the early hepatic diverticulum, endoderm cells become columnar and apically constricted, forming an expanded epithelial surface and, ultimately, an enlarged right liver lobe. In contrast, the cells on the left side become rounder, and rearrange into a compact, stratified architecture that produces a smaller left lobe. Side-specific gain- and loss-of-function studies reveal that asymmetric expression of the left-right determinant Pitx2c elicits distinct epithelial morphogenesis events in the left side of the diverticulum. Surprisingly, the cellular events induced by Pitx2c during liver development are opposite those induced in other digestive organs, suggesting divergent cellular mechanisms underlie the formation of different lateralities.

Program Abstract #293
Three Intermediate Filament Proteins Regulate Luminal Diameter in the Tubular Excretory Canals of C. elegans
Hikmat Al-Hashimi, Matthew Buechner
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The excretory canals of Caenorhabditis elegans are a fascinating and tractable model for understanding the formation and maintenance of tubule morphology in seamless single-celled polarized tubes. The canals regulate osmolarity in the worm in response to environmental conditions, and mutants in tube formation are sensitive to high-osmotic conditions. Light and electron microscopy show that mutants in the exc-2 gene start to form canals normally, but these swell to develop large fluid-filled cysts lacking a complete terminal web at the apical surface (lumen of the tube). In addition, filamentous material accumulates in the canal lumen. Through the use of whole-genome sequencing and gene rescue, we have found that exc-2 encodes a large intermediate filament protein. The EXC-2 protein, fluorescently tagged via CRISPR/Cas9, is localized to the apical surface of the canals together with, but independently of, two other intermediate filament proteins, IFA-4 and IFB-1. Tagged EXC-2 binds via pulldown assay to IFA-4. Overexpression of either protein results in narrow but shortened canals. These results are consistent with a model whereby these three intermediate filaments restrain swelling of narrow tubules in concert with actin filaments that guide the extension and direction of tubule outgrowth, while allowing the tube to bend as the animal moves. We are examining the effects of gene knockdown to examine the interactions of intermediate filaments with movements of endosomes previously shown to be essential for tubule formation in this tissue, as well as proteins found via an RNAi-knockdown screen. Funded in part by U. Kansas Graduate Research Fund to H.I.A.

Program Abstract #294
Cytotoxicity of silver nanoparticles (AgNPs) synthesized via completely “green” method on the epidermis of zebrafish (Danio rerio)
Grace Okuthe, Busiswa Siguba
Walter Sisulu University, ZA
Nanotechnologies are at the center of societal interest, due to their broad spectrum of application in different industrial products. Silver nanoparticles (AgNPs) in particular, are valuable metal nanoparticles that exhibit exceptional properties compared to their bulk counterparts. The pronounced surface area, complemented by small particle dimension and characteristics make them suitable in various applications. The therapeutic efficacy of AgNPs depends on their ability to escape the immune system by overcoming biological barriers of the body and concentrate at target tissues. Nanomaterials synthesized via completely green methods possess these qualities and utilize their targeting ability through cellular membrane interactions, making the targeting system biocompatible. Though the biosynthesis of these emerging materials has been proposed as a significant way of reducing their cytotoxicity, the current concern is the potential risks they carry for aquatic organisms. The aim of the present study was to evaluate the impact of AgNPs synthesized via a completely green method on zebrafish epidermis. Acute toxicity of AgNPs on zebrafish was investigated in a 96 h static renewal study. Effects of AgNPs were evaluated at a concentration of 0, 0.031 μg/L, 0.250 μg/L, 5.000 μg/L. Results indicate that AgNPs had negative impacts on the physiology of the epidermis. Lesions were observed only in the epithelial layer. Mucus secretion and swelling of goblet cells in the epidermal epithelium were also observed. The number of cells secreting acidic mucins increased with the increase of nano silver concentration; however at the highest concentration, the number of goblet cells was lower compared to control groups.

Program Abstract #295
MicroRNA-mediated control of lymphatic vessel development
Hyun Min Jung, Ciara Hu, Andrew Davis, Daniel Castranova, Brant Weinstein
Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, USA
MicroRNAs (miRNAs) are important post-transcriptional regulators that control precise gene expression program during development. It is estimated that about 30-60% of protein-coding genes harbor miRNA target sites indicating the extensive contribution of miRNAs in health and disease. Many miRNAs have distinct expression patterns and tissue-specific miRNA-mediated regulation is crucial for organogenesis. Our knowledge of lymphatic-specific miRNAs is still very limited and miRNA function in lymphatics is still largely unexplored. In this study, we used small RNA sequencing to identify miRNAs enriched in lymphatic endothelial cells. We identified an evolutionarily conserved miRNA highly enriched in human and zebrafish lymphatic endothelial cells compared to blood endothelial cells. We examined the functional role of this microRNA in zebrafish using morpholino injections and CRISPR/Cas9-mediated mutagenesis, performing high-resolution live imaging of lymphatic vessel development using a novel lymphatic transgenic reporter line in which EGFP expression is driven by the mannose receptor type C (mrc1a) promoter. We find that this novel microRNA is essential for development of primary lymphatic vessels including the parachordal line and the thoracic duct. We have uncovered a number of genes targeted for regulation by this microRNA, and we will report on the role of microRNA-mediated regulation of these genes in lymphatic development and lymphatic function. This work was supported by the intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (ZIA-HD008808 and ZIA-HD001011).

Program Abstract #296
A Novel Low-Density Lipoprotein Receptor Protects Against Ethanol Teratogenesis
Timothy Kuka, Johann Eberhart
University of Texas at Austin, USA
The manifestation of Fetal Alcohol Spectrum Disorders is highly variable, and this variability is influenced by genetics. We performed a forward genetic screen in zebrafish to identify loci involved in developmental ethanol sensitivity. One mutation identified in this screen was in a novel member of the low-density lipoprotein receptor-related protein (LRP) family. In this study, we have found the novel LRP protein lrp13b is essential for craniofacial and brain development and protects against ethanol sensitivity. lrp13b is expressed in the pharyngeal arches, otic vesicle, and neural floor plate of the developing zebrafish. Loss of this gene causes a fusion of jaw cartilages and a
slight reduction in jaw size. Exposure to a subteratogenic dose of ethanol further reduces the size of jaw skeletal elements and causes shape changes in the neurocranium. This phenotype is likely due to a reduction of *sox9a* expression in the pharyngeal arches. In the brain, loss of *lrp13b* causes a reduction in the number of oligodendrocyte precursor cells. Exposing these embryos to ethanol causes a further reduction in oligodendrocyte number. This phenotype is likely due to increased apoptosis in the ventricular layer. We have thus uncovered a novel locus which protects against ethanol teratogenicity in the brain and face.

**Program Abstract #297**  
*Activity of epigenetic regulator Brd2 is necessary for later development of the circulatory, excretory, and central nervous systems.*  
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Brd2 is a member of the bromodomain-extraterminal domain (BET) family of transcriptional co-regulators, which form histone-directed recruitment scaffolds for the assembly of chromatin modification complexes at promoters, thus controlling access of target genes to the transcriptional machinery. Brd2 is involved in the control of proliferation in adult mammalian tissues and in neuronal apoptosis during early vertebrate development. Its role in later development, when organ-systems other than the nervous system are being formed, however, is unknown. Previous studies in zebrafish have shown that complete *brd2a* knockout by Crispr-Cas9 is embryonic lethal, while *brd2a* knockdown by antisense morpholino results in a reduced hindbrain and abnormalities in the central nervous system associated with elevated levels of cell death. Since neither of these methods allows for temporal control of Brd2 deficiency, they are unable to reveal any later functions of Brd2 that may exist. In this study, we use a specific Brd2 BET small molecule inhibitor BIC1 to target Brd2 activity at different stages of development in zebrafish and find that later Brd2 deficiency results in abnormalities in the brain, trunk, and circulatory and excretory systems. BIC1 was administered at 3, 10, and 24 hpf, and fish were assayed at 24 hpf and 48 hpf for characteristic morphology. Early 3 hpf inhibition showed abnormalities in brain, trunk, and circulation when assayed at 24 hpf and 48 hpf, compared to wild-type. Treatment at 10 hpf showed pronephric duct abnormalities at 24 hpf assay, with recovery at 48 hpf assay. Late 24 hpf treatment showed circulatory and heart abnormalities at 48 hpf assay. Thus, Brd2 function is necessary both for early central nervous system development and for later circulatory and excretory system formation. These findings expand our understanding of the developmental role of Brd2, revealing its involvement in the proper formation of multiple organ-systems in vertebrates.

**Program Abstract #298**  
*Dullard, a phosphatase at the heart of outflow tract development and BMP pathway regulation*  
Jean-François Darrigrand¹, Vanessa Ribes², Bruno Cadot¹  
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The great arteries are pulsing blood in the systemic and pulmonary circulations to ensure oxygenation and nutrition of all tissues. They originally form in the embryo by septation of the heart outflow tract (OFT). The cardiac cushions, located along both sides of the OFT, are colonized by the cardiac neural crest cells (cNCCs), which condensate and trigger the individualization of the aorta and pulmonary artery by cleaving the endocardium. It is known that BMP signaling regulates cNCCs colonization and differentiation and the subsequent OFT septation, but the molecular mechanism tuning BMP levels remains to be established. By using mouse genetics, we show that the phosphatase Dullard is able to inhibit Smad phosphorylation following BMP pathway activation in the cNCCs. This control ensures the downstream balanced expression of Sema3c in the cNCCs, tuning their differentiation and condensation and ultimately leading to proper OFT septation. Deleting Dullard in the cNCCs causes premature and asymmetric septation of the OFT, pulmonary artery closure, haemodynamics alterations and early embryonic death. Thus, we show that Dullard’s fine tuning of BMP levels regulates NCCs condensation and OFT septation. Moreover, our results suggest that Sema3c, an oncogenic factor linked to angiogenesis, is a transcriptional target of BMP signaling. These results identify Dullard as a critical regulator of
BMP signaling and a potential therapeutic target in the many pathologies caused by BMP signaling impairment. This work is funded by Sorbonne University, the AFM (French Association for Myopathies) and the ANR (French National Research Agency).

Program Abstract #299
Inactivation of the minor spliceosome through Prx1-Cre mediated ablation of U11 results in limb defects
Kyle Drake, Christopher Lemoine, Anouk Olthof, Rahul Kanadia
University of Connecticut, USA
Post-transcriptional gene regulation actively regulates progenitor cell proliferation, migration, and differentiation in developing systems. I am interested in understanding the role of the minor spliceosome, a machinery which splices introns in a rare subset of genes (<2.5%), in developing tissues. The importance of minor intron splicing during development is underscored by the disease Microcephalic Osteodysplastic Primordial Dwarfism Type I (MOPDI), as single point mutations in U4atac snRNA, an essential component of the minor spliceosome, result in patients with shortened, malformed limbs as well as microcephaly. To understand how the minor spliceosome informs morphogenesis in developing systems, we engineered an Rnu11 conditional knockout mouse (cKO) to ablate the essential snRNA U11 and thus inactivate the minor spliceosome in a tissue specific manner. Using limb as a model, I employ Prx1-Cre, which is active in forelimb and hindlimb at E9.5 and E10.5 respectively, to remove U11 snRNA. These time points of Cre activity are coincident with forelimb development and delayed by one half day for hindlimb development. I report here that ablation of the minor spliceosome at the onset of forelimb development results in a severely truncated structure with no apparent organization. Interestingly, the hindlimb, which has one half day of normal development before minor spliceosome loss, has sufficient number of progenitor cells for a fully patterned, albeit smaller, limb. Thus, this phenotype suggests that progenitor cell number can be linked to scaling and patterning. We are currently investigating which model of limb development (progress zone model, two signal theory, and early specification/differentiation front model) most aptly predicts the phenotypes we observe. To do so, we are characterizing the cellular and molecular defects occurring within the U11-null limb buds. Funding from: NINDS NS101616, NSF GRFP 2018257410.

Program Abstract #300
Minor spliceosome is required for proper jaw development
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The minor spliceosome is responsible for splicing <0.5% of the introns found in genes that consist mostly of introns that are spliced by the major spliceosome. The presence of these introns requires coordinated action of both the major and the minor spliceosome for proper expression of minor intron-containing genes (MIGs). We are interested in understanding the mechanism of regulation of MIGs during development to inform cell cycle regulation and tissue development. The significance of proper minor spliceosome function and MIG-expression is underscored by the disease microcephalic osteodysplastic primordial dwarfism type 1, which is caused by point mutation in one of the crucial snRNAs, U4atac, of the minor spliceosome. The primary phenotypes include, microcephaly, micrognathia (small jaw), and short limbs. Since our objective is to understand the role of minor spliceosome in development, we have generated a conditional knockout mouse for U11, another crucial snRNA of the minor spliceosome. We employed Emx1-Cre to ablate U11 snRNA, thereby inactivating the minor spliceosome, in the developing cortex and the first branchial arch. The loss of minor spliceosome in the progenitor cells of the branchial arch resulted in compromised jaw development such that the mutant mice could not feed on solid food at weaning and had to be given wet food. The ability to masticate was further compromised in Emx1-Cre homozygous mice such that they could not get sufficient nutrition and had to be euthanized due to severe malnourishment. These findings suggest that the ablation of U11 in the progenitor cells of the branchial arch results in reduction of the jaw structures such that it prevents them from masticating solid food. Specifically, the mandible and the maxilla showed reduction in length. These findings reveal an important role of proper MIG-expression in regulating cell cycle and jaw development. Funded by NIH NS096684-01A1.
Program Abstract #301
Investigating the role of Tcf/Lef transcription factors in lung formation and fate maintenance
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Murine lung development begins at embryonic day (E) 9.5 with specification of NK2 homeobox 1 (Nkx2-1) positive epithelial progenitor cells in the anterior foregut. Such specification depends on Wnt2/2a and β-Catenin (Ctnnb1). Ctnnb1-mediated Wnt signaling activates the T-cell factor/lymphoid enhancer-binding factor (Tcf/Lef) family transcription factors, including Tcf7, Tcf7L1, Tcf7L2, and Lef1. Despite the importance of Wnt signaling in lung development, the unique identity and molecular mechanism of associated Tcf/Lef genes are unknown. We have shown that both Wnt signaling in an established human lung progenitor cell line and Ctnnb1 in vivo are required to specify and maintain lung identity while repressing GI fate. We generated a conditional knock out of both Tcf7L1 and Tcf7L2 or Tcf7 and Lef1 in the lung epithelium. Unlike the Ctnnb1 mutant where lung development does not occur, both double mutants develop lungs embryonically, suggesting significant redundancy among Tcf/Lef members. While expression levels of Tcf1 and Lef1 decrease in the lung epithelium to nearly undetectable levels at E14.5, all Tcf/Lef members are expressed in the lung epithelium during specification at E9.5, suggesting all Tcf/Lef family members are necessary for specification with only a few necessary for maintenance. Knowledge about the mechanism by which Tcf/Lef members are required for lung development and fate maintenance will improve the efficiency of generating lung cells in culture for regenerative medicine. This work was supported by the University of Texas MD Anderson Cancer Center Start-up Fund and National Institutes of Health grant R01HL130129 (J.C.).

Program Abstract #302
Cell-cell communication in the renal interstitium directs higher order structures along the cortico-medullary axis during mammalian kidney development
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The kidney contains higher order structures along the cortico-medullary axis. Although the recent studies have revealed factors for cell type specification during kidney development, regulatory mechanisms for development of higher order structure in the kidney are largely unknown. We previously discovered that Six2+ cap mesenchyme cells and Foxd1+ cortical stromal cells are progenitor cells for the nephron parenchyma and renal interstitium in the developing kidney, respectively, indicating a strict lineage boundary between the nephron parenchyma and interstitial compartments. We also showed that repression of interstitial identity in Six2+ nephron progenitor cells establishes the nephron-interstitium lineage boundary during kidney development. Our single-cell RNA-seq analysis showed that the renal interstitium is a highly heterogenous population. These different interstitial cell types were organized in multiple layers along the cortico-medullary axis of the kidney. We showed that, during earlier and later stages of kidney development, Foxd1+ interstitium progenitors generate medullary and cortical interstitial cell types, respectively. Although the nephron parenchyma is lost and kidney growth is arrested in the Pax2 mutant kidneys, the interstitial cell types were normally specified along the cortico-medullary axis, indicating that formation of the cortico-medullary axis in the renal interstitium is independent of the nephron parenchyma and kidney size. In ectopic renal interstitial cells induced by inactivation of Pax2, ectopic cortico-medullary axes were formed within each interstitial clone. Taken together, our observations suggest that cell-cell communication directs formation of the cortico-medullary axis in the renal interstitium. Reference: Naiman N, Fujioka K, Fujino M, Valerius MT, Potter SS, McMahon AP, Kobayashi A. Developmental Cell (2017) 41:349-365. Supported by grants from National Institutes of Health (NIH) DK094933 and OD021437.

Program Abstract #303
Scleraxis dependent recruitment of tenocytes is essential for tendon elongation
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Scleraxis dependent recruitment of tenocytes is essential for tendon elongation
The long tendons of the limb extend from muscles in the arm to their skeletal insertions in the hand. We previously showed that tendon formation occurs in two distinct stages, attachment of muscles via a tendon anlage to a skeletal insertion followed by tendon elongation to accommodate skeletal growth. Interestingly, tendon elongation in the arm and tail is prevalent in amniotes but does not happen in amphibians, suggesting it may represent a critical mechanistic adaptation for the coordination of soft tissue growth with the dramatic changes in skeletal size and shape in amniote evolution. Using Sox9Cre and Sox9CreERT2 to label the tenocytes of the tendon insertion we find that tendon elongation occurs by recruitment of new tenocytes and not by mere proliferation of the attachment tendon progenitors. Surprisingly, we also find that tenocyte recruitment is dependent on expression of the bHLH transcription factor Scleraxis in the recruited cells. To validate this model of tendon elongation we utilized transuterine injection of limb mesenchyme cells into the developing limb bud and found that injected cells indeed incorporated into elongating tendons. Moreover, we find that WT cells incorporated more readily into the tendons of Scx mutant embryos, reinforcing the notion that Scx mutant cells cannot be recruited into the tendon giving the WT cells a competitive advantage in the mutant environment. In this study we therefore demonstrate that tenocyte recruitment is essential for tendon elongation. Moreover, we find that tenocyte recruitment is dependent on Scx activity in the recruited cells, providing for the first time a mechanistic rationale for the tendon phenotype in Scx mutants. Improving our understanding of the mechanisms of tenocyte recruitment may shed light on a wide range of processes in tendon development and during tendon healing. Supported by Shriners Hospitals 85410-POR-14 grant to RS. and an Arthritis Foundation postdoctoral fellowship to AHH.

Program Abstract #304
**Defining Glucocorticoid Action in Primate Follicle, Oocyte and Embryo**
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IVF requires the administration of exogenous hormones to simultaneously recruit multiple ovarian follicles yielding a heterogeneous population of oocytes: only about ~35% of them successfully fertilize, cleave, and form blastocysts capable of implantation. To assess the role of intrafollicular microenvironment on IVF outcomes we used a high-throughput, non-targeted metabolomics platform to assess 74 follicular fluid (FF) samples from the highly translational rhesus macaque monkeys. We identified cortisone, an inactive metabolite of the biologically active glucocorticoid, to be higher and cortisol, the active form to be lower in the FF of the follicles that yielded an oocyte that did not form a blastocyst. Previous *in vivo* studies from our lab showed lower cortisone and higher cortisol:cortisone ratios during development of a dominant follicle. Cortisol binds to the glucocorticoid receptor (GR) and activates downstream signaling. Interestingly, GR immunolocalization revealed its expression in granulosa cells of the periovulatory follicle, as well as in nuclei of the oocytes that received the stimulation for re-initiation of meiosis and in the nuclei as well as cytoplasm of oocytes that did not receive the stimulation. Hence, we postulated that GR signaling within the oocyte is necessary for its maturation, fertilization, and/or blastulation. To assess the importance of GR in oocytes, we designed a morpholino to knockdown the GR in macaque oocytes. Knockdown was first optimized in macaque breast cancer cells with a 66% knockdown and then in macaque oocytes with 84% knockdown. Further, GR knockdown in rhesus macaque oocytes and the somatic granulosa cells will be performed to assess the GR dependent effect on maturation, fertilization and early embryonic development and the development of other follicle components during ovulation and fertilization. Funding: ONPRC Core Grant (OD011092), Jones Foundation Grant for Reproductive Media Funding to JDH and SLC

Program Abstract #305
**The role of oviductal cilia in gametes and embryo transfer**
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The Fallopian tube (oviduct) serves as the site for oocytes transport, fertilization, and early embryonic...
development. While research has shed light on the cellular and molecular mechanisms mediating these events, much of these data are derived from static histological analysis, low-resolution visualizations, and studies of invertebrate models (e.g. sea urchin). Therefore, any conclusions regarding mammalian fertilization, which takes place deep inside the body, are extrapolated and do not necessarily represent the native state. To overcome this technical limitation, we developed a 3D optical imaging approach combining optical coherence tomography (OCT) with an intravital dorsal imaging window, which allows for prolonged, functional, and quantitative analysis of the mouse oviduct in vivo. These methods provide information about transferring of oocytes/embryos, the contraction of the oviduct muscle, distribution of the frequency of cilia beat, as well as sperm behavior in the ampulla, revealing never-before-seen dynamic events. We observed very rapid and large bi-directional movements of oocytes and embryos through the oviduct presumably caused by muscular contractions during preimplantation pregnancy, suggesting that it is unlikely that the primary role of cilia is for directional movement of oocytes and embryos. Regarding sperm, volumetric imaging in the oviduct revealed novel sperm behaviors relative to the wall of the oviduct, suggesting a role for cilia dynamics in the regulation of sperm movements. We are now using these methods to elucidate the specific roles of the cilia beat in the gametes transport and fertilization. Funding: This work was supported by the National Institute of Health grant R01HL120140 (to I.V.L.) and by the American Heart Association grant 16POST30990070 (to S.W.).

Program Abstract #306
Expression of melatonin receptors MT1, MT2 and ASMT mRNA in equine ovarian follicle cells
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Melatonin is produced by the pineal gland and other organs expressing the enzyme acetylserotonin O-methyltransferase (ASMT), which is required for melatonin synthesis. The actions of melatonin on the reproductive system of mammals are mediated by receptors MT1 and MT2. In cows and swine, the role of melatonin on oocyte maturation has been explored while in the mare, only MT1 expression has been described in the CL and ovarian follicles. In equine, there is no published information regarding ASMT or MT2 expression in the ovarian follicle cells. Thus, the purpose of this study is to evaluate the expression of ASMT, MT1 and MT2 receptors by real-time PCR in the oocyte, cumulus cells, granulosa cells and theca cells of the mare ovarian follicle. Theca, granulosa and cumulus-oocyte complexes (COCs) were collected from ovaries obtained from the local abattoir. The expression of ASMT, MT1, and MT2 genes was evaluated by real time-PCR. Specific primers were designed, sequenced and optimized for each cell type. Our study revealed that MT1 was present in oocytes, cumulus, theca and granulosa cells. MT2 was not detected in theca cells but was expressed in oocytes, cumulus, and granulosa cells. ASMT was expressed in both theca and granulosa cells but was not detected in cumulus cells and oocytes. The present study provides evidence that melatonin can be synthesized in theca and granulosa cells as previously described in bovine cumulus cells and human granulosa cells. After secretion, melatonin would diffuse into the follicular fluid, modulating ovarian function and regulating specific functions of follicular development through of receptor activation. Another possible role during follicular development is the oocyte protection from the oxidative process by free-radical damage resulting in a positive effect during oocyte maturation. This work was funded by Fonds en Santé Équine, Université de Montréal, Zoetis, and CONACYT.

Program Abstract #307
Dead-End mimic helicase activity to promote nanos translation in the germline
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Primordial germ cells (PGCs) constitute the exclusive progenitors of the gametes in the developing embryo. PGCs maintain their totipotency even upon differentiation into highly specialized gametic cells. Preserving full potential is an essential attribute of PGCs and is required for the survival of all sexually reproducing species. The germ cell lineage in Xenopus is specified by the inheritance of germ plasm that assembles within the Balbiani body in stage I oocytes. Specific RNAs, such as nanos1, localize to the germ plasm. nanos1 has the central germline function of blocking somatic gene expression and thus preventing PGC loss and sterility. It is unclear how Nanos translation is regulated in developing embryos. We report here that nanos1 translation after fertilization requires Dead-end 1 (Dnd1), a vertebrate-specific germline RNA-binding protein. Dnd1 expression is restricted to the vertebrate germline where it is believed to activate translation of RNAs required to protect and promote that lineage. nanos1 is one such germline RNA whose translation is blocked by a secondary RNA structure in the ORF. Dnd1 contains a canonical RNA Recognition Motif (RRM1) in its N-terminus but also a less conserved RRM2. Here we provide a mechanistic picture of nanos1 RNA-Dnd1 interaction. Dnd1 possesses ATPase activity. Surprisingly this activity is mapped within the RRM2, different from the C-terminal region where it was first found in zebrafish. More importantly, we show that while RRM1 is required for binding nanos1, RRM2 is required for nanos1 translation and germline survival. Further, Dnd1 functions as a homodimer and binds nanos1 RNA just downstream of the secondary structure required for nanos1 repression. We propose a model where the RRM1 is required to bind nanos1 RNA while the RRM2 is required to promote translation through the action of ATPase. This work uncovers a novel translational regulatory mechanism that is fundamentally important for germline development.

Program Abstract #308
Understanding the migration of primordial germ cells in the avian embryo
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Primordial germ cells (PGCs) transmit genetic information to the progeny by giving rise to male and female gametes. In the avian embryo, PGCs get specified in the epiblast layer of an early developing bi-layered embryo. After specification, they migrate and accumulate at an anterior extracellular region by embryonic day 1 (E1). By E2, PGCs interact with the developing vascular plexus to get into blood circulation and eventually reached the gonads. The migration mode of PGCs and the molecular mechanisms by which they associate with endothelial cells to move into the vascular plexus have not been described. We are using time-lapse imaging to study how PGCs move through the gastrulating embryo to the germinal crescent niche where they soon associate and interact with nascent endothelial cells (EC) forming the vascular plexus. Single-cell RNA-Seq analysis of PGCs and ECs provide detailed molecular information about possible genes involved in PGC movements and PGC-EC interactions. Conventional RNAi experiments will be used to functionally analyze key genes and their products. Funding sources: CHLA TSRI Pre-Doctoral award (2016-2018).

Program Abstract #309
In vivo functional validation of germ cells derived from human embryonic stem cells
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Germ cells are special cells that give rise to gametes and play a crucial role during development by transmitting genetic information to the next generation. They are originated in the epiblast and must migrate across the embryo to settle in the developing gonads. Little is known of molecular requirements for human germ cells specification. Thus, identifying the factors that lead to proper germ cell differentiation will give significant insight into regulation of development as well as having important implication in infertility treatments, regenerative medicine and species conservation. Chick embryos primordial germ cells (cPGCs) present a unique migratory pathway. They circulate temporarily through the bloodstream to reach the gonadal ridges where they form the germ cells. Here, we generated human-chick chimeric embryos to address whether the human PGCs are specified...
and can migrate to their presumptive final location in an in vivo environment. hESCs were cultured in geometrically confined micropatterns and differentiated into PGCs. hPGCs were then dissociated and injected into the chick vasculature system at stage HH16-17 through the dorsal aorta. Control embryos were injected with saline solution. Embryos were collected 3 days after injection when the gonads were already established. To track the hPGCs, embryos were stained with human nuclear antigen (HNA) specific antibody and DAZL, a germ cell specific marker. Next, embryos were cleared using iDISCO protocol and imaged in the light sheet microscope. 3D images analysis shown that HNA is expressed in the developing gonads and colocalize with DAZL expression. In the control embryos, no expression of HNA is observed. Our preliminary results suggested that the hPGCs can respond to signals in vivo and home to the gonads. Further characterization is necessary to elucidate if the primordial germ cells that reached the gonads are proliferating and differentiating.

Program Abstract #310
Next-generation sequencing reveals novel maternal germline RNAs crucial for the wasp Nasonia germline development
Honghu Quan
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Many animals set aside primordial germ cells in the earliest stages of embryogenesis, using maternally provisioned and localized germ plasm. In holometabolous insects, germ plasm is assembled at the posterior of the oocyte. Typically, syncytial nuclei of the embryo that enter the germ plasm cellularize precociously (becoming pole cells), take on germ cell traits, and later migrate to the gonad. Surprisingly, germ plasm and pole cell features are quite diverse among the Holometabola. For example, the germ plasm (aka oosome) in the wasp Nasonia is spheroid, moves freely in the posterior half of the egg, and then produces a single large bud at the posterior pole which then subdivides to give multiple pole cells. This contrasts with the small, stationary polar granules and individual germ cell buds in Drosophila. Later, the Nasonia pole cells take a distinct migratory path to the gonads. To understand these differences, we have sequenced RNAs of anterior and posterior fragments of Nasonia embryos, and have identified more than 30 posteriorly localized mRNAs potentially involved in germ cell determination. Only a handful of the fly orthologs of these transcripts are localized or have a described germ cell role. Functional analysis has confirmed that several of the wasp specific transcripts are important for the unique properties of the Nasonia oosome. These include the release of the germ plasm from the cortex just after the first syncytial division, the coalescence of the germline material into a spherical mass, and the cell biology of generating an extremely large posterior pole cell bud.

Program Abstract #311
Identification of interacting partners of Tnpo-SR in the control of germline cell divisions in the Drosophila ovary
Virginia Vasquez-Rios, Taylor D. Hinnant, Elizabeth T. Ables
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Improving reproductive health requires a thorough understanding of the cellular processes that enable the continued production of gametes. For example, oocyte production requires carefully regulated germline precursor cell divisions balanced with the induction of the meiotic program. While many advances have been made in understanding the signaling pathways that underlie gametogenesis, it remains largely unclear which cellular signals are essential for proper germline cell division. To elucidate the genes necessary for proper proliferation and differentiation of germ cells, we focus on the multipotent germline stem cells (GSCs) and germline cysts in Drosophila melanogaster ovaries. Due to the similarity in genes and cell processes between Drosophila and humans, our findings in the Drosophila ovary may be applied to the human germline. We previously identified the nucleocytoplasmic transport protein, Tnpo-SR, in a genetic screen for novel genes regulating germ cell division in adult oogenesis. Here, we find that Tnpo-SR regulates GSC self-renewal and proliferation, along with early germ cell proliferation and oocyte fate. We identified putative Tnpo-SR protein cargoes and studied their role in germ cell function and division using RNAi knockdown. Similar to Tnpo-SR, we hypothesize these cargo proteins allow for proper GSC self-renewal, germ cell proliferation, and oocyte specification. This would suggest the transport of
these cargo proteins via Tnpo-SR is vital to germ cell production. By determining the role of nucleocytoplasmic transport in germ cells, we can further understand the intricacies of gametogenesis and germ cell divisions. This work is funded by the Office of Undergraduate Research at East Carolina University, Greenville North Carolina.

Program Abstract #312
Analysis of the gap junction protein Zpg in soma-germline communication in the Drosophila testis
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Gametogenesis is a developmentally conserved process in animals that requires coordinated signaling between germ cells, which will give rise to sperm and egg cells, and somatic cells, which surround and support the developing germline. It was previously shown in Drosophila that the communication between soma and germline regulates cell proliferation and differentiation, failure of which could lead to infertility or tumor formation. Gap junction channels are important for rapid cell-cell communication, as they allow the passage of small molecules between cells. Drosophila innexins constitute gap junction proteins and are structural homologs of vertebrate connexins. Previous studies in our lab showed that Innexin4/Zero population growth (Zpg) functions in the germline to mediate bi-directional signaling between soma and germline. Flies lacking zpg expression show rudimentary gonads and are sterile due to impaired germ cell differentiation and maintenance. Already during larval stages, germ cells in testes of zpg mutants fail to proliferate and differentiate, indicating the necessity of Zpg for gonad development. In addition to its function in the germline, Zpg also regulates proliferation and differentiation of somatic cells in the testis. However, the signals that pass through the gap junction channel consisting of Zpg on the germline side and Inx2 in the soma have not been identified and the molecular mechanism of Zpg function is poorly understood. To address this, several Zpg mutants were generated in which key residues of the protein are disrupted in order to perform classic structure function analysis. 3D protein structure predictions suggest that the N-terminus of innexins might be involved in regulating channel permeability. The analysis of N-terminal zpg and inx2 mutants will give us insight into how gap junctions linking soma and germline work and could hint at which signals pass through them for bi-directional soma-germline communication.

Program Abstract #313
Dynein independent role for dynein light chain in meiotic progression
Sara Fielder
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Homologous chromosome pairing and meiotic synapsis are essential processes that are required in both oogenesis and spermatogenesis to prevent aneuploidy and developmental defects in offspring. Despite the importance and high conservation of synapsis, not every aspect is the same between the two sexes. Heterogametic species have evolved less stringent regulation of meiotic pairing and synapsis in order to successfully pass on their genetic information. My preliminary results indicate that male and female C. elegans even have different requirements for dynein motor proteins in regulating synapsis. Dynein dependent forces have been proposed to test whether a potential homolog match is correct, and once a match has been established, synapsis (SYP) proteins are loaded between the homologs. Knockdown of the dynein light chain (DLC-1) at an elevated temperature results in formation of an abnormal SYP polycomplex away from chromatin in females. Unexpectedly, DLC-1 depletion in males at the same temperature shows grossly normal synapsis. Even more surprisingly, mutants in the heavy chain and dynactin components of dynein also do not show SYP polycomplexes in female meiosis. This indicates that there is a previously undescribed function for DLC-1 in synapsis initiation. There are many examples of dynein-independent functions for DLC-1, including stabilizing or interrupting dimer interactions. A consensus binding motif for the mammalian DLC-1 ortholog has been reported, and we identified a potential binding motif in one of the SYP proteins. Additionally, small polycomplexes have been observed at the beginning of meiosis before pairing has been completed, and knock down of an axis component of the
synaptonemal complex results in many small SYP protein polycomplexes instead of one large complex as in the DLC-1 knockdown. All this suggests that DLC-1 directly interacts with SYP proteins and may have a role in polycomplex regulation. Research supported under NIH F31GM123750.

Program Abstract #314

Dissecting the mechanism of Drosophila egg activation
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Cornell Univ, USA
At the end of oogenesis, mature oocytes arrest in meiosis; they need to be “activated” to transition to embryonic development. Major events in egg activation include resumption and completion of meiosis, and modification of maternally deposited proteome, transcriptome and egg coverings. These downstream events are conserved, as is their association with a rise in intracellular calcium in the egg. In vertebrates and echinoderms, the fertilizing sperm triggers egg activation, but in the arthropods studied activation is uncoupled from fertilization. For example, Drosophila egg activation is triggered by ovulation, due to mechanical pressure and entry of the oocyte into a new environment. In Drosophila, the calcium rise initiates at oocyte pole(s) and travels through the egg in a wave. In vitro studies suggest that mechanosensitive TRP family ion channels mediate influx of external calcium to initiate the wave, and IP3R-mediated calcium release is needed for wave propagation. We are screening TRP channels expressed in Drosophila oocytes to identify those that cause the calcium influx. The calcium rise is thought to trigger downstream events by activating calcium-dependent factors in the oocyte. A candidate factor is the conserved serine/threonine phosphatase calcineurin, which is crucial for resumption/completion of meiosis Drosophila, Xenopus and ascidians. Since there are large changes to the phosphoproteome during Drosophila egg activation (as in sea urchins and frogs), we investigated whether calcineurin activity is crucial for these phosphoproteomic changes. We quantified proteomic and phosphoproteomic changes during egg activation using quantitative mass spectrometry, in normal oocytes and ones whose calcineurin regulatory subunit was depleted by RNAi. We find that calcineurin modulates phospho-states of proteins needed for events such as meiosis completion and translation activation. QH & ZZ contributed equally. We thank NIH grant R21-HD088744 for support.

Program Abstract #315

Elucidating Novel Roles of the 19S Regulatory Particle subunits of the 26S Proteasome in C. elegans Gametogenesis and Fertilization
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The 26S proteasome is one of the major proteolytic machineries in the cell that selectively degrades polyubiquitinated proteins. Malfunction of the 26S proteasome is seen in many disease conditions such as neurodegenerative diseases and cancer. Recent studies demonstrate that the 26S proteasome can perform non-proteolytic functions in chromatin structure, transcription, mRNA export and cell cycle regulation. In 2014, our lab showed that when specific subunits of the Caenorhabditis elegans proteasome 19S RP lid were co-depleted via RNAi with the Myt1 ortholog WEE-1.3, this rescued the infertility of wee-1.3(RNAi) animals. Only a subset of the 19S lid subunits suppress the wee-1.3(RNAi) infertility. Therefore, we hypothesize that specific 19S lid subunits may play a non-canonical role in C. elegans reproduction, potentially through regulation of the cell cycle inhibitory kinase WEE-1.3. Additionally, we found that down-regulation of specific 19S RP subunits results in aberrant nuclear localization of WEE-1.3 in oocytes compared to the normal perinuclear localization. Down-regulation of the 19S subunit RPN-12 showed the highest nuclear expression intensity of WEE-1.3. We obtained and characterized a viable rpn-12(av93) mutant which we will show has defects in reproduction, including a low sperm count in hermaphrodites and reduced fertility. We can partially rescue the fertility defect of rpn-12(av93) by mating to wild type males, however not to wild type levels. Therefore, rpn-12(av93) fertility issues are most likely due to both a lack of sperm and oocyte defects. Interestingly, rpn-12(av93) males do not show fertility defects, and there are no visible developmental or growth defects in males or hermaphrodites. Future genetic and
biochemical studies will focus on the role of RPN-12 in oocyte quality and its interaction with WEE-1.3 in C. elegans hermaphrodite germline. Funding sources: HU start-up grant and DoD grant

Program Abstract #316
Novel functions for the RNA-binding protein ETR-1 in Caenorhabditis elegans male gametogenesis and discovery of ETR-1’s RNA targets
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RNA-binding proteins (RBPs) are essential regulators of gene expression that act through a variety of mechanisms to ensure the proper post-transcriptional regulation of their target RNAs. RBPs play crucial roles during development and have important functions in various adult organ systems, including the nervous, muscle, and reproductive systems. We previously showed that the Caenorhabditis elegans RNA-binding protein ETR-1 is essential for the full reproductive potential of hermaphrodites, and that ETR-1 is involved in the engulfment of physiological germline apoptotic corpses. Here we show that ETR-1 plays a significant role in male reproduction and have identified mRNA targets of ETR-1 via RIP-seq. Spermatogenesis genes in C. elegans are typically RNAi resistant and only a few spermatogenic genes, i.e.- gsp-3/4, are capable of being depleted via RNAi using an enhanced sensitivity to RNAi strain [rrf-3(pk1426)]. RNAi depletion of ETR-1 in rrf-3(pk1426) males results in a 64% reduction in fertility compared to control males. ETR-1-depleted males exhibit abnormal tails and copulatory structures, and defects in spermatid activation. Some spermatids exhibit "rod-like" projections, similar to phenotypes observed in spe-15(ok153), and suggestive of defects in Major Sperm Protein polymerization or cellular component sorting. Interestingly, ETR-1 is expressed in the cytoplasm of spermatids and then localizes to the pseudopod of mature spermatozoa. We identified previously unknown RNA targets of ETR-1 via RIP-seq, and will present those results. Initial analysis of the RIP-seq data indicates an enrichment of calcium binding and Major Sperm Protein genes, both of which are involved in sperm activation. The new knowledge of the ETR-1 mRNA targets, in conjunction with the characterized phenotype s, will enable us to elucidate the mechanism of ETR-1 action in gametogenesis. Funding: Howard University, DoD Grant #64684-RT-REP

Program Abstract #318
Elucidating the Role of Securin in Regulating Separase during Cortical Granule Exocytosis
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Separase (SEP-1) is a key player during meiosis, known for its role in chromosome segregation. SEP-1 also functions in vesicular trafficking during anaphase. We investigated whether the cell cycle machinery known to control SEP-1 during chromosome segregation also controls its localization and function during vesicular trafficking. Following spindle attachment and chromosome alignment, the anaphase promoting complex/cyclosome (APC/C) is activated, resulting in the degradation of the SEP-1 inhibitory chaperone securin (IFY-1) and entry into anaphase I. In C. elegans embryos, SEP-1 localizes to specialized vesicles called cortical granules and regulates their exocytosis during anaphase I. Cortical granule exocytosis is necessary for eggshell formation. Before SEP-1 appears on cortical granules, SEP-1 localizes to cytosolic filaments near the plasma membrane. SEP-1 colocalizes with IFY-1 on cytoplasmic kinetochore-based filaments during prometaphase, and both disassociate from these structures during anaphase I. Inhibition of APC/C prevents SEP-1 and IFY-1 from leaving the filaments, and depletiof IFY-1 prevents cortical granule exocytosis. This suggests degradation of IFY-1 regulates SEP-1 localization to vesicles. To address whether IFY-1 degradation is required to allow SEP-1 vesicle localization, we generated a non-degradable IFY-1 (IFY-1DM::GFP). IFY-1DM::GFP is not degraded following anaphase I onset, persisting on chromosomes and in the cytoplasm. IFY-1DM::GFP localizes to filaments normally and, in contrast to wild-type, localizes to vesicles during anaphase I. In the future we will investigate how IFY-1DM::GFP affects SEP-1 localization to cortical granules. This will provide insight into how regulatory components of the cell cycle control SEP-1 localization to promote cortical granule exocytosis during anaphase I. This work is funded by startup funds from UT Knoxville and by NIH R01 GM114471.
Program Abstract #319
PP1/SDS-22 phosphatase controls germ plasm segregation in the *C. elegans* zygote
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In many animals, maternally deposited germline proteins and RNAs (the “germ plasm”) concentrate in germ cells and specify germ cell identity. During the asymmetric division of the *C. elegans* zygote, the germ plasm RNA-binding protein POS-1 segregates to the posterior cytoplasm, leading to its preferential inheritance by the posterior germline blastomere. POS-1::GFP segregation is stimulated by the polo-like kinase PLK-1, which concentrates in the anterior cytoplasm and phosphorylates POS-1 to inhibit its retention in the anterior (B. Han et al, 2018). We hypothesized that a phosphatase might counteract PLK-1 to enable POS-1::GFP retention in the posterior cytoplasm. Through an RNAi screen, we identified the catalytic subunits GSP-1 and GSP-2 and the regulatory subunit SDS-22 of PP1 phosphatase as critical regulators of germ plasm segregation. In wild-type embryos, PLK-1 and POS-1::GFP asymmetries are established during interphase and are maintained for 10 minutes until cytokinesis. In *sds-22(RNAi)* embryos, PLK-1 segregates to the anterior cytoplasm as in wild-type embryos. In contrast, POS-1::GFP remains symmetrically distributed in *sds-22(RNAi)* embryos until late in mitosis (~3 minutes before cytokinesis), at which point it rapidly segregates to the posterior cytoplasm. Strikingly, we find that the retention of other germ plasm factors including PIE-1::GFP and germ (P) granules are similarly disrupted in *sds-22(RNAi)* embryos. Consistent with a critical role for SDS-22 in the development of the germline, *sds-22(null)* embryos develop into sterile adults. We propose that germ plasm retention is stimulated by PP1/SDS-22 phosphatase during interphase and by an unknown “backup” mechanism that acts during mitosis. Supported by NIH grant R01GM110194.

Program Abstract #320
Damage-Activated Regeneration Enhancers (DAREs) control the regenerative capacity of *Drosophila* tissues
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Many organisms lose the ability to regenerate as they mature, although the underlying causes remain unknown. The larval organs of *Drosophila*, known as imaginal discs, have significant regenerative capacity earlier in life, and can recover from substantial insults, including irradiation, physical wounding and genetically-induced damage. However, this regenerative ability progressively declines as the animal proceeds through development. This loss of regenerative capacity correlates with reduced damage-responsive expression of multiple genes, including those regulating the regrowth and re-patterning of the tissue, such as Myc, MMP1, and WNT signaling. These genes also have essential roles in the development of the imaginal disc, and so until recently it was unclear how their regenerative response can be selectively inhibited without compromising their normal developmental expression in undamaged discs. Our work has found that the expression of two WNT genes, *wg* and *Wnt6*, during regeneration requires a bipartite Damage-Activated Regeneration Enhancer, or DARE, whose activity declines during larval life as a result of highly localized epigenetic silencing at the enhancer, leading to a progressive loss of regenerative WNT expression. As this silencing is localized specifically to the DARE, this mechanism limits their activation solely in the context of regeneration, without affecting WNT expression directed by normal developmental signals. Building on this finding, we have now identified a comparable bipartite DARE regulating the key regeneration factor MMP1, showing that it has a similar regulatory signature of damage activation and epigenetic silencing. By analyzing whole-genome chromatin accessibility and transcriptional changes using ATAC-seq and RNA-seq we further show that potential DAREs are widespread throughout the genome, and likely regulate multiple factors that, similar to MMP1 and the WNT genes, have essential roles in both development and regeneration.

Program Abstract #321
Reawakening the Regenerative Potential of Mammalian Müller Glial Cells
Ross Poché, Elda Rueda, Benjamin Hall, Matthew Hill, James Martin

In many animals, maternally deposited germline proteins and RNAs (the “germ plasm”) concentrate in germ cells and specify germ cell identity. During the asymmetric division of the *C. elegans* zygote, the germ plasm RNA-binding protein POS-1 segregates to the posterior cytoplasm, leading to its preferential inheritance by the posterior germline blastomere. POS-1::GFP segregation is stimulated by the polo-like kinase PLK-1, which concentrates in the anterior cytoplasm and phosphorylates POS-1 to inhibit its retention in the anterior (B. Han et al, 2018). We hypothesized that a phosphatase might counteract PLK-1 to enable POS-1::GFP retention in the posterior cytoplasm. Through an RNAi screen, we identified the catalytic subunits GSP-1 and GSP-2 and the regulatory subunit SDS-22 of PP1 phosphatase as critical regulators of germ plasm segregation. In wild-type embryos, PLK-1 and POS-1::GFP asymmetries are established during interphase and are maintained for 10 minutes until cytokinesis. In *sds-22(RNAi)* embryos, PLK-1 segregates to the anterior cytoplasm as in wild-type embryos. In contrast, POS-1::GFP remains symmetrically distributed in *sds-22(RNAi)* embryos until late in mitosis (~3 minutes before cytokinesis), at which point it rapidly segregates to the posterior cytoplasm. Strikingly, we find that the retention of other germ plasm factors including PIE-1::GFP and germ (P) granules are similarly disrupted in *sds-22(RNAi)* embryos. Consistent with a critical role for SDS-22 in the development of the germline, *sds-22(null)* embryos develop into sterile adults. We propose that germ plasm retention is stimulated by PP1/SDS-22 phosphatase during interphase and by an unknown “backup” mechanism that acts during mitosis. Supported by NIH grant R01GM110194.
Vision loss, due to congenital retinal degeneration, aging, or traumatic injury, is irreversible. Current approaches to prevent retinal degeneration or restore lost photoreceptors are heavily invested in adeno-associated virus-mediated gene therapy and cell transplantation. However, such strategies require either a clear genetic etiology or efficient donor cell engraftment into the pre-existing retinal circuitry, thereby imposing significant technical limitations. An additional means of promoting mammalian retinal regeneration may derive from the finding that the Müller glial cells (MGCs) of the zebrafish retina have the remarkable ability of retinal regeneration. In doing so, these MGCs re-enter the cell cycle, acquire a multipotent, progenitor-like identity and differentiate into new retinal neurons including photoreceptors. Based on previous studies showing that the Hippo pathway negatively regulates mouse cardiomyocyte regeneration, we investigated whether the Hippo pathway may function in a similar capacity within mouse MGCs. We determined that quiescent mouse MGCs express the transcriptional coactivator YAP, a known target of the Hippo pathway. We also show that retinal damage leads to an increase in Hippo signaling and consequently YAP repression, which we hypothesize, functions as a block to MGC-mediated retinal regeneration. Consistent with this, transgenic expression of a Hippo non-responsive, constitutively active form of YAP in MGCs drove spontaneous proliferation and this response was further enhanced by the presence of retinal damage. Strikingly, we also observed that a subset of transgenic MGCs expressed markers of retinal neuron identity. These data raise the intriguing possibility that by bypassing Hippo regulation, MGCs develop the potential to regenerate retinal neurons akin to the zebrafish system. Funding: NIH R01 EY024906 and The Bright Focus Foundation

Program Abstract #322
Gradual expansion of RPE-like cells in chick Müller glial culture
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Müller glia in high vertebrate retina are known to possess certain stem cell properties. Previously we detected RPE-like cells in Müller glial cell cultures established with embryonic day 13 (E13) and E14 chick retina. To shed light on this presumed “Müller-to-RPE” transition, we examined whether it could occur with more developed retina and whether and how RPE-like cells amplified in the culture. Primary Müller cell cultures were established with central and central-peripheral regions of chick retina at E16, when cell proliferation has ceased, and at E18, when the retina becomes functional. After 7 days in vitro (DIV), Müller cells started to constitute a significant portion of the cell population. From 0 DIV to 25 DIV, the culture lacked cells containing the dark pigment granules typically present in RPE cells. By 28 DIV, darkly-pigmented cells were visible under microscope. More cells became melanized as the culture aged. Many of the melanized cells clustered as colonies visible to the naked eyes. Sequential photographs of the same culture over time showed that individual colonies of melanized cells increased in their sizes, but their pattern of distribution and their shapes (marked by melanin) remained more or less the same throughout the period. The presence of melanized cells in E18 culture suggests a Müller-to-RPE transition occurring in culture derived from well-developed, functional retina. The conservation of the shapes of melanized colonies and their overall distribution pattern implies a presence of localized factor(s) promoting RPE-like cell “recruiting” and that the enlargement of individual colonies likely due to existing, neighboring cells becoming melanized rather than to the proliferation of already melanized cells. Supported by NIH/NEI grant EY011640, Research to Prevent Blindness, EyeSight Foundation of Alabama, and NIH/NEI core grant P30 EY003039.

Program Abstract #323
Neural progenitors selectively recapitulate embryonic gene regulatory programs during vertebrate regeneration
Anneke Kakebeen, Alexander D. Chitsazan, Andrea Wills
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While some vertebrates can regenerate their spinal cord (SC), we still lack a fundamental understanding of how
they access a regenerative program that is not available in humans. Our lab takes advantage of the transient regenerative competency of *Xenopus tropicalis* tadpoles to reveal the molecular mechanisms driving SC regeneration. A critical cell population for SC regeneration is the neural progenitors (NP), which balance self-renewal and neurogenesis. During embryonic patterning, NP domain specification and subsequent neurogenesis are driven by opposing bmp and shh signaling and discrete transcriptional programs. I am interested in addressing whether SC regeneration recapitulates development and reuses these same processes, or applies them in a unique way to enable tissue remodeling. To get at this, I examined chromatin accessibility over a regeneration time-course to define the regulatory network governing neurogenesis and self-renewal in FACS purified NPs. ATAC-Seq libraries made from NPs in 0, 6, 24, and 72 hours post amputation (hpa) identified accessible regions of the chromatin specific to each regeneration timepoint. Gene ontology analysis of accessible regions revealed temporally distinct chromatin accessibility landscapes associated with tube morphogenesis at 6hpa, neurogenesis prioritized at 24hpa, and stem cell maintenance and proliferation prioritized at 72hpa in NPs. This temporal ordering reflects key differences from development, with differentiation prioritized earlier than proliferation. Analysis of accessible regions specific to each time-point also revealed that NPs differentially utilize embryonic transcription factors at each timepoint to drive these processes during regeneration. Our data provides critical insight into how regeneration exploits embryonic transcriptional regulatory programs during SC regeneration, but the ways regenerative programs may uniquely differ from embryogenesis. PHS NRSA T32GM007270 from NIGMS, NINDS R01NS099124

**Program Abstract #324**
**Making and remaking of the sea star larval nervous system**
Minyan Zheng, Gregory Cary, Veronica Hinman
*Carnegie Mellon University, USA*

Sea star embryos are an ideal model system to study developmental gene regulatory networks (GRNs). We now seek to extend on the well-studied GRNs and establish the starfish *Patiria miniata* as a model to study the cellular sources and the GRNs controlling regeneration. Starfish larvae have extraordinary regenerative capacity and can grow back their anterior structures, including the serotonergic ganglia within 7 days post decapitation. An important, open question is how and whether developmental GRNs are recapitulated for the reformation of structures during regeneration. To understand whether the neural regenerative processes recapitulate the developmental paradigm, here we examine the cell proliferation profile, expression patterns of transcription factors and signaling molecules that regulate neurogenesis at different time points over the course of regeneration.

**Program Abstract #325**
**Wnt signaling regulates head regeneration in the Starlet Sea Anemone Nematostella vectensis**
Yasuno Iwasaki, Matthew Lee, Gerald Thomsen
*Stony Brook University, USA*

The Starlet Sea Anemone, *Nematostella vectensis*, is an excellent model system for investigating anthozoan cnidarian embryonic development and regeneration. Like many cnidarians, Nematostella is highly regenerative and can renew any amputated portion of its body, but knowledge of the cellular and molecular mechanisms which regulate regeneration in Nematostella are only beginning to emerge. We are investigating how an amputated aboral fragment (the physa) of the adult Nematostella polyp is able to regenerate an entire polyp, and how bisected polyps regenerate missing structures with proper oral or aboral polarity. In the present study we have investigated the action of Wnt signaling in oral regeneration. The Nematostella genome encodes a nearly complete set of Wnt genes that are orthologous to bilaterian Wnts, and we find that in the amputated physa nearly all Nematostella Wnt genes are expressed in a temporal sequence during head and body regeneration. This sequence of Wnt gene activation is initiated by wound-induced MAPK signaling, and the early Wnt signals trigger cell proliferation at the regenerating oral pole of the physa. Blocking Wnt signaling inhibits oral and body regeneration, demonstrating that Wnt signaling is essential for oral regeneration. In the complementary, aboral
cut surface of the amputated polyp body, MAPK signaling is also triggered by amputation, but Wnt genes are not activated as the physa regenerates on the cut aboral end of the polyp. Our findings demonstrate that the Wnt pathway is essential for regeneration of oral and body structures in the amputated physa and specifies oral polarity. In Hydra, Wnt signaling is required for head regeneration, indicating an evolutionarily conserved mechanism for oral regeneration among hydrozoan and anthozoan cnidarians.

Program Abstract #326
Identifying conservation of metazoan features of regeneration in a larval echinoderm
Andrew Wolff, Gregory Cary
Carnegie Mellon University, USA
The process of regeneration is one that has both fascinated and puzzled researchers for decades. Across the metazoan phylogenetic tree, animals with varying degrees of regenerative capacity have been identified. Some common processes underlie regeneration in these model organisms, but the extent of conservation across more distantly related animals remains unknown. Understanding the mechanisms of regeneration in diverse animal models will aid in identifying the molecular machineries that prevent other species, including many vertebrates from regenerating complex structures. Echinoderms, a phylum of invertebrate deuterostomes, are well known to possess fantastic regenerative ability, but this process has not been studied extensively in these organisms. Here, we present an examination of the mechanisms of whole-body regeneration of a larval sea star Patiria miniata. Using EdU labeling, we identified the pattern of proliferating cells throughout regeneration, with global levels of proliferation diminishing before the emergence of a blastema. When labeled with BrdU and EdU before and prior to blastemal proliferation, we observed that these are two distinct populations, suggesting that normal proliferation is turned off while regenerative proliferation is specifically induced in this context. Perturbation of Wnt signaling prior to the formation of the blastema results in a reduction in blastemal proliferation, pointing to a role of this pathway in regenerative proliferation. RNAseq of regenerating sea star larvae identified the upregulation of MAPK signaling components early in regeneration, and perturbation of MEK at this time reduced proliferation later, suggesting that this pathway plays a role upstream of the induction of regenerative proliferation. Taken together, these results highlight the conservation of proliferation during regeneration in this Echinoderm model. Funding: Carnegie Mellon

Program Abstract #327
Scar-less whole body regeneration in the absence of a blastema
Mark Martindale, Julia Ramon
Univ of Florida, USA
Many animals are capable of regenerating massive regions of their adult bodies following surgical removal. One such group are lobate ctenophores or comb jellies, fragile holopelagic marine carnivores that represent one of the oldest extant metazoan lineages. It has been known for well over 50 years that these animals have the capacity to replace substantial missing parts, but the cellular and molecular basis that confers this ability is not understood. We are studying wound healing and adult regeneration in the ctenophore Mnemiopsis leidyi by time-lapse imaging and confocal microscopy. Animals cut in half along the oral-aboral axis complete wound healing by 4 hours after surgery and complete the regenerative process in 4 days. However, anlage for all missing structures (4 comb plates and the tentacle bulb) are already visible by 2 days after surgery. We used EdU incorporation to determine the source of the cells that form the missing structures, and determine the role of cell division in the ability to complete the regenerative process. EdU labeling in control animals indicates that the majority of S-phase cells are located in the tentacle bulbs, where they give rise to the feeding tentacles throughout their lifetime. Pulse-chase EdU labeling experiments indicates that cells in both the endodermal and ectodermal layers begin to cycle at the cut site 18 hours after surgery. The tentacle bulbs contain cycling cells that may act as a source stem cell population during regeneration, and our results suggest that cells from the tentacle bulb appear to migrate to the wound site and participate in the formation of new structures. We are currently investigating the molecular changes occurring during wound healing and regeneration, and have developed techniques to manipulate gene
expression in regenerating adult animals that will allow us to understand how these ancient animals can quickly regenerate their entire body plan. This work was supported by the NSF.

Program Abstract #328
**Bmi1-expressing intestinal stem cells drive epithelial development and harness a developmental program during regeneration in adult mice**

Nicholas Smith¹, Noelle McPhail¹, Sidharth Sengupta¹, Michael Parappilly¹, Catherine Beach¹, John Swain¹, Joshua Burkhart¹, Andrew Fields¹, Andrew Adey¹, Jared Fischer², Melissa Wong¹,²

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Multiple intestinal stem cell (ISC) populations contribute to homeostasis and injury-induced regeneration of the adult epithelium. Homeostatic renewal is driven by active-cycling ISCs expressing the Wnt target gene Lgr5, while a slow-cycling population expressing the epigenetic regulator Bmi1 has stem capacity after injury. Although stem state plasticity has been demonstrated between these two ISC populations, their relationships in developmental and regenerative contexts remain incompletely understood. Here, we use GFP-reporter and Cre-mediated lineage tracing to examine relationships between Bmi1⁺ and Lgr5⁺ cell populations during development and in adult mice during tissue regeneration. We found that Bmi1 expression preceded Lgr5 within the developing epithelium until transition to a Wnt-dependent growth state at E15.5. Clonal analyses and ATAC-Seq demonstrated that early development is driven by the Bmi1⁺ population, but later shifts to the Lgr5⁺ population. Further, lineage tracing in early development revealed that adult Lgr5⁺ ISCs arise from the Bmi1⁺ population. This developmental relationship is recapitulated in the regenerating adult epithelium. Ex vivo stimulated Bmi1⁺ ISCs grew as highly proliferative spheroids that expressed low levels of differentiated or active-cycling ISC markers consistent with a developmental-like cellular program. After injury, Bmi1⁺ cells expressed developmental markers prior to replenishing the Lgr5⁺ ISC population in vivo. Our data indicate that Bmi1⁺ cells transition from a proliferative developmental state to a slow-cycling adult state, which is likely affected by Wnt signaling within this population. Moreover, the capacity of adult Bmi1⁺ ISCs to adopt a developmental state may underlie their regenerative function.

Program Abstract #329
**Bmi1-expressing intestinal stem cells give rise to the active-cycling stem compartment**

Michael Parappilly, Nicholas Smith, Paige Davies, John Swain, Alexandra Gallagher, Melissa Wong

Oregon Health & Science University, USA

The continuous renewal and dynamic repair of the intestinal epithelium are driven by active- and slow-cycling intestinal stem cells (ISCs) respectively. Lineage tracing studies suggest that reciprocity in hierarchy exists between these distinct ISC types, however their relationships during tissue development, homeostasis, repair, and disease remain poorly understood. To investigate the relationship between the slow-cycling (Bmi1⁺) ISC and the active-cycling stem cell niche (Lgr5⁺ ISC and Paneth cell), in vivo lineage tracing using Bmi1-Cre mice was performed. These studies revealed that Bmi1⁺ ISCs can directly give rise to both Paneth cells and Lgr5⁺ ISCs in the adult homeostatic intestine and indicates that these slow-cycling ISCs harness this capacity to drive a program of emergency re-epithelialization for rapid restoration of the active-cycling niche cells after injury. Further, Bmi1⁺ ISCs exist as the sole proliferative stem cell in the developing intestine at early time points, and lineage tracing indicates that they give rise to Lgr5⁺ ISCs that can be tracked in the adult epithelium. This relationship was recapitulated during tissue regeneration. Using a panel of novel monoclonal antibodies that facilitate isolation of discrete ISC populations from non-reporter mice, the hierarchical relationships between active and slow-cycling ISCs were confirmed using in vitro enteroid culture assays. Our data indicates that Bmi1⁺ ISCs have the capacity to give rise to the active-cycling stem cell niche during development and in adult tissue at homeostasis, and call upon this program for rapid restoration of tissue homeostasis after injury.
**Program Abstract #330**

**Regulation of mucociliary epithelial stem cells - from frog embryos to diagnostic organoids**

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Mucociliary epithelia line the embryonic epidermis of many vertebrates as well as inner organs (e.g. the airways) and are equally important for animal development as well as human health. Nevertheless, our understanding of mucociliary cell type specification, morphogenesis and regeneration remains limited, which prevents the development of diagnosis and treatment strategies for airway disease patients and precludes personalized regenerative medicine approaches. To investigate the cell and developmental biology of mucociliary epithelia in an integrative and comparative manner, we are using a set of systems, including immortalized human airway stem cells, mice, and the embryonic epidermis of *Xenopus* embryos. Here, I will present our novel findings regarding the evolutionarily conserved reiterative roles of Wnt/beta-catenin signaling in mucociliary development and disease. Importantly, our data indicate that Wnt/beta-catenin signaling regulates mucociliary stem cells (basal cells) through the transcription factor ΔN-Tp63. Overactivation of Wnt/beta-catenin signaling causes excessive ΔN-Tp63 expression and prevents differentiation of stem cells into epithelial cell types leading to basal cell stacking. This mode of regulation is conserved in mucociliary epithelia from frogs to humans. Loss of differentiation and basal cell stacking are phenotypes commonly observed in chronic lung diseases, indicating that dysregulated Wnt signaling could contribute to the pathogenic changes. We applied this knowledge to the development of patient-derived mucociliary organoids, which can be used for diagnosis of human diseases and potentially for *in vitro* testing of therapies. Taken together, our data reveal an evolutionarily conserved mode of stem cell regulation in mucociliary epithelia with important translational impact and establish the *Xenopus* embryonic epidermis as model to study basal stem cell regulation in vivo. Funding: NHLBI (K99), DFG (Emmy Noether Program)

**Program Abstract #331**

**Development of cell-based therapies to treat congenital strial deafness**

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The stria vascularis is a specialized epithelial structure localized in the lateral wall of the cochlea. It generates the positive endocochlear potential that is essential for mechanotransduction by sensory hair cells. It is composed of four different cell types of distinct embryonic origins: marginal cells derived from otic epithelium, melanocyte-like intermediate cells derived from neural crest cells, basal cells that have a mesenchymal origin, and mesoderm-derived blood vessels that are interspersed throughout the lateral wall. During inner ear development, neural crest cells (NCC) migrate through the cochlea to form the glial cells of the spiral ganglion and the intermediate cells of the stria vascularis. This process requires transcription factors such as SOX10, MITF, and EDNRB. In the absence of these transcription factors NCC do not reach their target sites and this results in congenital strial deafness, due to the lack of intermediate cells in the stria vascularis. Knock out or conditional knock out mice for these genes should be useful models of congenital strial deafness. Our aim is to determine whether multipotent NCC and/or melanoblasts can replace the missing intermediate cells in a strial deafness mouse model, and restore strial function and thus hearing. For this project, we decided to study the injection of GFP-labeled cells into neonatal, EdnrB KO mice. We first characterized and confirmed the absence of intermediate cells in the cochlea of KO mice by immunostaining; and measured the elevation of the hearing threshold by Auditory Brainstems Response. We also confirmed the location of the injection site and the survival of injected cells by immunostaining two days post injection. Here we show that we have optimized the protocols to deliver cells with potential therapeutic effects to the lateral wall of the cochlea of neonatal mice. This research was supported by the Hartwell Foundation.
Program Abstract #332
Live imaging of emergence, expansion, and diversification of founder osteoblasts during skeletal regeneration in zebrafish
Ben Cox1, Alessandro De Simone1, Valerie Tornini1,2, Sumeet Singh1,3, Stefano Di Talia1, Kenneth Poss1
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Osteoblasts are matrix-depositing cells that can divide and heal bone injuries. Their deep tissue location and the slow progression of bone regeneration challenge attempts to capture osteoblast behaviors in live tissue at high spatiotemporal resolution. We have developed an imaging platform to monitor over long periods (up to 24 hours) the individual and collective behaviors of osteoblasts in regenerating zebrafish scales, which regenerate rapidly after loss to maintain integrity of the body armor. Using a panel of transgenic lines that mark and manipulate osteoblasts, we show that a founder pool of osteoblasts emerges through de novo differentiation within one day post-scale plucking (dpp). We imaged regenerating scales over multiple time windows beginning with the appearance of new osteoblasts and continuing until initial morphogenesis of mature scale features at 3 dpp. We find that early in regeneration, osteoblasts undergo divisions that are largely uniform in frequency and stochastic in orientation to establish a primordium. By two days after injury, osteoblast proliferation slows significantly, and population dynamics diversify across the primordium. Cell divisions become focused near and with orientations parallel to the scale periphery, creating a dense outer ring of osteoblasts. This change in proliferation mirrors changes in localization of fgf20a gene expression, and we demonstrate that FGF signaling perturbation restricts scale growth. As osteoblast proliferation slows, conspicuous grooves in the scale called canals form by recruitment and selective death of osteoblast subsets in posterior regions, while surviving osteoblasts elongate to form the canals. By imaging live developmental events at single-cell resolution, we detail acquisition of spatiotemporally distinct cell division, shape, and death dynamics within a founder osteoblast pool during bone regeneration. Funding: NSF GRFP 2014175655 to BDC; NIH R01 GM074057 to KDP; NIH R01 GM122936 to SDT

Program Abstract #333
Generation of iPSC-derived limb progenitor-like cells for stimulating phalange regeneration in the adult mouse
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The capacity of digit tip regeneration observed both in rodents and humans establishes a foundation for promoting robust regeneration in mammals. However, stimulating regeneration at more proximal levels, such as the middle phalanges (P2) of the adult mouse, remains challenging. Having shown the effectiveness of transplantation of limb progenitor cells in stimulating limb regeneration in Xenopus, we are now applying the cell transplantation approach to the adult mouse. Here we report that both embryonic and iPSC-derived limb progenitor-like cells can promote adult mouse P2 regeneration. We have established a simple and efficient protocol for deriving limb progenitor-like cells from mouse induced pluripotent stem cells (iPSCs). iPSCs are cultured as three-dimensional fibrin bodies, followed by treatment with combinations of Fgf8, CHIR99021, Purmorphamine and SB43542 during differentiation. These iPSC-derived limb progenitor-like cells resemble embryonic limb mesenchyme cells in their expression of limb-related genes. After transplantation, the limb progenitor-like cells can promote adult mouse P2 regeneration, as embryonic limb bud cells do. Our results provide a basis for further developing progenitor cell based approaches for improving regeneration in the adult mouse limbs.

Program Abstract #334
Reprogramming non-limb fibroblasts into limb bud progenitor-like cells
Yuji Atsuta, Alan Rodrigues, Changhee Lee, Charlotte Colle, Cliff Tabin
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The limb bud progenitors (LPs), which originate from the somatopleural layer of the lateral plate mesoderm, give rise to the majority of tissues within the mature patterned limb including bones, cartilage and tendons. While LPS have such differentiation and patterning potentials, lateral plate-derived cells outside of the limb buds do not
exhibit these properties. To identify factors involved in establishing limb progenitor properties in lateral plate mesoderm, we took a reprogramming approach, reasoning that the full set of the factors specifying “limbness” might be sufficient to convert non-limb fibroblasts into LP-like cells. First, we attempted to establish a robust culture system for endogenous mouse LPs (mLPs) that would underpin reprogramming assays. By taking advantage of hyaluronan-based hydrogels, we have succeeded in maintaining LP marker expression in cultured mLPs for 10 days. To screen a set of potential candidates, we generated a list of transcription factors expressed specifically in the early limb fields by using RNAseq. As a result, 18 factors were deemed as candidates, and overexpression of them was indeed sufficient to induce the expression of LP marker genes such as Sall4 and Lhx9 in 3D-cultured mouse embryonic fibroblasts (MEFs). We further narrowed down a minimum set of factors by examining the effect of withdrawal of individual factors, and identified that the combination of Prdm16, Plzf and Lin28a greatly upregulated the expression levels of LP genes. Moreover, single-cell RNAseq analysis revealed that a fraction of the reprogrammed cells had quite similar transcriptome profiles to legitimate mLPs. Strikingly, when placed in high-density “micromass” culture, the cells differentiated into alcian blue-positive cartilage as mLPs do. Together, these results suggest that the three factors could convert MEFs into LP-like cells, and they may play pivotal roles in cell specification of LPS during limb development. Funding: JSPS fellowships, NIH R01

Program Abstract #335
How Do Stem Cells Find Their Niche?
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Stem cells reside in specialised cellular niches that regulate their function. Wnt ligands are niche signals that are essential for the maintenance of many types of stem cells. Localised Wnt signals can induce self renewal by orienting asymmetric cell division to produce one stem cell and another lineage restricted daughter cell. We perfumed a large-scale quantitative analysis of single embryonic stem cells that revealed a mechanism employed by stem cells to recognise and actively recruit directional Wnt signals that drives asymmetric cell division. We found that the stem cells detect localised Wnt proteins by specialized nanoprotrusions which are actin-enriched and beta-catenin dependent. To characterise further the interaction of the cells and localised Wnt, we apply the theory of simple liquids bridging the gap between living and dead matter. We quantify the biophysics and the dynamics of this interaction in terms of effective potentials and pair correlation functions. Our findings show a positive correlation between the cellular levels of beta catenin and the affinity to the localised Wnt. Additionally, beta catenin levels influence the location and duration of the interaction between localised Wnt and the plasma membrane. Importantly, localised Wnt modulates the fluidity of the plasma membrane to initiate the polarisation of the stem cell. Finally we demonstrate that in the presence of localized Wnt signals, beta catenin is dispensable for polarizing the receptor Lrp6 but essential for polarizing the down stream effector APC, spindle orientation and consequently asymmetric stem cell division. Our results provide a novel mechanism for the stem cell-niche recognition and can be extended to other stem cell types and niche signals.

Program Abstract #336
Novel strategies for induced in vivo transdifferentiation across germ layers
Duc Dong, Clyde Campbell, Joseph Lancman, Jonatan Matalonga
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The extent to which differentiated cells, while remaining in their native microenvironment, can be reprogrammed into unrelated cell types will reveal fundamental insight into cellular plasticity and impact regenerative medicine. To investigate in vivo lineage potential, we developed a novel in vivo discovery platform for lineage conversion. Using this platform, we have identified a cocktail of transcription factors that, when mis-expressed in several non-endoderm lineages including skeletal muscle, are able to specifically and cell-autonomously trigger the endoderm genetic program. These endoderm induced muscle cells can proceed to lose muscle gene expression and morphology, while gaining key endoderm organogenesis markers, such as the pancreatic specification genes,
Endoderm markers appearing prior to loss of muscle cell morphology, a lack of dependence on cell division, and a lack of dedifferentiation, mesoderm, ectoderm, and pluripotency gene activation, together, suggests that reprogramming occurred by direct lineage conversion. Mechanistic studies reveal broadly applicable new strategies for enhancing lineage reprogramming efficiency by leveraging synthetic and xenotic factors, as well as genetic loss-of-function. Our work demonstrates that within a vertebrate animal, differentiated cells originating from one germ layer can be induced to directly adopt lineages of a different germ layer – suggesting that differentiated cells in vivo may have unrestricted lineage potential. Our technology may pave the way towards a vast new in vivo supply of replacement cells for injuries and degenerative diseases such as diabetes. These studies were funded by the NIH Director’s New Innovator Award and by the W. M. Keck Foundation Award.

Program Abstract #337
Robust differentiation of functional glial cells from human neural crest cells
Rebekah Charney, Maneeshi S. Prasad, Samantha Bruinsma, Martin Garcia-Castro
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Spinal cord injury (SCI) leads to impairments of considerable proportions and presents major clinical challenges. A central focus of SCI research has been to overcome the failure of axons to regenerate. Due to their innate potential to clean and remove dead cells, and myelinate and repair damaged neurons, glial cells – in particular, Schwann cells (SC) and olfactory ensheathing cells (OEC) – are a promising tool for SCI repair. However, current methods used to generate these cells are lengthy and expensive, and produce a mixed population of cells yielding inconsistent regenerative outcomes. Therefore, there is a critical need for rapid and efficient methods that will generate unlimited, pure populations of SC and OEC. A key consideration in stem cell therapy is to start with a developmentally correct path towards a cell fate, and neural crest cells (NCC) are natural progenitors of SC and OEC. We established a rapid and robust protocol to generate human NCC from human embryonic stem cells or induced pluripotent stem cells in only 5 days. These cells display all the hallmarks of NC, and importantly, differentiate into expected NC derivatives, including glia precursor cells. Here, we present the robust derivation of SC precursors and mature SC from human NCC. We show that, using human NCC, we can generate a synchronized population of unlimited SC in less than 3 weeks. We have further validated the functionality of these SC to myelinate neurons in vitro, as well as in vivo using chick xenografts. Further, as specific markers for OEC are lacking, we are pursuing the molecular characterization of NCC-derived OEC using the chick model system. These findings will be invaluable towards the in vitro derivation of human OEC. Taken together, our work represents the generation of powerful tools which can be harnessed for regenerative medicine and the elucidation of the molecular mechanisms underlying hNCC-derived glial cells. Funded by NIH 5R01DE017914 to M.I.G.C.

Program Abstract #338
Restoration process of cryoinjury in the early chick embryonic heart
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Adult mammalian heart is non-regenerative because cardiomyocytes do not re-enter the cell cycle. In contrast, urodele amphibians and teleost fishes retain a capacity for cardiac regeneration by the dedifferentiation and proliferation of preexisting cardiomyocytes. To date, only a few literatures were published on the embryonic heart regeneration, so that it is uncertain whether the heart is capable of regenerating before the formation of epicardium. The aim of this study is to establish the cryoinjury model in early chick embryonic heart and examine the restoration process after injury. The heart apex was injured by cryoprobe cooled in liquid nitrogen at HH stage 16, embryos were reincubated, and examined the restoration process of the lesion. Immediately after injury, cardiomyocyte defect was closed by endocardium herniated through the defect followed by the formation of scar tissue containing epicardium, mesenchymal cells and blood cells. Five hours after injury, cardiomyocytes surrounding the cryoinjury-induced defect showed a reduced BrdU labeling and an up-regulation of Cdk inhibitor protein p27 suggesting that G1-to-S arrest occurred at the acute phase after injury. After 12 hours, there is no significant difference in the rate of proliferation markers in cardiomyocytes between injured hearts and sham-
operated control hearts. Furthermore, no cardiac progenitor cell was found in either the scar tissue or remaining cardiomyocyte. These observations suggested that the reactive cardiomyocyte proliferation as well as dedifferentiation did not occur in the heart injured before the formation of epicardium.

**Program Abstract #339**

**Gap junction-mediated regulation of hematopoiesis in Drosophila**  
Kevin Ho, Rohan J. Khadilkar, Lulu Yang, Guy Tanentzapf  
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Stem cells are essential for animal development and tissue maintenance. When stem cells divide daughter cells can either self-renew to replenish the stem cell population or differentiate to give rise to a particular cell type. Stem cell differentiation and self-renewal are tightly regulated since producing too many stem cells can result in tumor formation while producing too many differentiated cells depletes the stem cell population. Our work focuses on hematopoiesis, the production of new blood cells from hematopoietic stem cells (HSCs). Hematopoiesis in *Drosophila* occurs in the larval organ called the lymph gland (LG). HSCs, housed in the LG exist primarily in a quiescent state but are activated in response to infection. Signaling between the different compartments of the LG is essential to regulate HSC function. We will present evidence that Gap Junctions, intercellular channels that mediate direct communication between neighboring cells, play an important role in regulating stem cell behavior in the LG. Gap junctions, composed of innexins in invertebrates and connexins in vertebrates, mediate the exchange of small metabolites, secondary messengers, and ions between adjacent cells. We performed comprehensive analysis of the requirement for gap junction proteins in the LG and identified key roles for a number of innexins in specific cell types of the LG. We will present our analysis of the role innexin4/zero population growth (Zpg) in regulating the differentiation of HSCs and controlling the size of the stem cell niche. Future work will focus on elucidating the mechanism by which Zpg regulates the HSC and niche cells in the LG and analyzing the precise role of Zpg in the immune response induced by bacterial infection and wasp infestation. This work is supported by grants from the Canadian Institute of Health Research [PJT-156277 to G.T.].

**Program Abstract #340**

**Macrophages are Required for Proper Tail Regeneration in Xenopus tropicalis**  
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Macrophages play a critical role in the balance between scarring and regeneration. When macrophages are removed from regenerative species such as zebrafish, axolotls, or spiny mice, they lose their regenerative capacity. More detailed comparisons of the innate immune response in regenerative animals and their non-regenerative counterparts have revealed crucial differences in the acute response of macrophages to injury. In non-regenerative species the majority of the macrophages recruited to the wound are pro-inflammatory macrophages that act to fight pathogens by secreting proteases and pro-inflammatory mediators. Comparatively, regenerative species show higher levels of IL-4 and IL-13 activated macrophages that act to suppress the immune response and initiate tissue growth. Despite the emerging significance of the innate immune system in regenerative versus non-regenerative healing, there are multiple unknowns about how macrophages are affecting regeneration. The role of macrophages in *Xenopus* regeneration has not yet been described but this is an excellent model system for this research area. *Xenopus* can regenerate their limb buds and tails as tadpoles but lose this ability as adults which allows for within-species controls between regenerative and non-regenerative stages. To date, we have established a reliable macrophage depletion protocol in *Xenopus* tadpoles and shown that *Xenopus* tadpoles require macrophages for proper tail regeneration. Now we are exploring gene expression changes following tail amputation in macrophage depleted animals as compared to controls. This research may help clarify why *Xenopus* are strong but stage-specific regenerators and allow us to better understand the role macrophages play in regeneration.
Program Abstract #341
Importance of injury induced senescence during regeneration in Acomys compared to fibrotic repair in Mus
Sandeep Saxena, Ashley W. Seifert
University of Kentucky, USA
During epimorphic regeneration, blastemal cells re-enter the cell cycle and proliferate to form new tissue. Cell cycle re-entry or arrest is at least partly regulated by cellular senescence which negatively impacts the proliferative pool of cells during regeneration. What remains unclear is whether cells in regenerating systems possess an increased propensity to proliferate and are refractory to signals that induce senescence. To test this idea we used spiny mice (Acomys), a mammalian taxa that exhibit enhanced regenerative ability and are able to replace skin and musculoskeletal tissue. Using an ear hole injury, Acomys show resident cell activation, cell cycle progression and proliferation in contrast to Mus musculus (Mus), who heal identical injuries via fibrotic repair and exhibit limited cell cycle progression. Culturing primary ear pinna fibroblasts in vitro we found that Acomys cells proliferated longer in culture with significantly more cell population doublings (PDs) compared to Mus cells before these cells senesced. Where Mus cells experienced crisis in ~40 days, Acomys cells proliferated for 90 days in culture. This difference was further magnified under physiologically relevant oxygen (3%) where Acomys cells proliferated for ~140 days. We also analyzed replicative capacity in another regenerating (Oryctolagus cuniculus-rabbit) and non-regenerating (Rattus rattus) mammal. Surprisingly, fibroblasts from both species showed no signs of senescence after 150 days. To ascertain if Acomys cells are more resistant to oxidative or inflammatory-mediated stress we exposed these cells to H2O2 and LPS at sub-lethal doses that induce senescence in Mus. Remarkably, Acomys fibroblast exhibited normal proliferation and resisted senescence as determined by SA-βgal staining. Together, our data suggests that resident cells in regenerating species possess higher intrinsic proliferative capacity and a higher threshold to resist stress-induced senescence. Funding: NIH

Program Abstract #342
Transpositional scaling and niche transitions restore organ size and shape during zebrafish fin regeneration
Scott Stewart, Gabriel Yette, Astra Henner, Heather Le Bleu, Kryn Stankunas
University of Oregon, USA
Adult zebrafish fins perfectly regenerate regardless of the nature or position of the injury, providing a striking example of organ size control. Thomas Hunt Morgan pursued this mystery of appendage regeneration over a century ago, showing that the initial rate of fin regeneration correlates with the amount of tissue removed and decreases as the fin is restored. Prevailing models explaining robust fin size restoration speculate that fin cells establish and maintain a multitude of “positional identities” that instruct differential outgrowth after injury. We propose a distinct and straightforward model that neatly explains how fin size and shape is restored without invoking molecularly encoded positional information. Revisiting Morgan’s experiments, we show that the amount of Wnt that sustains progenitor cells and is produced by distal “niche” cells determines the extent of regeneration. We identify Dachshund transcription factors as novel niche markers and show that the niche uniquely forms from intra-ray mesenchyme that populates the inside of the cylindrical, differentially sized, and progressively tapered fin rays. The niche, and therefore Wnt, steadily dissipates by re-differentiation as regeneration unfolds; once exhausted, growth stops. We show that longfin2 zebrafish regenerate exceptionally long fins due to a broken niche “countdown timer”. We identify the long mysterious gene responsible for longfin2 and demonstrate it likely promotes overgrowth by inhibiting Ca2+/calcineurin signaling and disrupting niche cell transitions. We conclude that regenerated fin size is dictated by the amount of niche formed upon damage – which is simply dependent on the availability of intra-ray mesenchyme defined by bone width at the injury site. This “transpositional scaling” model contends that macro-scale fin size and shape is determined by ion signaling-dependent mesenchyme-niche state transitions and self-restoring bone geometry rather than individual cell identities.
Program Abstract #343
Detection of bone mineralization in larvae and adult zebrafish using Carbon nanodots
Esmail Miyanji, Lesly Corado-Santiago, Bryle Barrameda, Isaac Skromne
University of Richmond, USA
Bone weakness and deformity arise from abnormal growth, development, remodeling, or disease. Towards developing new strategies to mitigate bone-related health problems, we are investigating the bone-binding properties of Carbon nanodots (C-dots) in larvae and adult zebrafish, as a method for the targeted delivery of drugs to bones. Our published work shows that C-dots are non-toxic to larvae, and that they bind with high affinity and specificity to mineralized bones: manipulations that increase or decrease calcium mineralization, respectively increase or decrease C-dots deposition in bones. These bone-binding properties are not affected by the derivatization of the C-dots with amine, fluorescein or biotin groups. Preliminary research shows that C-dots can also bind to exposed bones after fin amputation. Further work will determine the bone-binding properties of C-dots to adult bones during remodeling, wound healing and regeneration. (Support: NIH-NIAMS R21AR072226)

Program Abstract #344
The importance of dorsal root ganglia in mediating movement-dependent forebrain neurogenesis in zebrafish larvae
Zachary Hall, Vincent Tropepe
University of Toronto, CA
During postembryonic development, the brain exhibits substantial experience-dependent neuroplasticity, in which sensory experience guides normal brain growth. Traditionally, experience-dependent neuroplasticity is thought to occur primarily via structural and functional changes in pre-existing neurons. Whether neurogenesis also mediates the effects of experience on early brain growth is unclear. Here, we characterized the importance of motor experience on postembryonic neurogenesis in larval zebrafish. We found that swimming is critical to maintain an expanded pool of forebrain neural precursors by promoting progenitor self-renewal over the production of neurons. Physical cues associated with swimming (tail movement) increase forebrain neurogenesis and these cues appear to be conveyed, at least in part, by dorsal root ganglia (DRG) in the zebrafish body: DRG-deficient larvae exhibit attenuated neurogenic responses to changes in swimming and targeted photoactivation of DRG in immobilized larvae expands the pallial pool of proliferative cells. Our results demonstrate the importance of movement in neurogenic brain growth and provide a fundamental sensorimotor association that may couple early motor and brain development. Research funded by the Natural Sciences and Engineering Research Council of Canada.

Program Abstract #345
Predicting Neuroblastoma Using Developmental Signals and Logic-Based Modeling
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Genomic information from human patient samples of pediatric neuroblastoma cancers and known outcomes have led to specific gene lists put forward as high risk for disease progression. However, the reliance on gene expression correlations rather than mechanistic insight has shown limited potential and suggests a critical need for molecular network models that better predict neuroblastoma progression. In this study, we construct and simulate a molecular network of developmental genes and downstream signals in a 6-gene input logic model that predicts a favorable/unfavorable outcome based on the outcome of the four cell states including cell differentiation, proliferation, apoptosis, and angiogenesis. We simulate the mis-expression of the tyrosine receptor kinases, trkA and trkB, two prognostic indicators of neuroblastoma, and find differences in the number and probability distribution of steady state outcomes. We validate the mechanistic model assumptions using RNAseq of the SHSY5Y human neuroblastoma cell line to define the input states and confirm the predicted outcome with antibody staining. Lastly, we apply input gene signatures from 77 published human patient samples
and show that our model makes more accurate disease outcome predictions for early stage disease than any current neuroblastoma gene list. These findings highlight the predictive strength of a logic-based model based on developmental genes and offer a better understanding of the molecular network interactions during neuroblastoma disease progression.

Program Abstract #346
Agent-based modeling as a method to explore mechanisms of developmental patterning
Daniel L. Lakeland, Francesca Mariani
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A longstanding challenge in developmental biology is to understand how pattern can arise in the context of exponential growth. Here, we present the use of Agent-Based Modeling (ABM), a simulation method based on a cell’s ability to make decisions in response to stimuli. Based on biological observations, we show how agents can be programmed to behave according to simple rules that model cell behaviors such as proliferation, movement, and cell death, keeping in mind that cells are only capable of responding to cues in their local environment. As an example, we show how ABM can be used to study the impact of growth factor signaling during skeletal patterning. Using this method, we conclude that patterning and size of skeletal elements arises in large part due to the self-organizing properties of cells which includes their response to local signals and their proliferation and cell death rates. Interestingly, adjustments to simple parameters can give rise to a wide variety of skeletal patterns and thus ABM may be useful for understanding different phenotypic outcomes in human skeletal dysplasias. Our hope is that this approach can provide a framework that is broadly applicable for understanding mechanisms of patterning in a variety of developmental and regenerative contexts. For instance, ABM may provide insight into how compartments are patterned initially and if cells could be cooperating to generate compartment boundaries. In addition, ABM may open up interesting new lines of inquiry by providing a method for evaluating the combined impact of cell movements, multiple signaling pathways, and biomechanical forces on patterning. This work was supported by the University of Southern California and NIAMS/NIH.

Program Abstract #347
Toward measuring tissue-level mechanical stresses in branching embryonic epithelia
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Tree-like networks form the basic architecture for many organs, including the lung, kidney, and prostate. In the embryo, these structures are sculpted by a process known as branching morphogenesis, in which a simple epithelial tube is shaped into a ramified network by a sequence of branching events. In the embryonic lung, physical cues have been shown to regulate the branching process, but the patterns of mechanical stress that produce these changes in form remain poorly characterized. Here, we developed a three-dimensional (3D) traction force microscopy (TFM) assay for embryonic airway epithelial explants, cultured in gels of reconstituted basement membrane protein. Briefly, whole lung explants were dissected from early chicken embryos at embryonic day 5. The pulmonary mesenchyme was removed using fine tungsten needles, and the airway epithelium was embedded in 3D Matrigel containing a suspension of fluorescent microspheres. The explants were then cultured ex vivo, and time-lapse microscopy was used to quantify the branching dynamics, as well as the motion of the embedded microspheres. Traditional TFM assays use the elastic deformations of compliant substrata to compute the mechanical stresses exerted by cells. These calculations rely upon a knowledge of the mechanical properties of the substratum. We thus used microindentation testing and microrheology to measure the mechanical properties of Matrigel in our mesenchyme-free culture assay. The tracked motion of the fluorescent microspheres was then used to compute the gel deformations during the branching process. These deformations were decomposed into a component due to gel swelling and a component due to elastic deformation. We then used finite-element modeling to estimate the mechanical stresses exerted by the airway epithelium during morphogenesis. Our results can be combined with future studies of cell proliferation and growth-factor signaling to uncover how mechanical cues regulate epithelial branching.
Program Abstract #348
Quantifying endodermal tissue deformations during heart tube formation in the early chicken embryo
John Jackson, Meagan Furth, Victor Varner
The University of Texas at Dallas, USA
The heart is the first functioning organ to form during development and arises as a straight tube situated along
the embryonic midline. In the avian embryo, cardiac progenitor cells reside in the lateral plate mesoderm and are
organized as a pair of epithelia, which move toward the midline and fuse above the anterior intestinal portal (AIP)
to form the heart tube. Throughout this process, the cardiac progenitors remain in close contact with the
underlying endoderm. We have shown previously that contractile forces exerted by cells within the endoderm
pull the cardiac progenitors toward the midline to form the heart tube. How these forces are spatially distributed,
and how they drive the tissue-level deformations that sculpt the embryonic heart tube, however, remain unclear.
Here, we fluorescently labeled sub-populations of endodermal cells in early chicken embryos and tracked their
motion during development to quantify the morphogenetic deformations that occur during heart tube assembly.
Fertilized White Leghorn chicken eggs were incubated in a humidified, forced draft incubator to yield embryos at
HH stage 7. Whole embryos were harvested using a filter paper carrier method, and endodermal cells around the
AIP were labeled using DiI. Briefly, magnetic iron particles were soaked in a solution of DiI and distributed across
the ventral surface of the embryo. A magnet was then used to remove the iron particles, leaving fluorescently
labeled cells behind. Embryos were then cultured in a stage-top incubator, and bright field and epifluorescence
images were captured at 20 min intervals. Fluorescently labeled cells were tracked in time and used to compute
Lagrangian strain fields along the endoderm. Mediolateral strain gradients were observed around the AIP and
were disrupted when cellular contractility had been inhibited. These data suggest that spatial gradients of
contractility within the endoderm pull cardiac progenitor cells toward the midline to form the heart tube.

Program Abstract #349
Epithelial polarity proteins regulate the mechanics of invagination
Melisa Fuentes, Bing He
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Epithelial folding mediated by apical constriction provides a fundamental mechanism that converts flat epithelial
sheets into multilayered tissues. It remains elusive how forces generated near the apical surface drive tissue
folding in 3D. During Drosophila gastrulation, prospective mesoderm cells constrict apically and subsequently
invaginate into the interior of the embryo to form a ventral furrow (VF). Previous studies have suggested that
apical constriction by itself cannot fully account for invagination. To elucidate the additional requirements for
tissue invagination, we performed an RNAi-based candidate screen to identify genes that specifically regulate the
invagination phase of VF formation. We found that knockdown of apical basal polarity (ABP) determinants results
in epithelial folding defects. Despite undergoing normal apical constriction, ABP RNAi mutants exhibit abnormal
VF morphology and a reduced invagination rate. Interestingly, the rate of invagination negatively correlates with
the extent of stretching of the neighboring non-constricting cells during apical constriction. In ABP RNAi mutants,
the flanking cells seem more fluid-like and exhibit an altered spatial distribution of cortical actin. Together, our
findings suggest that successful invagination depends on the mechanical integrity of the flanking cells. Further
studies will be focused on understanding how cell rigidity is regulated by ABP determinants and how such
regulation may contribute to coordinated tissue folding. This study was supported by the GAANN Fellowship, the
Albert J. Ryan Foundation, and the American Cancer Society Research Grant #IRG-82-003-33.

Program Abstract #350
Extracellular matrix remodeling and activation of focal adhesion kinase direct airway epithelial branching
morphogenesis
James Spurlin, Micheal Siedlik, Mei Fong Pang, Sahana Jayaraman, Celeste Nelson
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Integration of biochemical and biomechanical cues influence the development of several organs, including the
airways of the lung. To understand how cells in the developing lung sense and respond to these cues from the local microenvironment, we carried out RNA-sequencing analysis of critical stages of branching of the embryonic chicken lung. This analysis revealed changes in the expression of genes associated with integrin signaling and extracellular matrix (ECM) remodeling. Therefore, we sought to define the relationship between ECM synthesis, remodeling, and integrin signaling during airway branching morphogenesis. We found that prior to branching, the basement membrane (BM) is a uniform sheath that wraps the airway epithelium and there is no observable BM turnover. However, the BM is significantly reduced at the tip of elongating airway branches after branch initiation. Cultures of embryonic lung explants reveal that BM thinning at branch tips is mediated by matrix metalloproteinases (MMPs). Inhibiting MMPs suppresses branch extension, but does not prevent the initiation of new branches. As epithelial branches extend, cells in the surrounding mesenchyme become highly elongated in shape and activate the integrin signaling molecule, focal adhesion kinase (FAK). This local activation of FAK is required for the synthesis of tenascin-C (TNC) at branch tips. Moreover, disrupting FAK activity blocks initiation of new branches. Based on these data, we propose a model by which the airway epithelium initiates branches in a FAK-dependent manner. While matrix remodeling is not required for branch initiation, BM depletion at branch tips is required for branch extension. As branches extend, neighboring mesenchymal cells are deformed, which promotes a change in local ECM composition via FAK activation, ultimately shaping the growing branch. This work was supported by NIH (HL118532, HL120142, HL137273, and CA187692) and the NSF (CMMI-1435853).

Program Abstract #351
The FERM domain-containing Protein Merlin is Required for Endometrial Gland Morphogenesis
Erin Williams Lopez, Zer Vue, Russell Broaddus, Richard Behringer, Andrew Gladden
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Disruption of endometrial gland formation or function can cause female infertility. Formation of endometrial glands via tubulogenesis of luminal epithelial cells requires the establishment and maintenance of cell polarity and cell adhesion. The FERM domain-containing protein Merlin coordinates epithelial cell polarity and cell adhesion and is critical for epithelial tissue function in the skin and kidney. We now demonstrate a requirement for Merlin in endometrial gland development. Conditional deletion of Merlin (Nf2lox/lox; Wnt7a-Cre and Nf2lox/lox; PR-Cre) in the endometrium results in female infertility caused by the absence of gland formation. Interestingly, we observed glandular epithelial markers within discrete groups of cells in the Merlin-deficient luminal epithelium. Wnt signaling, a pathway necessary for endometrial gland development, is maintained in Merlin mutant endometrium suggesting the glandular fate program is intact. Instead, we observe increased levels of apical actin and markers indicative of high membrane tension on the basal surface of the mutant luminal epithelium. These findings suggest that the structural integrity of the luminal epithelium during gland formation is necessary for appropriate endometrial tubulogenesis and tissue function. Moreover, our work implicates Merlin-dependent regulation of morphological involution in the proper formation of endometrial gland architecture. Supported by the Department of Defense grant (W81XWH-14-1-0053) to Andrew B. Gladden

Program Abstract #352
Reductions in hemodynamic loading impairs proper morphogenesis of the mouse embryonic heart
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In addition to the response of embryonic cells to traditional ligand-receptor signaling pathways, many studies have revealed that developing tissues can respond to biomechanical force. Of particular note, several studies in chick and zebrafish embryos have revealed that alterations in hemodynamic loading (shear stress and pressure) results in abnormal development of the heart—from alterations in heart size, myocardial thickness, looping, trabeculation, septation and valvulogenesis. Though much knowledge has been gained from chick and zebrafish models, there may be slight differences in the response of mammalian hearts to hemodynamic loading conditions, given that placental mammals, like the mouse, become embedded into the uterus and eventually gain additional
nutrient support, oxygenation and waste exchange through a placental connection. Thus, to determine the impact of hemodynamic loading on mammalian heart development, we isolated post-implantation mouse embryos (embryonic day [E] 8.5), manipulated them to reduce blood hematocrit, and cultured them for 24 hours. The purpose of lowering hematocrit was to reduce blood viscosity which resulted in the formation of low-hemodynamic loading embryos. We then performed optical projection tomography microscopy on the embryos, and performed morphometric analysis to compare embryonic hearts between the low-loading and normal-loading control hearts. Our study revealed that low-hemodynamic loading resulted in a reduction in heart size, and myocardial thickness. Low-loading hearts also exhibited defective looping and trabeculation. These results show a similar outcome to what has been reported in chick and zebrafish embryos which further validates the importance of biomechanical forces on regulating cardiac development, and further supports the idea of a conserved mechanism that spans across three different taxonomic classes. Funding: Missouri State University, Faculty Research Fellowship, Tri-Beta Undergrad Scholarship

Program Abstract #353
Interphase Cell Shape Does Not Predict Division Orientation in a Developing Drosophila Epithelium
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Epithelial tissues are remodeled during animal development to shape body structures. We investigated the cell behaviors that drive morphogenesis in the Drosophila follicular epithelium, a simple monolayer that undergoes elongation and expansion. Through live-imaging and semi-automated image analysis, we observed that cell division drives the early Drosophila follicular epithelium towards optimal geometric packing. This increase in regularity demands that cells break Hertwig’s rule, a long-established principle of cell biology which holds that divisions orient along the cell shape long axis. We show that there is a bias planar division orientation towards the elongating axis of the egg chamber, and that division orientation is not predicted by interphase cell shape. In contrast to other systems, planar division orientation is not achieved through the asymmetric enrichment of the Pins/Mud spindle-orienting machinery. However, we find that the bias in cell division is lost in the absence of Canoe, a scaffolding protein that links cell adhesion molecules to the actomyosin cytoskeleton. Through biophysical manipulation, we observe that this tissue is under anisotropic stress. We therefore conclude that spindle orientation in the apical-basal and planar axes are independent and suggest a model whereby tissue-scale tension provides a cue to orient cell divisions. This work highlights the diversity of division control in epithelial tissues, even within the same organism, and reveals that tissue-level mechanical forces influence cell behavior to drive morphogenesis of this tissue.

Program Abstract #354
Reconstituting embryonic tissue folding through programmed cell traction networks
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Many shape transitions at tissue interfaces during development are preceded by mesenchymal condensation, where populations of mesenchymal cells dispersed in an extracellular matrix coalesce to form dense aggregates. These aggregates engage in reciprocal signaling with overlying epithelial tissues through chemical and mechanical signals that drive differentiation. The presence of mesenchymal condensates is also associated with structural transitions such as bending, coiling, invaginations and branching in diverse tissues such as the gut, hair follicle, and kidney. The process of condensation can itself alter the mechanical microenvironment at tissue interfaces, which has been proposed to be sufficient to drive the folding of tissue interfaces. To test this, we used next-generation cell patterning techniques to produce synthetic mesenchymal condensates consisting of arrays of contractile cell clusters in fibrous collagen gels. We find that single clusters strain and radially align the surrounding gel, and that arrays of clusters can strain the gel with local directional bias depending on their geometric arrangement. The locally aligned and concentrated collagen bears elevated stresses, driving the out of plane buckling of nearby
interfaces along trajectories predictable by modeling. To demonstrate the versatility and robustness through which mesenchymal condensations drive diverse structural transitions, we built a diversity of folded architectures of biological relevance, including invaginations, evaginations, tubes, coils, and corrugations. We also demonstrate tessellation of these patterns, for example into a zig-zag architecture mimicking the E15 chick gut. These studies demonstrate the sufficiency of mesenchymal condensates to alter the mechanics and 3D shape of nearby tissue interfaces, and provide a fundamentally new strategy for controlling the architecture of organoids and engineered tissues across the millimeter and centimeter length scales.

Program Abstract #355
The role of hyaluronic acid in regulating palatal shelf elevation during secondary palate development
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Palatal shelf elevation is an essential morphogenetic process during secondary palate development. Palatal shelf elevation is achieved through shelf movements that are driven by an elevating force within the shelf itself. It has been proposed that the elevating force results from accumulation of hyaluronic acid (HA) in the extracellular matrix of the palatal shelves, but how this force leads to shelf elevation remains unclear. In mammals, HA is synthesized by hyaluronic acid synthase (HAS) that is encoded by three genes (Has1, 2 and 3). In this study, we used Wnt1-Cre driver to conditionally disrupt Has2 in the craniofacial mesenchyme, which significantly reduced HA content in cranial neural crest-derived mesenchymal tissues. Wnt1-Cre, Has2 conditional knockout (cKO) mice developed cleft palate and also showed severe mandibular hypoplasia. The palatal shelves of Wnt1-Cre, Has2 cKO mice were reduced in size and failed to elevate during palatogenesis. To detect shelf movements during elevation, we developed an in vitro assay that uses the technique of dynamic palate culture but leaves the tongue and mandible in place to maintain the integrity of the oral cavity. Palate explants were incubated in serum-free medium with rotation for 24 hours. The palatal shelves of control explants elevated after incubation through morphogenetic movements that closely resemble those of in vivo elevation. In the meantime, the tongue protruded out of the mouth and displayed concomitant morphological changes, suggesting that the palatal shelves interact with the tongue during elevation. The palatal shelves of Wnt1-Cre, Has2 cKO explants did not elevate after incubation and tongue morphology was not changed. These results suggest that the function of HA is to control shelf size and to provide a force that drives shelf movements and also mediates the interaction between the palatal shelves and tongue during elevation. This study is supported by NIH grant DE025077.

Program Abstract #356
Genetic and mechanical regulation of intestinal smooth muscle development
Tyler Huycke, Bess Miller, Clifford Tabin
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Beginning as a simple tube of endoderm surrounded by mesenchyme, the gut is patterned early in development to generate discrete layers of smooth muscle with distinct cellular alignments. Correct organization of these muscles into circumferential and longitudinal layers is required for peristalsis and to generate the appropriate physical forces that drive buckling morphogenesis of the gut lumen. How smooth muscle is patterned along the radial axis of the gut tube and the mechanisms controlling its alignment across the length of the tissue are poorly understood. We find that the precise radial pattern of the circumferential muscle layer is controlled by threshold levels of Hedgehog and BMP signaling, which act to place the muscle at an exact distance away from the endoderm. Differentiation of the later-forming longitudinal muscle layer depends on localized BMP inhibition, mediated by Noggin derived from both the circumferential muscle layer and enteric neural crest cells. The fact that the layers of smooth muscle form sequentially allows them to be exposed to unique mechanical environments present in the growing embryo at distinct developmental stages. In the early gut tube, differential growth along the radial axis places the outer mesenchyme under tension and causes the differentiating muscle cells to align circumferentially. Once formed, cells of this circular layer begin to spontaneously contract, and the
cyclic strain generated by these contractions orients cells of the outer layer longitudinally. Together, our findings provide a model for how gene-regulated patterning is coupled with mechanical forces to control the development of gut smooth muscle.

Program Abstract #357
Tissue surface mechanics drive mesenchymal-to-epithelial transition on embryonic aggregates
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The mesenchymal-to-epithelial transition (MET), an ability of loosely associated migratory cells to form a compact epithelium, is a conserved motif occurring throughout organogenesis and plays a key role in cancer metastasis and the generation of iPSCs. Here we introduce a 3 dimensional (3D) tissue model to study MET using Xenopus laevis embryonic mesenchymal cell aggregates and demonstrate a key role for tissue mechanics to initiate and regulate MET. Within 5 hours post aggregation, mesenchymal cells along the aggregate surface form a cohesive epithelium complete with apicobasal polarity and epithelial markers including keratin and ZO-1 before completing their differentiation into mucus-secreting, epidermal goblet cells. Live imaging of GFP-ZO-1 reveals a stochastic transition of individual mesenchymal cells which assemble into multi-cell clusters that join to cover the entire aggregate surface. This 3D tissue platform enables quantitative assessments of tissue compliance, YAP nuclear localization, and MET progression that allow us to propose cell contractility and cell-cell adhesion modulate the physical properties required for MET. Acknowledgments: This work is supported by National Institute of Health (R56HL13419) to L.A.D. H.Y.K. is supported by Young Scientist Fellowship from the Institute for Basic Science.

Program Abstract #358
Cellular mechanisms of Eph/ephrin mediated cell segregation and boundary formation in development
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Cell segregation, or cellular self-organization, leads to boundary formation and is critical for the organization of morphogenetic movement and tissue patterning. Signaling between membrane bound ephrins and Eph receptor tyrosine kinases is essential in boundary formation, driving segregation between ephrin-expressing and Eph-expressing cells. Utilizing mice that are mosaic for expression of X-linked ephrin-B1 and a cell culture system we demonstrate that Eph/ephrin signaling results in cell segregation and the generation of a cortical actin differential, with increased cortical actin specifically in EphB2 expressing cells. Prevention of signaling through Rho-kinase (ROCK) disrupts cell segregation in vivo and in cell culture indicating that actomyosin contractility is critical for this process. Conversely, disruption of cadherin-mediated adhesion has little effect on self-organization. By analyzing the contact angle of cells we estimated the interfacial tension of EphB2-ephrin-B1 heterotypic pairs relative to EphB2-EphB2 or ephrin-B1-ephrin-B1 homotypic interactions. Contact is dramatically reduced in heterotypic pairs, indicating an increase in the interfacial tension, and inhibitors of actomyosin contractility significantly diminish this increase, demonstrating that actomyosin contractility drives heterotypic interfacial tension. Additionally, using a 3D aggregate approach to observe multicellular scale organization we see that Eph/ephrin cell segregation affects large-scale tissue morphology dependent on actomyosin contractility. These data suggest a model for cell segregation in which Eph/ephrin signaling results in a cortical actin differential that prevents cells from making stable contacts and drives cell segregation, and ultimately affects tissue morphology by modulating interfacial tension. Funding: NIH/NIDCR: R01DE023337 and R21DE025923 to J.O.B.

Program Abstract #359
Myosin-dependent accumulation and spatial confinement of Rab11-positive vesicles
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During tissue morphogenesis, cell shape changes driven by mechanical forces usually involve changes in cell surface area, which often require active regulation of intracellular membrane traffic. It is not fully understood
how membrane traffic is modulated by mechanical stimuli. To address this question, we investigated the behavior of Rab GTPases, the master regulators of membrane traffic, during apical constriction-mediated mesoderm invagination in *Drosophila*. We found that the recycling endosome marker Rab11 undergoes dynamic reorganization during apical constriction. Prior to apical constriction, Rab11 is enriched at the perinuclear region. As the cells constrict apically, the cells elongate, and Rab11-positive vesicles become enriched near the apical membrane. While these vesicles undergo constant movement, their average distribution is confined near the contractile myosin that drives apical constriction. The apical enrichment of Rab11 vesicles is sensitive to disruption of myosin activity. In contrast, inhibiting endocytosis does not prevent vesicle accumulation. Interestingly, when we used an optogenetic approach to acutely inhibit myosin activity after Rab11 vesicles accumulate, the vesicles dispersed immediately from their confined locations and eventually disappeared. These results suggest that apical myosin activity not only induces the formation of Rab11 vesicles but also confines them to the vicinity of myosin. When Rab11 is locked in its GTP-bound form, however, a large fraction of the protein is instead localized at the cell-cell boundaries, and this localization becomes more prominent as the cells undergo apical constriction. We hypothesize that the accumulation of Rab11 vesicles facilitates membrane addition to the lateral membranes, allowing the cells to elongate as they constrict apically.

Program Abstract #360
An orthogonal switch in biomechanical forces during zebrafish brain morphogenesis
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The accessibility and transparency of the zebrafish embryo make it an excellent model system for investigating the morphogenesis of complex vertebrate organs *in vivo*. Recent work has highlighted the role of biomechanical forces in embryo morphogenesis. Whilst most studies have focused on superficial tissues, we investigate biomechanical forces during the formation of a complex 3D structure – the zebrafish neural tube. Zebrafish neural tube formation involves *de novo* apicobasal polarisation and complex cell rearrangements at the tissue midline, prior to lumen opening. Cells from each side of the neural primordium initially interdigitate before rearranging to meet at a distinct left-right interface. This interface is initially sinuous before straightening out so that left and right cells adhere precisely at the tissue midline. We seek to characterise the role of forces in this changing interface morphology. UV laser cuts were used to reveal biomechanical tensions in the complex 3D tissue of the neural rod. Apicobasal tension decreased during left-right interface straightening, whilst the orthogonal nascent apical plane tension increased. This ‘tension switch’ suggests that these orthogonal tension vectors are more influential at different stages. Pharmacological treatments showed that both nascent apical plane and apicobasal tension are actomyosin-dependent. During straightening, relative N-cadherin intensity at the interface increased, before decreasing following straightening and prior to lumen opening. This is suggestive of a change in cell-cell junction strength. Global reduction of actomyosin contractility reduced the relative N-cadherin intensity. This suggests that the ‘tension switch’ from across the midline to along the nascent apical plane could mediate this junction remodelling, and favour the weakening of junctions across the left-right interface at late stages, thus enabling lumen opening. Funding for this work was provided by BBSRC and Wellcome Trust

Program Abstract #361
Graded mechanical strain directs cilia differentiation in the *Xenopus* left-right organizer
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The Xenopus left-right organizer(LRO) breaks left-right symmetry by using different ciliated cell types to produce and sense flow. Cells within the center of the LRO extend a long motile cilium that moves to a posterior planar position to create leftward effective flow, while cells within the lateral LRO sense this flow by extending short,
immobile cilia that remain in a central planar position. The positioning of cilia in the LRO requires the core planar cell polarity (PCP) pathway, but how this pathway is aligned to the anterior-posterior (A-P) body axis is still unclear. Our previous work showed that an A-P PCP axis is established on the ventral side of the embryo in response to mechanical strain produced by gastrulation. In this study, we find that presumptive LRO is also subjugated to oriented mechanical strain during gastrulation and that this strain is both necessary and sufficient to promote A-P planar axis requiring for cilia positioning. We also find that strain is graded in magnitude along the medial-lateral axis with the highest levels occurring in the middle, tapering to lower levels laterally. Surprisingly, when strain is blocked in LRO explants, cilia not only fail to polarize along a planar axis, they also become short and immobile. Conversely, cilia length, motility, and planar polarity can be restored by treating LRO explants with exogenous physiological strain. Cilia length is graded in LRO and cilia length that forms in response to strain is proportional to the strain level. Finally, we show that strain directs cilia differentiation based on a prepatterning in presumptive LRO involving Foxj1, the master regulator of motile cilia differentiation. We proposed that the differential tissue strain works as a developmental cue, together with the Foxj1, to instruct cilia differentiation during the formation of the *Xenopus* LRO. This work was supported by an NIH grant R01HD092215

**Program Abstract #362**  
**Function shapes cardiac outflow tract dimensions by modulating endocardial growth**  
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Mechanical cues, such as fluid forces, have been shown to influence the shape, size, orientation and number of endothelial cells during cardiovascular morphogenesis. Little is understood about how these biomechanical inputs are sensed and how they are translated into cellular behaviors that govern three-dimensional changes in morphology. Here, we address these questions in the context of the cardiac outflow tract (OFT), a tubular extension of the heart that functions as a pivotal portal of blood from the ventricle to the vasculature. Importantly, the OFT is formed by the addition of late-differentiating cells to the arterial pole of the heart after blood flow and contractility have already initiated, pointing to a potential role of cardiac function during the earliest steps of OFT assembly. Using high-resolution morphometrics in the zebrafish embryo, we have found that the OFT endocardial lumen and its overlying myocardium widen as blood flow increases through the heart. This tissue expansion is accompanied by an increase in OFT endocardial cell number and a concurrent decrease in cell size. This growth of the endocardium can be attributed, at least in part, to proliferation of the OFT endocardial cells. In contrast to the wild-type scenario, mutants defective in cardiac function exhibit a collapsed OFT endocardial lumen; in addition, the number of OFT endocardial cells does not increase, and these cells remain relatively large. Interestingly, this endocardial collapse is paired with a significant reduction in the endocardial proliferation index. Finally, experiments using pharmacological inhibitors implicate mechanosensitive channels in the expansion of the endocardium. Overall, our data suggest a model in which cardiac function leads to the activation of mechanosensitive channels within the endocardium and trigger its proliferation, thereby inducing the expansion of the OFT lumen and shaping the dimensions of the OFT. This work is supported by NIH R01 HL108599.

**Program Abstract #363**  
**Pronephros, heart, and nervous system development are disrupted by acute knockdown of Tinagl1, an extracellular matrix glycoprotein, in early zebrafish embryos**  
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Tinagl1 is a basement membrane glycoprotein that modulates cell adhesion *in vitro*. Mammalian Tinag or Tinagl1 appear to function in renal and vascular development, in myofibroblast function after injury, and to alter metastatic potential. Their pathway and molecular interactions are poorly defined, except that *Drosophila* Tinagl1 binds tightly to the Wnt, Wg, and is required for Wg stability in vitro. Like *Drosophila*, zebrafish has only the *tinagl1* gene, yet has organogenesis more closely related to mammals. My lab, with early assistance from D.
Kozlowski, J. Mumm, and S. Dougan, examined expression and phenotypic consequences of MO and mosaic CRISPR acute knockdown in early zebrafish embryos. We have published on craniofacial defects (CPCJ 54:381-90, 2017) and will soon submit a manuscript on the following observations. *tinagl1* is expressed in pronephric duct and glomerulus, bands ventral to the hindbrain, and cells along the spinal cord. Heart L/R defects were accompanied by shorter and fewer cilia in the Kupffer’s vesicle; these findings together with hydrocephalus, renal cysts, pronephric cilia defects, small eyes, and ventral body curvature suggest a general defect in cilia. The *tinagl1* morphant pronephros defects were enhanced by co-injection with *wnt3a* MO but not control MO, and partially rescued by *tinagl1* and *ctnnb2* mRNA but not control mRNA. Overall, these results suggest that, in zebrafish, Tinagl1 is broadly required for motile cilia function and may interact with Wnt signaling pathways. In the spinal cord, losses of some dorsal cells and defects in motor axon outgrowth could also implicate roles in adhesion and migration. This work was supported by Vanguard Charitable Gifts Foundation and internal AU pilot grants. I have recently received approval for a NIAMS Re-Entry to Biomedical Research Supplement to pursue related ECM and cilia studies using mutant lines in collaboration with Rebecca Burdine.

**Program Abstract #364**

**Short range Sonic Hedgehog signaling promotes heterotypic cell interactions underlying branching morphogenesis of the zebrafish fin skeleton**

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Resected adult zebrafish fins perfectly regenerate including their extensive branched bony ray skeleton. Ray branching during regeneration occurs by Hedgehog/Smoothened (Hh/Smo)-promoted interactions between migrating, “split pools” of Sonic hedgehog a (Shha)-expressing basal epidermal cells and progenitor osteoblasts. However, mechanisms of *shha* induction, the splitting of *shha*+ epidermal cells, and how tightly restricted Hh/Smo activity in adjacent osteoblasts and epidermal cells promotes their heterotypic interaction are unresolved. Towards answering these questions, we explored if Hh/Smo signaling and epidermal dynamics also underlie developmental ray branching. We found that *shha* is expressed initially in basal epidermal cells along the entire length of forming fin bones in juvenile fish. As bones progressively mature, *shha* becomes distally restricted to epidermal cells neighboring Runx2+ pre-osteoblasts (pOubs). We used TgBAC(*ptch2:Kaede*) fish and photoconversion to show Hh/Smo signaling is restricted to these pOubs and immediately adjacent, distal-migrating epidermal cells. *shha*+ epidermal cells split into two groups immediately preceding ray branching. By live imaging, we found that basal epidermal cells continue migrating distally, cease Hh/Smo signaling, and are then shed. Small molecule inhibition of Hh/Smo using BMS-833923 shows that the pathway is largely dedicated to ray branching during fin development. We conclude that instructive Shha+ epidermal movements and Shh/Smo-promoted adhesion between epidermal cells and pOubs direct branching morphogenesis to pattern the fin skeleton during both development and regeneration.

**Program Abstract #365**

**Muscle Degeneration in a Zebrafish Model of Accelerated Aging**

Elizabeth Coffey, Chaya Karunasiri, Clarissa Henry

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Skeletal muscle serves many physiological functions; therefore, progressive loss of muscle with age, known as sarcopenia, negatively impacts health. Sarcopenia affects 50 million people, is associated with increased fracture risk and functional decline, and increases healthcare costs. Since augmenting muscle has the potential to delay age-related functional decline, it’s important to elucidate mechanisms underlying sarcopenia. Our approach is to leverage zebrafish displaying accelerated aging to study the first consequences of aging on muscle structure/function. This information may help delay the onset of muscle loss. Spinster mutant zebrafish express biomarkers of aging in muscle during embryonic stages and are an ideal model for studying the initiating events of muscle decline with age. Skeletal muscle consists of myofibers that attach to surrounding laminin-rich basement membranes (BMs). This attachment is critical for muscle structure/function and is disrupted in degenerative
muscle diseases. We’re interested in understanding how aging affects muscle-BM interactions. Our ultimate goal is to potentiate muscle-BM adhesion to treat or prevent sarcopenia. We used immunohistochemistry and confocal microscopy to characterize the effects of aging on muscle in spinster mutant zebrafish. We found that the developmental isoform of laminin is re-expressed in spinster mutant muscle at a time when the mature laminin isoform is normally expressed. Ongoing experiments will determine the phenotypic consequences and genetic regulation of this laminin isoform switch in aging muscle. Knowledge of how aging affects muscle may provide new therapies to prolong muscle function in aging and disease. This work was supported by the NIH and March of Dimes.

Program Abstract #366
Muscle precursor cell migration is specified by six1/six4 genes and initially guided by the fin bud in zebrafish
Jared Talbot¹, Emily Teets¹, Danni Ratnayaki², Duy Phan¹, Zachary Morrow¹, Peter Currie², Sharon Amacher¹
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Muscle precursor cells migrate from the trunk to form a subset of the hypaxial musculature. Here we investigate how these precursors are specified and how fin-derived signals control their migration in zebrafish. We find that four six family homeobox genes (six1a, six1b, six4a, six4b) redundantly specify muscle precursors for migration. Quadruple mutant precursors are immotile and fail to express met, which encodes a cell-guidance receptor. Loss of met function causes delayed muscle precursor motility and smaller hypaxial muscles. However, muscle precursors eventually migrate without met function, suggesting that other cues also guide the cells. These cues may change over time. For instance, using time-lapse microscopy we find that one cell stream that initially migrates into the fin bud will later change course towards a more posterior hypaxial muscle. The entire stream reroutes posteriorly when fin bud development is completely blocked, indicating that muscle precursors are guided by both fin bud-dependent and fin bud-independent cues. Work was supported by NIH grants GM061952, GM088041, and GM117964 (to S.L.A.), The Australian Regenerative Medicine Institute is supported by grants from the State Government of Victoria and the Australian Government. P.D.C. is supported by National Health and Medical Research Council Senior Principal Research Fellowship APP1136567 and this work was funded by Australian Research Council, Grant LP120100281 (to P.D.C.), and by an NIH loan repayment program contract, NIH T32 training grant NS077984, and Pelotonia Postdoctoral Fellowship (to J.C.T.).

Program Abstract #367
Cadherin-mediated coordination of chemokine signaling in collective cell migration
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Coordinated migration of cell collectives is essential in development, homeostasis and disease. Cells moving in groups adhere to each other while responding to attractant cues. In many cases, leader cells guide the follower cells. However, whether the follower cells also contribute to group migration is less clear. To understand how attractant sensing and cell-cell adhesion in leader and follower cells contribute to group migration we used the zebrafish lateral line primordium (primordium) as a model. The primordium is a collective of about 140 cells that express the chemokine receptor Cxcr4 and migrate in response to a self-generated Sdf1 gradient. This gradient is linear and stretches across the primordium, suggesting that each cell in the group perceives directional information and could contribute to the group movement. Consistent with this idea, we found that ccr4 mutant primordia migrate faster as we increase the number of Sdf1 sensing wild-type cells. Although the wild-type cells need to be present in the front to restore migration of the ccr4 mutant primordia, the increase in speed correlates only with the overall wild-type cell number. Also, the ability of the wild-type cells to restore migration of ccr4 mutant primordia suggests that the cells adhere to each other such that wild-type cells can “pull” their ccr4 mutant neighbors along. Likely candidates for cell-cell adhesion in the primordium are cadherins. Consistent with previous reports, we found that E- and N-cadherin are expressed in the primordium. Moreover, we found that these cadherins are required for retaining cells in the primordium and, intriguingly, are essential in wild-type cells to pull ccr4 mutant primordia along. Together these observations suggest that each cell in the primordium
extracts directional information from the Sdf1 gradient, contributes to the directional movement of the group and is coupled to its neighbors through Cadherins to ensure efficient migration. Funded by NIH.

Program Abstract #368
Differential Regulation of Protrusive Behavior During Collective Cell Migration
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Collective cell migration is important both during development and adulthood in processes such as blood vessel branching, neural crest migration, wound healing, and cancer invasion. During this process cohorts of cells maintain cell-cell contacts, group polarization and exhibit coordinated behavior. Migrating cells extend protrusions to interact with the extracellular environment, sense chemotactic cues, and act as points of attachment. The regulation of protrusive behavior has been widely studied in individually migrating cells; however, how this behavior is regulated throughout collectives is not well understood. To study protrusive behavior during collective cell migration, I am using the zebrafish posterior lateral line primordium (pLLP) as a model. The pLLP is a cluster of ~100 cells that migrates along the zebrafish trunk, depositing groups of cells that will become sensory organs. These organs make up the posterior lateral line, a mechanosensory system that senses changes in water current. To define protrusive behavior during pLLP migration, I performed mosaic analysis to sparsely label cells within the pLLP with a transgene marking filamentous actin. This approach revealed an abundance of brush-like and filopodia protrusions throughout the pLLP that are differentially regulated within the collective. Filopodia are more numerous and less persistent in the leading region, whereas brush-like structures are larger and more dynamic in the leading versus trailing region. To identify genes that regulate this behavior, I examined expression of candidate genes, including those regulated by canonical Wnt signaling, a pathway important for pLLP migration. We found a number of Wnt target genes known to regulate actin dynamics that show regional specific expression patterns in the pLLP. Our results suggest that protrusive behavior is spatially regulated throughout the pLLP by region specific expression of genes known to regulate actin dynamics. Funding: OHSU.

Program Abstract #369
LRP5 regulates collective cell migration during posterior lateral line development in the zebrafish
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Embryonic development requires the precise coordination of multiple cellular behaviors such as proliferation, migration, and differentiation. Canonical Wnt signaling has emerged as one the key pathways that regulates cellular behaviors during development and is often a target of mutation in may disease states including birth defects and invasive cancers. Wnt/b-catenin signaling has been shown to be required for development of the posterior lateral line (pLL) mechanosensory system, specifically, for regulating collective cell migration of the posterior lateral line primordium (pLLP). The pLL forms from the pLLP, a cohort of ~100 cells which collectively migrate along the trunk of the developing zebrafish embryo. The pLLP is comprised of proliferative progenitor cells and organized epithelial cells that will form the hair cell-containing mechanosensory organs of the pLL. Wnt signaling is active in the leading progenitor zone of the pLLP and regulates cellular proliferation, survival and maintenance. We have identified a mutation in the Wnt receptor LRP5 that results in defective collective migration of the pLLP and premature truncation of the pLL. Understanding the mechanisms underlying the defects in collective migration in the LRP5 mutants will allow a more complete picture of how canonical Wnt signaling regulates embryonic development and how it is misappropriated during disease.

Program Abstract #370
Draxin: a novel player in the molecular control of neural crest EMT
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Neural crest (NC) cells undergo a spatiotemporally regulated epithelial-to-mesenchymal transition (EMT) to delaminate from the neural tube, with a sequence of initiation that progresses from rostral to caudal. Here, we show the secreted molecule Draxin acts to modulate Wnt signaling downstream of NC specification to control the timing of cranial NC EMT. Chick draxin is expressed in a coordinated wave along the axis, where its expression initiates after neural crest specification and is downregulated just prior to cranial NC delamination. Functional experiments showed that Draxin controls the timing of cranial NC EMT via canonical Wnt signaling modulation. Ectopic maintenance of Draxin expression resulted in reduced NC emigration from the neural tube, suggesting a repressive role for Draxin in controlling NC EMT. Reciprocally, loss of Draxin triggered premature NC EMT. Interestingly, we show these phenotypes resulted from aberrant regulation of factors associated with EMT, including Snail2, Cadherin6B, and laminin. Modulating Draxin expression also altered canonical Wnt reporter activity in vivo, in a manner consistent with Draxin acting as a Wnt antagonist. Accordingly, co-expression of Draxin with canonical Wnt pathway components rescued NC EMT and emigration. Taken together, these data indicate that Draxin acts as an essential molecular rheostat of canonical Wnt signaling to regulate the precise timing of cranial neural crest EMT. This work was funded by an NIH NIDCR grant (R01DE024157 to MEB) and NRSA (F32DE026355 to EJH).

Program Abstract #371
Aquaporin-1 promotes cranial neural crest migration by stabilizing filopodia and influencing extracellular matrix degradation
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Cranial neural crest cells migrate dynamically through the embryonic microenvironment to form discrete streams of cells in the head. Using multiple gene profiling approaches, we have identified aquaporin-1 (AQP-1), a water channel protein, which is highly expressed by neural crest cells in the invasive front of the migrating stream. Functional in vitro and in vivo experiments suggest that AQP-1 is involved in neural crest cell motility. Knockdown of AQP-1 reduced neural crest cell speed, directionality, and thus migration, in both in vivo and in vitro assays. Overexpression of AQP-1 increased speed and invasion of neural crest cells. At the cellular level, perturbations in AQP-1 significantly affected the length and stability of filopodia in vivo. Furthermore, AQP-1 co-localized with the EphB1 and EphB3 receptors, at both the mRNA and protein levels, indicating that this water channel is interacting with guidance receptors known to be necessary for directed cell migration. In addition, we have evidence that AQP-1 co-localizes with phosphorylated focal adhesion kinase as well as increases MMP activity, suggesting AQP-1 is involved in cell adhesion and ECM degradation during neural crest cell migration. Together, these data strongly suggest that AQP-1 promotes directed neural crest cell migration through filopodia dynamics, complex protein interactions and increases in MMP activity. Funding Sources: The Stowers Institute for Medical Research; National Institute of Child Health and Human Development (R03HD089190)

Program Abstract #372
Modulation of cellular rearrangements and intercellular adhesion by the Wnt/PCP formin Daam1 during nephron morphogenesis
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Kidneys are critical for filtering blood and concentrating metabolic waste into urine. Kidneys, like most organs in our body, consist of a network of epithelial tubules. These tubules make up the basic structural unit of the kidney,
the nephron, whose morphology is vital for kidney function. During nephron development, the cadherin family of cell adhesion proteins are thought to mediate cell interactions and sorting of loosely associated progenitor mesenchymal cells into a tightly organized tubular epithelium. The role of cadherins in these processes depends on the subcellular organization of the actin cytoskeleton. However, the mechanisms that govern the organization of the actin cytoskeleton and intercellular adhesions during nephron morphogenesis are poorly understood. Dishevelled-associated activator of morphogenesis 1 (Daam1) is a formin protein that facilitates actin polymerization as a part of the Wnt/PCP pathway. Previous work from our lab showed that Daam1 is important for nephron morphogenesis in the *Xenopus* embryonic kidney. Therefore, we are investigating the mechanism by which Daam1 regulates nephron shape and development. Our preliminary data show that Daam1 localizes to the plasma membrane and the cell-cell contacts of the renal progenitor cells. Furthermore, our results suggest that Daam1 promotes F-actin and E-cadherin localization at the cell-cell contacts and influences positioning of the renal progenitor cells within the nephrogenic primordia. These results suggest that Daam1, by regulating the actin cytoskeleton, mediates intercellular adhesion and cellular rearrangements during nephron morphogenesis. This work was supported by a National Institutes of Health (NIH) KO1 grant (K01DK092320 to R.K.M.) and startup funding from the Department of Pediatrics Pediatric Research Center at the University of Texas McGovern Medical School (to R.K.M.).

**Program Abstract #373**

Examine the intersection of cell migration, cell cycle regulation and differentiation using *C. elegans* sex myoblasts

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Cell invasion and migration are essential cell behaviors in many normal developmental and physiological contexts; they are also defining processes for cancer metastasis. Here we explore cell migration and differentiation by studying the sex myoblasts (SMs), a cell lineage that migrates during larval development and differentiates into the adult vulval muscles. Two SMs travel anteriorly in the second larval stage until they flank the gonad and begin to divide. Work by others has shown that exogenously secreted fibroblast growth factor (FGF) is an important cue, but the genetic and kinetic mechanisms underlying SM cell migration remain largely unknown. We visualized SM migration and pre-differentiation behaviors *in vivo* using spinning disk confocal microscopy following SMs transgenically labeled with membrane bound and F-actin reporters. Following migration, we observed SMs forming dynamic protrusions, a behavior yet unreported in the literature. Our current work aims to understand their function in response to extracellular and autonomous signals. We present early findings from an RNAi screen designed to identify cytoskeleton-associated proteins and cell signaling factors that may regulate SM migration and differentiation. Lastly, we used an *in vivo* cell-cycle indicator to observe the cell cycle state of migrating versus stationary SMs. Our results indicate that the SM cells may be in G1/G0 cell cycle state during migration. These findings lay the groundwork for establishing SMs as a model for cell migration and for studying the relationship between migration and cell-cycle state.

**Program Abstract #374**

Niche Cell Wrapping Depends on Basement Membrane Cues in the *C. elegans* Embryonic Gonad

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Somatic niche cells are often critical regulators of germline stem cells; in various contexts controlling survival, proliferation and differentiation. Using the *C. elegans* embryo, we have investigated how those cells interact with the surrounding basement membrane (BM). We show that BM plays a critical role in establishing and maintaining niche cell wrapping during embryogenesis. We study the gonad primordium in *C. elegans* - a simple, 4-cell structure formed during embryogenesis that will give rise to the entire germline in the adult worm. It is comprised of two primordial germ cells (PGC), each of which interacts with a single somatic gonadal precursor cell (SGP).
Each SGP extends its cell membrane around the body of a PGC, so as to completely enwrap it. This wrapping both establishes a morphology that is carried into larval development and provides a protective niche for the PGCs in the embryo. The value of this niche is demonstrated since unwrapped PGCs die unexpectedly when they are cannibalized by a neighboring endodermal cell. We show that the ability of the SGPs to enwrap PGCs depends in part on production of the BM first by surrounding tissues and subsequently by the SGPs themselves. Laminin is first recruited to surrounding tissues, and in animals where this process is blocked, a minority of SGPs fail to wrap leading to PGC cannibalization. Next the SGPs are required to recruit laminin to the basal surface of the primordium. If this BM is disrupted, SGP wrapping is not maintained. This leads to a disorganized larval gonad and a tumorous, sterile germline. Finally, we find that dystroglycan is expressed by the SGPs and is required to maintain wrapping. Dgn-1 null mutants exhibit occasional PGC cannibalization, frequent unwrapped SGPs, and a disorganized larval germline. Thus we conclude that SGP interaction with the BM ensures the integrity of the niche throughout embryogenesis.

Program Abstract #375

**Novel mechanisms of Ephrin reverse signaling in the control of neuronal migration**

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Dynamic interactions between Ephrins and Eph receptors regulate many aspects of cell migration, but the mechanisms controlling these responses are only partially understood. Ephrin ligands stimulate conventional “forward” signaling by activating Eph receptors, but Eph receptors can also induce “reverse” signaling via their cognate Ephrins. Using Manduca sexta (tobacco hornworm) as a model system, we discovered that reverse signaling via Manduca Ephrin (a type-A, GPI-linked Ephrin) regulates the collective migration of neurons that innervate the gut. During the formation of the enteric nervous system, groups of neurons (EP cells) undergo chain migration along specific visceral muscles while avoiding adjacent midline regions. The migratory EP cells express MsEphrin, while the midline boundary cells express the sole Manduca Eph receptor (MsEph). Unexpectedly, studies in embryo culture showed that reverse signaling via MsEphrin prevented inappropriate midline crossing by the migratory neurons, whereas forward signaling (via MsEph receptors) played no role in this process. We also showed that MsEphrin reverse signaling in the neurons was transduced by Src42, a Src family kinase (SFK) that induced RhoA-dependent retraction responses away from the midline. In a screen for MsEphrin-interacting proteins that might directly activate Src42, we identified RACK1 (Receptor of Activated Protein Kinase C), a reciprocal regulator of Src family kinase (SFK) activity. We now have shown that MsEphrin requires RACK1 to activate Src42, while Src42 is required for the inactivation of RACK1 during MsEphrin signaling. These results support the model that a Src-RACK “rheostat” plays a central role in the transduction of Ephrin-A reverse signaling. We are currently testing candidate co-receptors that may couple MsEphrin to the RACK1-Src42 complex, with the goal of fully understanding how Ephrin-A reverse signaling regulates cell migration during embryonic development. Funding: NSF 1557414

Program Abstract #376

**The basolateral Scribble Complex promotes cooperativity during collective migration**

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The evolutionarily conserved Scribble complex is required in polarized epithelia to define basolateral membrane identity, antagonize the Par and Crumbs complexes and scaffold a variety of signaling molecules. Interestingly, in some systems, Scribble is required for cell motility, while in others, motility is enhanced in the absence of Scribble. Precisely how Scribble functions to promote or inhibit collective cell migration is unknown. Using the border cell migration model in the *Drosophila* egg chamber, we found that cells migrating collectively require the scribble complex. To explore the function of Scribble in border cell clusters, we created GFP knock-in lines to the Scribble locus by CRISPR. Using the Degrade-GFP system to knockdown GFP-tagged Scribble, we found that Scribble is required in the migratory border cells for the cluster to migrate to the oocyte and the removal in these cells miss-
localizes the apical protein, aPKC throughout the cluster. RNAi mediated knockdown of either Scribble, Discs Large or Lethal Giant Larva in both migratory and non-migratory cells of the cluster causes a significant increase in cluster dissociation and failure to detach from the follicular epithelium. Over-expression of Scribble similarly causes detachment defects and increases the number of protrusions such that all cells in the cluster form multi-branched protrusions. Our preliminary data using a Rac and Cdc42 FRET biosensor indicates that Scribble overexpression increases the overall level of active RhoGTPases in border cell clusters. Taken together, our data suggest the Scribble Complex likely has multiple functions in migrating collectives. First, Scribble modulates cluster cohesion through maintenance of apical/basal membrane identity. Second, it can coordinate RhoGTPase signaling such that multi-cell clusters coordinate their front/back polarity and move directionally. This work is supported by the American Cancer Society and National Institutes of Health.

Program Abstract #377
Unraveling the role of Yorkie-interacting proteins during Drosophila oogenesis
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The Hippo pathway controls organ size through regulating cell proliferation and programmed cell death in a conserved manner. Core components of the Hippo pathway are two Ser/Thr kinases Hippo (Hpo) and Warts (Wts), as well as a transcription co-activator Yorkie (Yki). Although most of the core components have been identified, the whole picture of this pathway remains incomplete. Given that a recent study has reported the Hippo protein-protein interaction network (Hippo-PPIN) based on physical interaction, I utilized the Drosophila ovary as a model to examine whether proteins of the Hippo-PPIN functionally interact with the Hippo pathway. Previous studies have shown pleiotropic roles of the Hippo pathway during oogenesis in Drosophila, including orchestrating tissue homeostasis and border cell migration. Knockdown of two candidate genes encoding Yki-interacting proteins attenuated border cell migration. The border cell fate, however, was correctly specified in the manipulated egg chambers. I am now investigating roles of these candidate genes in border cell migration. This research was funded by the National Science Council grant and by the Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University.

Program Abstract #378
Protein Kinase C delta regulates the structure and dynamics of cellular protrusions during collective cell migration
Felix Gunawan, Syed Saad Husainie, Adam Kramer, Jing Lu, Amad Bhatti, Dorothea Godt
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Actin-based membrane protrusions are essential for many cell migration processes. These include the collective migration of the border cell cluster (BCC) during Drosophila oogenesis. In a transcriptome-wide screen, we identified Protein Kinase C delta (PKCδ) as a putative downstream target of the large Maf transcription factor Traffic Jam, which we had previously shown to regulate BCC migration. Here, we show that the serine/threonine kinase PKCδ regulates the formation and organization of actin filament bundles in migrating border cells. PKCδ appears not to co-localize with F-actin bundles but is enriched adjacent to them at the plasma membrane and in the leading protrusion of the BCC. Examination of CRISPR-generated PKCδ mutants revealed that PKCδ is required for normal F-actin organization and extension/retraction dynamics of the leading protrusion of the BCC, and for limiting tumbling movements of border cells. Increased PKCδ expression caused delays in BCC migration by disrupting the formation of actin filament bundles and reducing the length of cellular protrusions. Expression of catalytically inactive PKCδ affirmed that the kinase activity of PKCδ is necessary for the effect of PKCδ on the F-actin network. In summary, our analysis identifies PKCδ as an important regulator of F-actin organization and cellular protrusion dynamics during collective cell migration.
Program Abstract #379
CK1α decreases β-catenin levels at the adherens junctions to facilitate wound closure in Drosophila larvae
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Skin wound repair is essential for organisms to restore barrier function and prevent infection after tissue damage. The wound-edge epidermal cells migrate as a cell sheet to close the wound gap. However, the detailed mechanisms of how cells interact with each other during wound healing in vivo are still not clear. We tested the functional role of adherens junctions during epidermal wound healing in Drosophila larvae. Interestingly, epidermal overexpression of an adherens junction component, β-catenin (β-cat), significantly delays wound closure, suggesting β-cat levels need to be tightly regulated during this process. Indeed, around wounds in control larvae β-cat protein is reduced at the cell-cell junctions of wound-edge epidermal cells in the early stages of healing. The wound closure defect seen upon overexpression of β-cat is related to its function at adherens junctions because loss of E-cadherin strongly abolishes the observed wound closure defect. What factors might regulate β-cat during wound healing? Casein kinase 1α (Ck1α) phosphorylates β-cat and promotes its degradation (Yanagawa S et al., 2002). Therefore, we tested if Ck1α regulates β-cat levels or localization in the larval epidermis. Strikingly, loss of Ck1α dramatically increases β-cat at the adherens junctions and also causes a strong wound closure defect. Loss of Ck1α-induced wound closure defects can be partially rescued by silencing β-cat, indicating that β-cat is a critical substrate of Ck1α during wound closure. Collectively, we showed that Ck1α reduces β-cat levels at the adherens junctions to facilitate epidermal cell migration during wound healing. Regulation of adhesive strength may thus be a critical mechanism to coordinate forward cell movement during wound-induced cell sheet migration. I am currently trying to measure the adhesive forces during this process. Funding: CRT was support by American Heart Association predoctoral fellowship 16PRE30880004, MJG was supported by NIH R01 GM083031.

Program Abstract #380
Epithelial Cell Reintegration: The Ins and Outs
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Epithelial tissues perform a range of specialized functions, including secretion, absorption, and protection. All of these functions require that the component cells remain tightly packed. This is a particular challenge during development, when new cells are being added to the tissue. Work in a number of systems shows that one answer to this challenge is cell reintegration: epithelial cells can be born protruding from the sheet, then reincorporate into it. Our previous work demonstrated that reintegration in the Drosophila follicular epithelium relies on Fas2 and Neuroglian, homophilic adhesion molecules that promote axonal growth and pathfinding. Our current model is that reintegration is driven by remodeling of the cortex after mitosis, and requires coordination between cell-cell adhesion and the juxtamembrane spectrin-based cytoskeleton. Our work in Drosophila is now directing experiments in other epithelial systems, including mammalian epithelial organoids.

Program Abstract #381
Analysis of Proliferation and Migration in Phenylalanine, Retinoic Acid, and 4-diethylaminobenzaldehyde Treated Cells
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Maternal phenylketonuria [MPKU] is a syndrome of multiple congenital anomalies including cardiovascular malformations [CVMs], and brain and growth restriction when a mother with Phenylketonuria [PKU] does not control her dietary intake of Phenylalanine [Phe]. However, the mechanisms responsible for Phe-induced CVMs are poorly understood. Our lab has preliminary evidence that high levels of Phe could inhibit Retinoic Acid [RA] signaling, which typically promotes the expression of genes such as proliferation, migration, and differentiation.
Proliferation and migration of the neural crest cells are important in formation of the outflow tract (OFT) and aortic arch arteries (AAA). We hypothesize that Phe inhibits migration and proliferation, which may contribute to the defects seen in MPKU. We also looked at the effects of exposure to RA and 4-diethylaminobenzaldehyde (DEAB), a known RA inhibitor. We conducted in-vitro proliferation and migration assays on several cell types to determine if proliferation and migration was affected by Phe, RA, and DEAB exposure. Images were analyzed with ImageJ and GraphPad Prism. Present research suggests that Phe exposure causes a significant decrease in proliferation of cells. It is also shown that RA increases or does not affect proliferation, and that DEAB decreases cell proliferation. In this way, Phe is similar to DEAB, which suggests that it also acts as an RA inhibitor. This could contribute to the CVMs observed in MPKU. The results of migration studies showed that Phe acted similar to DEAB, where the rate of migration was slower than that of RA, which is known to increase migration. This suggests that Phe may act as an inhibitor of migration. This is significant because it eludes to a potential mechanism that Phe could affect RA signaling, thus resulting in the types of defects observed in human MPKU.

Program Abstract #382
Mispositioned nuclei during myogenesis: Many mechanisms drive similar phenotypes
Torrey Mandigo, Mary Ann Collins, Elizabeth Wynn, Eric Folker
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Nuclei undergo several long-range movements during skeletal muscle development. Mispositioned nuclei are a hallmark of aging and diseased skeletal muscles, suggesting that nuclear movement and position are critical to muscle development and function. To determine how nuclei move during muscle development, we disrupted the expression of several genes linked to different muscle diseases, and measured nuclear movement during Drosophila myogenesis. The genes mutated in patients with Emery-Dreifuss muscular dystrophy (dEmerin), Centronuclear myopathy (Amphiphysin), and Duchenne muscular dystrophy (Dystrophin) regulated nuclear movement by distinct mechanisms. dEmerin is required to separate neighboring nuclei, Amphiphysin promotes interactions between nuclei, and Dystrophin is involved in the final anchoring of nuclei. Phenotypically, dEmerin mutant embryos resemble embryos that lack the microtubule binding protein Ensconsin, suggesting cooperation between microtubule organization and Emerin. However, time-lapse microscopy suggested distinct mechanisms. In Ensconsin mutants, nuclei were spherical and only rotated in place. In dEmerin mutants, nuclei were stretched and did not rotate, but escaper nuclei moved rapidly to the proper position. These data suggested that in Ensconsin mutants the machinery to move the nuclei was disrupted whereas in dEmerin mutants the nuclei could not separate from their neighbors. To test this, we mechanically removed nuclei via laser ablation and measured the responses of the neighboring nuclei. In Ensconsin mutant embryos, nuclei remained stationary suggesting that the nuclei were under little mechanical strain, consistent with a defect in the force-producing machinery. In dEmerin mutants, nuclei recoiled indicating that force production was in-tact and interactions between nuclei prevented movement. Altogether, these data indicate that multiple mechanisms underlie the mispositioned nuclei that are evident in poorly functioning muscles.

Program Abstract #383
Asymmetric cell division: the driver of Ciona notochord tapering.
Konner Winkley, Spencer Ward, Wendy Reeves, Michael Veeman
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Tapering of the notochord towards both the anterior and posterior tips is common across the chordates, yet the mechanisms driving tapering are still largely unknown. The Ciona notochord forms a tapered rod consisting of only 40 cells, and therefore presents a uniquely tractable system for studying how taper arises. Previous work suggested that unequal cleavage during notochord cell division might play an important role in creating the AP cell volume differences that explain much of the notochord’s taper. To test this, we used a genetic fate mapping approach relying on clonal propagation of electroporated transgenes to identify sibling cell pairs for all notochord cells after all the relevant divisions. We then measured cell volumes in 3D by confocal imaging and computational image analysis to quantify volume asymmetries between sibling cells at multiple stages of notochord...
development. We found that there are distinctive patterns of asymmetric cell division (ACD) in different notochord sublineages. These unequal cleavages are relatively subtle, but they are iterative and finely spatially patterned such that cells at the front and back of the notochord primordium become smaller than cells in the middle. By comparing sibling cell volume ratios both immediately after division and also several hours later we find that these ratios are stable over time, indicating that patterned cell volume changes apart from ACD do not contribute to notochord taper. Taken together, these findings show that finely patterned ACDs have an unexpectedly important role in controlling the shape of an entire chordate organ. To determine the mechanisms underlying these ACDs, we are currently testing potential polarity cues as well as the hypothesis that mother cell shape may play an important role. This work was supported by NSF IOS 165455 “Linking Cell Shape and Asymmetric Division in Ciona Notochord Tapering” to MV

Program Abstract #384
Mutant Cell Fate Analysis in Zebrafish Embryogenesis Using Single Cell RNA Sequencing
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Cell fate specification is a fundamental process during metazoan development, and many insights into its molecular mechanism have been gained through analyses of developmental mutants, often by classic approaches that focused on a few genes and cell types at a time. The recent advent of high-throughput single cell RNA sequencing (scRNA-seq) allows characterization of mutants on whole transcriptome level and at single-cell resolution. Here, we explore the power of scRNA-seq to characterize cell fate changes in the well-studied zebrafish MZoep mutants, which lacks an essential developmental signal, Nodal. We generated MZoep and wild-type single cell transcriptomes at the onset of gastrulation, identified modules of covarying genes using Non-negative Matrix Factorization (NMF), and inferred the spatial origin of the transcriptomes using Seurat. Our analysis identified missing gene modules and cell types in the mutant, and revealed an spatial shift of remaining cell types in the mutant compared to the wild type. In addition, we combined our mutant analysis at the onset of gastrulation with wild-type gene module analysis at later developmental stages. From the modules missing at the onset of gastrulation, we were able to predict the modules and associated cell types that would be missing at later stages in the mutant; additional mutant transcriptomes sequenced at a later stage verified our predictions. Our results agreed with classic fate mapping and phenotyping results, and further suggested that no new cell type was created in the mutant; instead, mutant cells were all canalized into a subset of wild-type fates, even on the whole transcriptomic level. This research was supported by the NIH (A.F.S., J.A.F., A.R.), the Allen Discovery Center for Cell Lineage Tracing (A.F.S.), Jane Coffin Childs Memorial Fund (J.A.F.), Charles A. King Trust (J.A.F.), Howard Hughes Medical Institute (A.R.), and the Klarman Cell Observatory (A.R.).

Program Abstract #385
Dynamic analyses of germ cell development in gastrulating amniotes
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Germ cells are the source of heritable genetic variation and are responsible for the propagation of a species. Amniote germ cells appear to derive from a few pluripotent epiblast cells, while most of their neighboring epiblast cells become somatic embryonic cells. The cellular processes involving the specification and commitment of these epiblast cells to germ versus soma cell fates, the timing over which the processes occur, and the changes induced in transcriptome profile remain unclear. We are using innovative advances in lineage tracing, time-lapse imaging,
and molecular profiling within living transgenic quail embryos to elucidate how pluripotent epiblast cells are induced to become and maintain a germ versus somatic cell fate. The integration of dynamic imaging and single cell RNAseq allow us to envision a future in which we understand cell types not just as static molecular snapshots of individual cells but more broadly as cellular life histories unfolding in time.

Program Abstract #386
Is there a conserved neural crest cell invasion signature throughout the embryo?
Jason Morrison
Stowers Institute for Medical Research, USA

Morrison JA, McLennan R, Gogol MM, Chen S, Peak A, Perera AG, Kulesa PM.

Neural crest cells are a multipotent and highly migratory population that invade the embryo all along the vertebrate anteroposterior axis to contribute to nearly every organ. We recently discovered by single cell analysis a novel transcriptional signature associated with the most invasive (Trailblazer) cranial neural crest cells that is consistent throughout their long distance migration (Morrison et al., eLife 2017). Although we analyzed a typical cranial neural crest cell migratory stream at the axial level of the hindbrain and target second branchial arch (BA2), it remained unclear whether this Trailblazer signature is conserved within the invasive fronts at different axial levels throughout the embryo. To address this, we expanded our single cell transcriptional analysis to include other neural crest cell migratory streams and their surrounding microenvironments including axial levels from BA1-BA4, using 10X Genomics. We will describe the extent of conservation of invasion signatures between the different axial levels and identification of unique receptor-ligand pairs in neural crest and neighboring cell types. Together, these data will help to identify a focused list of genes critical to neural crest migration independent of axial level and provide unique insights into specific peripheral target invasion. This research was supported by the generosity of the Stowers Institute for Medical Research.

Program Abstract #387
Delineating the mouse liver bud and early embryonic liver diversification through single-cell expression profiling
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In the embryo, hepatic progenitor cells, known as hepatoblasts, evaginate from the endoderm, and invade the surrounding septum transversum mesenchyme to form the embryonic liver bud. The hepatoblasts give rise to the main functional cells of the liver, differentiating into cholangiocytes, the bile duct epithelial cells, and hepatocytes, which are responsible for blood detoxification and bile production. Besides mesenchymal cells, endothelial cells and hematopoietic cells are also important players in the hepatic niche to promote liver development. However, the detailed landscape of this microenvironment remains elusive. In addition, the remaining cell types are poorly characterized and under-explored. To delineate cell diversity in the mouse liver during its early stages, we employed 10x Chromium high-throughput single-cell 3' RNA-seq to generate libraries of 9252 cells from E8.5 (15ss) endoderm, 2643 cells from E9.5 liver buds, and 9117 cells from E10.5 livers. To visualize cell diversity, we use Phenograph, a previously developed graph-based clustering algorithm that is based on Wishbone developmental trajectory positioning and MAGIC data imputation algorithms. Further, we employed MAST and Monocle to explore differential gene expression within cell types at different time points and developmental trajectories within lineages, respectively. Together, we unveil the dynamic gene expression patterns in the hepatic
Program Abstract #388
**Single-cell RNAseq transcriptomics reveals novel embryonic cell populations in embryonic male gonads.**
Kathryn McClelland, Oswaldo A. Lozoya, Brian Papas, Jian-Liang Li, Richard Woychik, Humphrey H-C Yao
*National Institute of Environmental Health Science, USA*

Profiling transcriptomic changes has provided important insights into fate determination and lineage specification processes that define gonadal development. To overcome limitations of bulk-RNAseq approaches, we implemented single cell RNA-seq to reconstruct transcriptional portraits of cell lineages in embryonic testes. Using two single cell sequencing platforms, DropSeq and single-cell combinatorial indexing (sciRNAseq), we extracted sets of differentially expressed genes (DEGs) using a novel statistical workflow and found that most (>57%) were shared across multiple specimens under each platform (N=3 murine embryonic testes each). By DropSeq, we captured >18K cells across biological specimens, with over 600 DEGs spread among 8 separate cell groups with distinct transcriptional patterns; similarly, we captured >30K cells with 517 DEGs across 8 cell clades by sciRNAseq technology (log2 ratio FDR p<0.05 v. bulk average, post hoc statistical power β>90%). We then integrated data from the 670 DEGs represented in either platform and identified 163 consensus DEGs insensitive to cross-specimen and cross-platform batch effects. These consensus DEGs are robust transcriptional markers for one of 8 transcriptionally coordinated cell profiles. The 8 transcriptional profiles showed expression patterns and gene ontologies consistent with existing microarray data, some of which was validated against mRNA and protein expression assays. In summary, we present reproducible transcriptional evidence for at least 8 distinct cell subpopulations in embryonic testes based on single-cell RNAseq assays. Here, we outline a robust experimental workflow to exploit the novel capabilities of single cell RNA-seq as a powerful discovery tool to interpret transcriptional dynamics in developing tissues. This study was supported by the NIH Intramural Research Program. KSM and OAL equal contribution.

Program Abstract #389
**SALSA: a cross-platform integration workflow for single-cell RNAseq data from developing reproductive tissues**
Oswaldo Lozoya, Kathryn McClelland, Brian Papas, Jian-Liang Li, Richard Woychik, Humphrey H-C Yao
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Embryonic cell populations rely on keenly concerted and highly dynamic transcriptional events that lay the structural foundations of early anatomical systems. The reproductive system stands out not only as an environment that nurtures the germline into adulthood, but also as one which, by the process of sex determination, leads to distinct anatomies from bipotent germ layer precursors. We can now unravel the transcriptional patterns at play during embryonic sex determination by single-cell transcriptomics; yet, insight from single-cell RNAseq data remains plagued by reproducibility issues. Here, we present a novel statistical workflow, single-cell amalgamation by latent semantic analysis (SALSA), that integrates data from multiple biological specimens, and demonstrate its performance by profiling embryonic mouse testes via DropSeq and single-cell combinatorial indexing RNAseq (sciRNAseq) alike. Unlike other statistical tools, SALSA exploits data sparsity in gene-cell expression matrices to produce replicate-specific sets of differentially expressed genes (DEGs), and then combines information from DEGs across specimens and platforms to infer minimal sets of highly reproducible consensus DEGs. Using 14.5-dpc embryonic testes from CD-1 mice (N=3 independent biological replicates per platform) we show that DEGs detected from individual specimens exhibit matching ontologies and pathway enrichment networks when analyzed by SALSA regardless of platform. Also, we report 8 mutually exclusive cell profiles from >40K single cells overall across platforms, based on 168 consensus DEGs, and insensitive to cross-replicate and cross-platform batch effects (FDR p<0.05, within-gene post hoc adjusted power β>90%). In summary, we demonstrate SALSA as a powerful analytical tool to interrogate lineage determination in embryonic tissues when data sparsity in gene-cell expression matrices is treated as information in single-cell transcriptomics.
Program Abstract #390
Single-cell RNA-sequencing implicates brain vasculature in the pathogenesis of human cerebellar malformations
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Cerebellar malformations are phenotypically heterogeneous birth defects that are frequently associated with significant developmental disabilities. Both non-genetic prenatal and genetic factors have been implicated in the cause of cerebellar malformations. However, a fundamental question is whether specific cerebellar malformations represent causally heterogeneous disorders in which multiple genetic and/or environmental risk factors disrupt common molecular pathways. To address the genetic basis of cerebellar malformations, we performed exome sequencing in 100 families with one or more children diagnosed with Dandy-Walker malformation (DWM) or diffuse cerebellar hypoplasia (CBLH). We identified 33 genes associated with DWM or CBLH. Most of these genes were associated with known neurodevelopmental disorders, but the cerebellar anomaly was an underappreciated feature of the syndrome. To investigate whether these cerebellar malformation genes converge on specific cell types in the developing cerebellum, we used single-cell RNA-sequencing to profile the transcriptomes of ~5,600 cells collected from 5 cerebella ranging in age from 12 to 21 post conception weeks. Surprisingly, among the 33 genes associated with DWM or CBLH, few were expressed in the primary cell types of the cerebellum, granule cell precursors and Purkinje cells. Instead, ~30% of the genes were expressed in endothelial or mural cells. Together, these data suggest that defects in cerebellar vasculature underlie some genetic causes of cerebellar malformations, which further intersects with non-genetic causes of CBLH including cerebellar hemorrhage.

Program Abstract #391
Bootstrapping Biology: Quick and easy de novo genome assembly to enable single cell gene expression analysis
Jamie Schwendinger-Schreck1, Stephen Williams1, Ian Fiddes1, Stephane Boutet1, Nikka Keivanfar1, Donald Miller2, Doug Antczak2, Deanna M. Church1
110x Genomics, USA; 2Baker Institute for Animal Health, USA
The availability of a high-quality draft assembly is a critical component for addressing biological questions about an organism. Until recently, development of such an assembly has been costly and time intensive. We recently described a protocol for simple genome assembly from a single library that utilizes cost-effective short read sequencers (Weisenfeld et al., 2017). Here we create both a reference assembly, as well as perform single cell gene expression (scRNA-Seq) analysis, on a single sample from an individual. Using peripheral blood from a male Equus asinus (donkey), we first generate a phased, diploid assembly using Chromium Linked-Reads and the Supernova assembler. Our assembly has superior scaffold N50 [Supernova: 49.6 Mb; reference: 3.8 Mb] and contig N50 [Supernova: 494 Kb; reference: 66.7 Kb] compared to the E. asinus reference. Next, we use Cactus, a multi-genome alignment tool, followed by the Comparative Annotation Toolkit (CAT), to generate an annotated draft reference. CAT leverages the comprehensive annotation of horse to generate high-quality annotation on the E. asinus genome, keeping track of orthology relationships. Overall, our annotated assembly has extremely high (>91% of horse genes in Ensembl) high quality copies of orthologous vertebrate genes. We next performed scRNA-Seq on peripheral blood lymphocytes from the same donkey, resulting in analysis of over 7000 cells, which are clustered based on gene expression profiles. Further, CAT-based annotation enables annotation of clusters representing all expected major cell types, including subsets present at less than 1%. This dual approach brings scRNA-Seq to organisms that were previously inaccessible, while simultaneously generating a high-quality phased assembly that enables genomic interrogation and population-based studies. Funding: This work was supported in part by the Dorothy Russell Havemeyer Foundation, Inc. and by the Zweig Memorial Fund for Equine Research in New York State.
Program Abstract #392
The role of reactive oxygen species in the regulation of anoxia tolerance during development
Amy Seufert
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Completion of embryonic development is dependent on aerobic metabolism. Across most species dependence on aerobic metabolism increases during development, with early embryos more tolerant of hypoxia and anoxia than later stage embryos. The development of metabolic and physiological traits is poorly understood, and connections between metabolic pathways and developmental outcomes have received very little attention. All aerobic organisms produce ROS, and some are often exposed to high ROS levels during anoxic to normoxic transitions. While ROS have critical roles in cell signaling, excess ROS levels lead to the damage and destruction of proteins, lipids, and nucleic acids that can disrupt normal development. Embryos of the annual killifish Austrofundulus limnaeus are the most anoxia tolerant vertebrates yet discovered, with the ability to survive for months without oxygen. These embryos also possess effective mechanisms of protection against ROS damage induced by exogenous hydrogen peroxide. To study the unique metabolic abilities of these embryos a neural progenitor cell line has been established that has a time to 50% mortality (LT50) of 26.5 days at 30°C. This cell line provides a powerful system for understanding the role of ROS production and detoxification in regulating cell survival in the early nervous system. We will quantify ROS levels within A. limnaeus cells exposed to anoxia and recovery from anoxia, and compare these to mouse and human cell lines. We will also identify the sites of ROS production within A. limnaeus cells in order to pinpoint potential protective mechanisms they possess against ROS production. Identifying these mechanisms may help to highlight the unique metabolic adaptations in this species, and may also point to pathways that make typical embryos so sensitive to oxygen limitation. Funded by NSF IOS-1354549 to JEP.

Program Abstract #393
High Resolution Promoter Analysis Using Random Mutation and Sequencing
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Accurate analysis of transcription factor binding to promoter and enhancer regions is essential for understanding the gene regulatory events driving development. High resolution promoter analysis is necessary because transcription factors bind to DNA regions between 6 and 10 base pairs in length. However, traditional methods of promoter bashing are limited to much larger regions. Thus, we are developing a technique capable of characterizing promoters at a one-base pair resolution. In our method, error prone PCR is used to generate a pool of mutated promoters with 1-2 mutations per promoter variant. We then Gibson clone these promoters upstream of a transcribed region containing a GFP gene and a unique barcode. The resulting library is sequenced by PacBio sequencing to match each barcode to its corresponding mutations in the promoter. These results are entered into a database for analysis. At the same time, we inject zebrafish embryos with the Gibson cloned library, extract RNA, and PCR amplify the barcode region of the mRNA for RNA-seq analysis. We then use the RNA-seq data and PacBio sequencing to calculate the relative expression of each barcode region, which will correlate to the level of expression of each mutant promoter. This method shows the relative importance of each individual base pair of a promoter for expression of a gene. This technique will increase the efficiency of studies on gene regulation, thus allowing us to understand and treat congenital defects with currently unknown causes.
NHLBI 2U1HL098160

Program Abstract #394
Elucidating the role of the transcriptional coactivator Camta1 in cardiac development
Lindsey Madsen, Zachary Frederich, Evangeline Friedbaum, Morgan Fronk, Colby Nielsen, Trenton Meline, Mary Taylor, Jonathan Chapple, Jonathon T. Hill
Brigham Young University, USA
Congenital Heart Disease (CHD) is the most common type of birth defect in the United States, causing more first-
Identifying the genes controlling embryonic heart development, especially those involved in stress responses, is essential to improving CHD treatment and diagnosis. Camta1 is transiently expressed during heart looping in zebrafish, suggesting a role in heart morphogenesis. Additionally, it has been shown that Camta2, the only other member of the Camta family in vertebrates, responds to increased cardiac stress to activate a hypertrophy gene program during adult heart failure. Thus, Camta1 may act as a sensor for stress as part of normal heart development or in response to abnormal cardiac loads in the embryo. In silico homology analysis has predicted that Camta1 binds Calmodulin in the cytoplasm when Ca++ concentrations increase, translocating Camta1 into the nucleus and activating genes repressed by Nkx2.5. To establish the role of Camta1 in heart development, we are mutating zebrafish using CRISPR and characterizing the transcriptomic effects of Camta1. Preliminary results show that the Camta1 phenotype is similar to the Nkx2.5 phenotype, which has an enlarged atrium and small ventricle. Furthermore, we are currently determining whether Camta1 binds to calmodulin in the presence of calcium and relocates to the nucleus. These analyses will demonstrate the calcium-dependent translocation of Camta factors in vertebrates for the first time, determine the role of Camta1 in cardiac development, and potentially provide an important link between stress and cardiac morphogenesis in normal and abnormal development.

Program Abstract #395
Characterizing the Early Transcriptional Response to Ethanol Teratogenesis
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Alcohol consumption during pregnancy is the most preventable cause of birth defects, yet approximately 2-5% of children are afflicted with Fetal Alcohol Spectrum Disorders (FASD). Twin studies suggest a genetic predisposition, contributing to the variation in risk for FASD. Despite this, we lack a basic understanding of the factors that protect or predispose an individual to FASD. To assess the relative level of variation of the transcriptional response to ethanol, we performed single embryo RNA-seq during early embryonic stages. Individual zebrafish embryos were exposed to a subteratogenic dose of 1% ethanol in embryo media. Our data suggests that the effect of ethanol is subtle; time is the most important variable driving variation in fold coverage across all samples. Despite this, we found two modules that positively (mediumpurple4) and negatively (darkolivegreen4) correlate with ethanol exposure using Weighted Gene Co-expression Network Analysis (WGCNA). Nearly all genes in darkolivegreen4 are located on Ch4 and many of these genes encode zinc finger proteins. We validated gene expression changes in both modules using qRT-PCR. We also found more upregulated than downregulated genes among ethanol-treated individuals, implying a stress response. Transcriptional changes due to ethanol are indicative of increased oxidative stress and ion transport and reduced DNA replication and cell division, resulting in developmental delay. We utilized the LINCS L1000 dataset to query drugs that exacerbate or rescue ethanol-induced phenotypes in our ethanol-sensitive zebrafish mutants. Future analyses will include ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) to validate the accessibility of Ch4q after ethanol exposure.

Program Abstract #396
Spatio-temporal reconstruction of gene expression during embryogenesis based on live imaging and transcriptome data
Yasuhiro Kojima
The University of Tokyo, Japan
Embryogenesis is achieved by gene expression and cell movements highly regulated in terms of spatial and temporal contexts. Due to recent advancements of sequencing technology and computational powers, researchers succeeded in reconstructing spatial expression pattern of numerous genes at some stages of embryogenesis. However, from only such spatial expression pattern at one time point, it is difficult to reveal the process of generating the spatial expression pattern, which require the continuous spatio-temporal gene expression pattern during embryogenesis. On the other hand, the technological advancements of live imaging enable researchers to capture cell movements during early embryogenesis. We developed computational
methodology to calculate the continuous spatio-temporal gene expression pattern by combining the spatial expression pattern with the spatio-temporal cell coordinates during embryogenesis. Specifically, we infer the gene expression at arbitrary spatio-temporal coordinate using a machine learning methodology, Gaussian process regression, assuming that gene expression is a continuous spatio-temporal function, and observed gene expression is derived from the function. Applying this method to the existing data of spatial gene expression pattern and spatio-temporal cell coordinate for early zebrafish embryogenesis, we reconstructed the spatial expression pattern of a gene, admp, at an intermediate time point (10 hpf) from spatial gene expression pattern at the two different time points. We confirmed that the reconstructed spatial expression pattern at the unobserved time point is similar to the empirical spatial expression pattern derived by in situ hybridization. To future, we are going to validate our method for other genes. We believe that our method have a potential to provide the dynamics of genome wide gene expression behind various morphological process captured by microscopy. This work was supported by JSPS KAKENHI Grant Number 17J09326.

Program Abstract #397
Sox9 defines cell-specific targets of Wnt signalling
Aravindabharathi Ramakrishnan, Kavya Adiga, Ken M. Cadigan
University of Michigan, USA
Continuous Wnt signaling in essential for the maintenance of both intestinal stem cells and the terminally differentiated Paneth cells that flank them. However, the two cell types express different genes in response to Wnt/β-catenin signaling. We examined the promoters of Paneth cell-specific Wnt target genes to explore the mechanisms of cell type-specific gene expression. As expected, these promoters contain binding sites for TCF/LEF family transcription factors, the primary effectors of Wnt/β-catenin-dependent transcription. In addition, they contain putative binding sites for SOX9, a transcription factor required specifically for Paneth cell differentiation. Luciferase reporters generated from these promoters are synergistically activated by Wnt signaling and SOX9 overexpression in multiple cell lines. Reporters containing mutations in the putative TCF or SOX9 binding sites fail to be activated in this manner, suggesting direct regulation by these factors. We also found that SOX9 binds to multiple TCF/LEF family members in vitro. TCFs and SOX proteins bind to DNA through an HMG domain, and these domains on the two proteins also mediate TCF-SOX interactions. The requirement of SOX9 for the activation of Wnt targets is surprising given the wealth of literature on SOX9 as an antagonist of Wnt signaling. The expression of SOX9 at levels which activate Paneth cell reporters also strongly suppresses the activity of Wnt-regulated enhancers lacking SOX9 binding sites. Taken together, our data support a model of SOX9 as a switch factor that defines the Paneth cell transcriptome by not only activating the expression of Paneth cell Wnt target genes, but also simultaneously suppressing the expression of stem cell Wnt targets. Funding: This work is supported by an NIH R01 grant to Prof. Ken Cadigan.

Program Abstract #398
SoxE paralogs are direct targets of Tfap2 paralogs in the gene regulatory network governing neural crest specification
Andrea Hallberg¹, Christopher Dooley², Ruth Williams³, Gregory Bonde¹, Tatjana Sauka-Spengler³, Elisabeth Busch-Nentwich⁴, Robert Cornell¹
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How are pluripotent precursor cells specified? We are exploring this question by dissecting the gene regulatory network (GRN) governing specification of neural crest in the neural plate border. Many transcription factors contributing to this GRN have been identified, but the direct connections within it are poorly understood. Zebrafish embryos doubly homozygous for loss of function alleles of tfap2a and tfap2c lack expression of all neural crest markers tested. We have shown that Tfap2a binds near the transcriptional start site of sox10, but there are likely to be other direct targets of Tfap2a/c in this GRN because sox10 loss-of-function mutant embryos retain neural crest-derived ectomesenchyme. To identify these targets, we generated expression profiles of
Among Tfap2 dependent genes, these are several genes encoding transcription factors, including SoxE family members sox9b and sox10 and SoxD family member sox5. We reasoned that for an enhancer to be active, it must be a nucleosome-free region (NFR). To identify NFR in neural crest, we performed Assay for Transposase Accessible Chromatin Sequencing (ATAC-seq) on isolated neural crest. In addition, we performed ATAC-seq on lysates of wild-type or tfap2a/c double mutant embryos. Tfap2-dependent NFR are present near the sox genes mentioned above, and some other proposed members of the NC specification GRN. In vivo reporter assays on a subset confirm they are neural crest enhancers. Ongoing chromatin immunoprecipitation assays are testing whether Tfap2a directly binds such elements. We are also examining the set of Tfap2-dependent NFR for shared sequence features. This work indicates the SoxD and SoxE family members are key effectors Tfap2 family members in the neural crest GRN, and illuminates the direct connections in the GRN governing NC specification in the neural plate border.

Program Abstract #399
Finding the switches that activate animal genes through a combined in silico and in vivo approach
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The DNA sequences of genomes encode the recipes for making functional cellular products, notably proteins, and switches that regulate when these products are made. While the genetic code for proteins has been known for decades, a similar code for the regulative switches is lacking. This presents a major challenge to understanding the genetic basis of life, as these switches (called cis-regulatory elements or CREs) may outnumber protein-coding genes by 20-50 fold. Both in vivo and in silico approaches exist to study CREs, but the former approaches are generally low throughput and not up to the scale of vast genomes, and the latter lack validation of predictions. We are merging in silico and in vivo approaches to identify the CREs controlling genes responsible for a fruit fly pigmentation trait. Here, we are leveraging the knowledge of five CREs that switch on the transcription of five different genes from a fruit fly tergite pigmentation gene regulatory network (GRN). We are using the SCRMshaw bioinformatic tool to identify novel predicted CREs controlling genes within this GRN based on underlying similarities in the DNA sequences of the known CREs. From this novel list, we will test 24 for CRE activity in in vivo reporter transgene assays. The results from these tests will reveal to what extent the in silico method succeeded. Novel validated CREs will be compared with the known five to reveal what the molecular functions are for the common DNA motifs as the next stage of this research project. The encoding of information in CREs is a universal feature of life, so these results bear upon life at every level, including the betterment of the human condition. This project has received funding support from the Berry Summer Thesis Institute and the Biology Department at the University of Dayton, and the National Science Foundation (1555906).

Program Abstract #400
A supply and demand model for accumulation and loss of free nuclear histones in the early embryo
Yuki Shindo, Amanda Amodeo
Princeton, USA
In Drosophila, a newly fertilized embryo undergoes thirteen rounds of rapid, synchronous, syncytial nuclear divisions to generate approximately 6,000 nuclei in the first two hours of embryogenesis. Remarkably, the embryo does not increase in volume during this period and all the materials required for the processes are maternally deposited as RNA and protein during oogenesis. Therefore many nuclear proteins are supplied in the cytoplasm and must be rapidly partitioned into the exponentially increasing number of nuclei in order for proper development. Among these, histones are one of the most abundant proteins in the early embryo and must be rapidly transported into nuclei to chromatinize the thousands of newly synthesized genomes. However, regulation of the histone nuclear import and their dynamic properties in vivo is not yet well characterized in the early Drosophila embryo. Here, we constructed a novel endogenously-regulated histone H3 reporter tagged with a green-to-red photoswitchable fluorescent protein Dendra2. Using this system, we are able to measure in vivo
*vivo* parameters of histone dynamics in the embryo, including nuclear import rates, export rates, and the fraction of DNA-bound/unbound histones within the nuclei at various stages of development. Surprisingly, we found that is early cell cycles more than half of the total H3 is not bound to DNA. However, by later cycles this pool of “free” nuclear histone becomes depleted consistent with a simple titration of maternally provided stores. These findings have profound implications for the chromatin environment during early embryogenesis leading up to zygotic genome activation. This work was funded by the Lewis Sigler Institute for Integrative Genomics and JSPS Overseas Research Fellowship.

**Program Abstract #401**

**Nanoscale visualization of cis-regulation in development using ORCA**  
Leslie Mateo  
*Stanford University, USA*

Many of the differences in cell-type arise from specific interactions between distal enhancers and their target promoters. Both long-range activity and target specificity are believed to arise from the cell-type specific three-dimensional (3-D) genome organization. Yet, we have not directly observed genome folding *in situ*. Our limited understanding of 3-D genome structure has improved due to Chromosome Conformation Capture (3C) technology; however, sequencing based approaches lack information pertaining the spatial organization of cells in tissues, and largely provide population level information, not single cell data. I will describe a novel microscopy approach: optical reconstruction of chromatin architecture (ORCA), to follow the nanoscale DNA path in steps as small as 3 kb, giving an unprecedented view of chromatin organization at the single cell level. Our method allows measurement of 3-D trajectories from 1000s of cells to compute the pairwise contact frequencies between any two loci and quantitatively recapitulates measurements from high read-depth Hi-C. In addition, to single cell DNA structure, we can simultaneously image both the nascent RNA transcript and mature cytoplasmic RNAs of 30 genes within the same cells. I applied our method to study the 3-D organization of the bithorax complex (BX-C) within the developing *Drosophila* embryo. I find cell type specific differences in chromatin structure between the anterior and posterior cells that correlate with differences in segment specific enhancer activity and expression changes of BX-C genes. I will present our data and analyses as well as the genetic perturbations we are using to study the mechanisms of chromatin organization. This work was supported by a Burroughs Wellcome CASI grant and Searle Scholar’s grant to Dr. Alistair Boettiger.

**Program Abstract #402**

**Run transcription factor’s identity crisis in the *Drosophila* embryo: temporal switch from repressor to activator impacts dorsal-ventral patterning**  
Theodora Koromila, Angelike Stathopoulos  
*California Institute of Technology, USA*

In the early *Drosophila* embryo, we have focused on how genes are temporally regulated, as for many genes several enhancers act sequentially to support continuous gene expression. Our recently published study showed that the broadly-expressed transcription factors Suppressor of Hairless [Su(H)] and Run can coordinate the timing of enhancer action. Here we have used several approaches to provide insight into transcription factor activity by focusing on regulation of the gene *short gastrulation* (*sog*). Expression of the *sog* gene is controlled by two enhancers: *sog* Distal (*sogD*) and *sog* Intronic. ChIP data supports a direct role for Su(H) and Run in regulating expression from the *sogD* enhancer. Predicted binding sites for both factors were identified in *sogD* and upon mutagenesis the gene expression patterns exhibit alteration in fixed, as well as in live embryos visualized using the MS2-MCP system. Both live imaging and chromatin accessibility data verify that Su(H) functions as a dedicated repressor to regulate *sogD* activity. Additionally, we defined a dynamic role for Run in supporting *sogD* expression, finding that it switches from repressor to activator at early to late syncytial blastoderm stages, respectively. To provide additional insight, we turned to previous studies of anterior-posterior patterning where Run is known to exhibit spatially-changing roles, influenced by the Odd paired (Opa) transcription factor. Interestingly, Opa binding motifs are present in *sogD* and it is expressed at cellularization, when Run acts as an activator. Currently, we are
testing the idea that Opa is required for the temporal switch in Run’s role. These experiments provide insight into the mechanism by which ubiquitous factors act at particular enhancers demonstrating that a single factor can change its role, temporally, based on the everchanging landscape of co-factors that co-occupy flanking DNA sequences. This study was supported by NIH Grant R35GM118146.

Program Abstract #403
Regulation of Hox gene expression by the leg gene regulatory network
Arjun Balyan, Sebnem Ece Eksi, Teresa Orenic
University of Illinois at Chicago, USA
Hox genes are implicated in generating the diverse morphological features of structures along the anterior/posterior body axis of insects, vertebrates and other animals. The function and regulation of embryonic expression of these genes have been extensively investigated. However, Hox genes also function during later development stages in patterning and morphogenesis of limbs and other organs, and this process is not well understood. Previous studies have shown that modulated expression of Hox genes in insect limb fields is important for generating diverse limb patterns. For example, we have found that modulated expression of Drosophila Hox genes in developing legs underlies the formation of segment-specific sense organ patterns among the legs. The Ultrabithorax (Ubx) Hox gene, for instance, is expressed at low levels throughout the legs of the third thoracic segments (T3 legs), but high-level Ubx expression in defined domains of T3 leg alters the leg sense organ patterning pathway, resulting in development of T3 leg-specific sense organ patterns. Understanding the regulation of modulated Hox gene expression is essential for gaining insight into the generation of diverse morphological features of limbs. We, therefore, have investigated the regulation of Ubx expression in T3 legs and have determined that modulated Ubx expression is regulated by several components of the leg proximal/distal patterning pathway, including Distalless (Dll), Dachshund and bric-a-brac 1 and 2 and that Dll acts through a cis-regulatory module (CRM) of Ubx (Ubx-1kb-CRM) to activate Ubx expression in the T3 leg. Our findings indicate that generation of segment-specific sense organs involves modulation of Ubx expression within the leg primordia via integration of Ubx into the intrasegmental leg patterning pathways. Thus, Ubx acts to link the leg regulatory network to sense organ patterning pathways.

Program Abstract #404
Pax9 modulate Wnt signaling during palate development
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Using the genetic model of the Pax9-/- mouse that displays a consistent secondary cleft palate, we have recently shown that the Wnt signaling pathway is involved in Pax9-mediated signaling within the posterior palatal domain. Such a molecular circuit is critical for the morphogenetic gradient that defines the medial-lateral (ML) axis during palate morphogenesis. The functionality of this unique molecular relationship is underscored by the genetic rescue of palatal clefts in mice that lack both Pax9 and Dkk1, a proven antagonist of canonical Wnt signaling. Exciting is the high fidelity correction of palatal clefts in Pax9-/- embryos that received small-molecule and antibody-based agonists through placental circulation (Development, 144, 3819-28, 2017). Although our molecular and genetic data suggest that Pax9 functions upstream of the Wnt pathway in posterior palatogenesis, it is unclear whether Pax9 interacts directly with inhibitors of Wnt signaling or Wnt ligands. The objectives of these studies were to assess the nature of the molecular relationship between Pax9 and specific Wnt pathway genes so as to shed insight into their roles in palatogenesis. Our ChIP-qPCR analyses revealed specific binding activities of Pax9 near the transcription start sites of Dkk1, Dkk2 as well as the intergenic region of Wnt9b and Wnt3. In pCMV-Pax9 transfected cells, expression levels of Dkk1 and Dkk2 were down-regulated while Wnt9b and Wnt3 were upregulated. The dual relationship of Pax9 and Wnt genes was underscored by data from the expression analysis that revealed an overlapping expression pattern between Pax9 and Wnt ligands. In contrast, the patterns of Dkk1 and Dkk2 expression was inversely related to that of Pax9. Taken together, these results
suggest that Pax9 modulates Wnt signaling activity through its dual role in inhibiting expression of Dkk1 and Dkk2 while increasing Wnt ligand levels during posterior palatogenesis.

Program Abstract #405
GLI transcriptional repression regulates enhancer activity and chromatin accessibility for Hedgehog target genes
Kristin Falkenstein1, Zhicheng Ji2, Rachel Lex1, Weiqiang Zhou1, Hongkai Ji2, Steven Vokes1
1University of Texas At Austin, USA; 2Johns Hopkins Bloomberg School of Public Health, USA
The Hedgehog (HH) signaling pathway is a major system for cell-cell communication that is essential for the development of most organs. Its transcriptional output is primarily mediated by de-repressing target genes but the mechanisms by which GLI proteins repress transcription remain unknown. Here we report that GLI repressors regulate enhancer activation and chromatin accessibility at a subset of their binding regions, termed HH-responsive enhancers. These regions are highly enriched around HH-responsive genes and drive tissue-specific enhancer activity within HH-responsive regions. They also lose enrichment of the active enhancer marker Histone H3K27ac in Shh−/− limb buds though they retain markers of poised enhancers. Conversely, Gli3−/− limb buds lacking GLI repression have increased acetylation. Contrary to previous findings, the Polycomb repression complex is not associated with most HH-responsive enhancers. Interestingly, HH-responsive enhancers have reduced chromatin accessibility in Shh−/− limb buds. Our results suggest that GLI represses target gene expression by altering the chromatin environment within a specific subset of its own enhancers.

Program Abstract #406
Controlling tissue patterning by translational regulation of signaling transcripts through the core translation factor EIF3C
Kotaro Fujii
Stanford University, USA
The impact and dynamics of translational control during mammalian development remain poorly understood. Here we monitored translation of the mammalian genome using state-of-the-art ribosome profiling in mid-gestation mouse embryos as cells become specified and organize into tissues. We identified differential translational regulation between neural tube and limb for hundreds of mRNAs guiding critical tissue-specific functions as well as developmental signaling cascades, demonstrating that translation regulation acts to diversify gene expression. Unexpectedly translation of the core development signaling circuitry including Shh, Wnt, Hippo, PI3K and MAPK pathways are widely repressed in mesoderm lineage, revealing pervasive translational regulation for signaling transcripts. To further extend molecular understanding into this layer of gene expression regulation, we identified and functionally characterized a complex landscape of uORFs across 5′-UTRs of key signaling components. Using CRISPR-mediated mutagenesis of ES cells, coupled with in vitro differentiation into neurons, we demonstrate the significance of uORF-mediated translational repression within the major SHH receptor, PTCH1, in control of cell signaling and neuronal differentiation. Finally, phenotypic analysis revealed that the core component of eukaryote translation initiation factor 3 subunit c (EIF3C) is specifically required for Shh-mediated tissue patterning. The eIF3 complex has been thought to be required for general translation initiation. However, Eif3c heterozygous embryos show a selective reduction of Ptc1 mRNA translation in the developing neural tube and EIF3C has a specific requirement in limb and neural tube patterning. Altogether, this work unravels the designing principles of mammalian embryogenesis at the step of translation and reveals surprising specialization of housekeeping translation initiation machinery in regulating fundamental aspects of the developmental signaling circuitry.

Program Abstract #407
Developmental signals, topological landscapes and three-dimensional cis-communication between enhancers and promoters during development
Roel Neijts
Institut Pasteur, France
Awarded this year by the 'Developmental Biology Outstanding Paper Award' for my graduate work performed on Cdx/Hox regulation in the lab of Jacqueline Deschamps (Hubrecht Institute, Utrecht, the Netherlands), I will discuss the regulatory mechanisms behind the initiation and progression of Hox gene expression during mouse gastrulation and thereafter. Hox genes are organized in clusters, and are known for their colinear expression in time and space. Transcriptional activation starts on the 3’ side of a cluster and gradually progresses towards the 5’ end. Progenitors of anterior embryonic tissues express 3’ Hox genes exclusively whereas progenitors of more posterior tissues express in addition more and more 5’ genes of the clusters. These progenitors transmit their Hox codes to their differentiated tissues during axial elongation. We have found that the very first expression of the 3’-most Hox genes is regulated by Wnt signals. We have identified some early Wnt-responsive Hox enhancers, located in a 3’ Hox genomic segment (a ‘sub-TAD’). After this initial Wnt-dependent phase of Hox expression, more 5’-located Hox genes are gradually activated. We show that Cdx, shown earlier to regulate Hox genes, is required for chromatin opening of regulatory sequences in the trunk Hox genes genomic segment. Thus, Wnt signals and Cdx transcription factors play consecutive roles on enhancers in the process of Hox colinear expression during axial elongation. Approaches such as ATAC-seq and 4C-seq have contributed to the identification of regulatory elements – like the Hox enhancers above – and their topological context. Understanding the functional three-dimensional dynamics of (distant) enhancers within a regulatory landscape however is challenging. In the group of François Spitz (Institut Pasteur, Paris, France) I am currently developing tools that are expected to provide more information of the spatiotemporal ‘behavior’ of cis-regulatory regions in finding their target promoters.

Program Abstract #408
Discovery of notochord enhancers in Ciona intestinalis
Granton Jindal, Emma Farley
University of California, San Diego, USA
During embryonic development, precise patterns of gene expression enable a single cell to develop into a multicellular embryo with many different tissues. Enhancers are the genomic elements that control the precise tissue-specific gene expression patterns required for successful development. Here, we study how enhancers control gene expression in the notochord, a major signaling center in the developing embryo. The transcription factors Brachyury (Bra) and FoxA1/2 (FoxA) are necessary and sufficient for correct notochord development, however, we do not fully understand how they regulate notochord-specific gene expression. Even though there are some notochord-specific genes with enhancers co-regulated by Bra and FoxA, enhancers co-regulated by Bra and FoxA have not been found for other notochord-specific genes. Recently, many genes expressed specifically in the notochord of the Ciona intestinalis embryo were found using RNA sequencing. Focusing on transcription factors expressed in the notochord of Ciona, we find enhancer elements near these genes that drive notochord-specific expression. We also evaluate the necessity of Bra and FoxA transcription factors for this notochord-specific expression by systematically evaluating the necessity of Bra and FoxA sites of all affinities, not just ones of high affinity. This study provides insight into how enhancers are structured to activate notochord-specific transcription factors and further illuminates the developmental program of notochord development. G.A.J. is supported by NIH grant T32HL007444, Chancellor’s Research Excellence Scholarship, and NIH grant DP2HG010013. E.K.F. is supported by NIH grant DP2HG010013.

Program Abstract #409
Extreme nuclear morphology in Xenopus
Hannah Arbach, Marcus Harland-Dunaway, Andrea Wills
University of Washington, USA
An ellipsoid nuclear shape is highly conserved across cell types and species. Deviations from ellipsoid morphology through modulation of nuclear lamina and other nucleoskeletal components regulate complex cellular properties such as differentiation and tissue elasticity. Most perturbations of nuclear morphology are associated with pathologies, including progeria, cancer, and muscular dystrophy. The dearth of healthy models of nuclear shape...
variation has limited the understanding of the mechanisms that govern nuclear shape. Here, we introduce nuclear branching in the epidermal fin cells of *Xenopus* as a model for extreme nuclear morphological variation in a healthy, genetically tractable, easily manipulated and visualized tissue. We find that nuclear branching arises and elaborates during development but diminishes at the onset of metamorphosis. Cells with branched nuclei have active cell cycles and contain marks of transcriptionally active chromatin and heterochromatin throughout the nucleus. Disruption of actin filaments and the nuclear lamina protein, LaminB1 decreases nuclear branching. We have also found that LaminB1 is necessary for proper fin formation, suggesting that the loss of nuclear branching compromises the integrity of this highly specialized epithelium. Overall, this study establishes a new model for extreme nuclear morphological variation in a healthy tissue, which may provide insight into the range of structural constraints that can influence nuclear shape, how nuclear morphology can be perturbed, and the how nuclear morphological variation interrelates with other parameters of cell health. Funding: 5R03HD091716-02

**Program Abstract #410**

Progenitor expansion and competence are controlled by Lsd1 and three associated non-coding RNAs during fly follicle cell development  
**Ming-Chia Lee¹, Allan Spradling²**  
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Progenitors must expand sufficiently to achieve final tissue size, while retaining chromatin flexible enough to produce multiple cell types yet receptive to external signals effectively. The Drosophila follicle stem cell generates daughters that divide nine times to produce a large progenitor pool ideally suited for studying progenitor differentiation. Previously, we showed that lysine-specific demethylase 1 (Lsd1) functions as a histone 3 lysine 4 (H3K4) demethylase to dynamically maintain plastic epigenetic states in early progenitors that are crucial for proliferation and to time differentiation (Lee and Spradling, 2014, Genes Dev. 28, 2729). In order to understand how Lsd1 is able to program progenitor competence mechanistically, here we identified novel Lsd1 interacting non-coding RNAs (LINRs) that we propose acting as molecular scaffolds to modulate Lsd1 action on chromatin states. Studies of CRISPR-mediated deletions of individual LINRs suggest that LINRs play antagonistic roles in Lsd1-mediated follicle cell differentiation. While LINR1 and LINR3 promote epigenetic plasticity to sustain progenitor proliferation, LINR2 seems to drive stable epigenetic states that enable competence permitting progenitor differentiation. Moreover, our preliminary results suggest that LINR1 bridges an Lsd1 complex to Polycomb repressive complex 2 (PRC2) to dynamically yet precisely modulate poised chromatin states that regulate follicle progenitor competence during fly follicle cell development.

**Program Abstract #411**

Identifying a role for gene body methylation during zygotic genome activation  
**Deanna Arsala, Jeremy A. Lynch**  
University of Illinois at Chicago, USA  
Changes in global DNA methylation patterns occur during the maternal to zygotic transition and are necessary for zygotic genome activation (ZGA) in vertebrates. However, the modes of methylation in vertebrates are complex, and phenomena such as promoter methylation are restricted to vertebrates. Gene body methylation (GBM) is thought to be the ancestral form of methylation and is prevalent throughout Eukarya. Despite its broad conservation, the importance of GBM is still unclear, largely due to the complexity of the vertebrate methylation repertoire. Unlike the invertebrate models *D. melanogaster* and *C. elegans*, the wasp *Nasonia vitripennis* possesses a complete DNA methylation toolkit. Unlike vertebrate models, the methylation machinery appears to exclusively mediate GBM thus simplifying interpretation of the role of GBM in development. We confirmed that a knockdown of DNMT1 (*Nv-dnmt1a*) in *Nasonia* leads to a failure of gastrulation. We also found *Nv-dnmt1a* is not required to activate early embryonic patterning genes. Instead, *Nv-dnmt1a* is required for the activation of a subset of late zygotic genes involved in morphogenesis. We hypothesize that *Nv-dnmt1a* is required to activate genes that coordinate gastrulation and other morphogenetic movement dependent on ZGA. To identify the *Nv-dnmt1a* morphogenetic targets, we are taking a whole-genome approach. Mock-treated and *Nv-dnmt1a*-RNAi
embryos were aged to time points that correspond with the early onset of ZGA, major wave of ZGA when cellular patterning and cell-fate specification take place, and the morphogenetic stages of gastrulation. RNA and DNA from these embryos are currently being processed for mRNA-seq and whole-genome bisulfite sequencing. By using a genome-wide approach to investigate the role of Nv-dnmt1a during ZGA, we will provide a direct role for GBM in the activation of the zygotic genome and earliest aspects of morphogenesis. Funding: NIH 1R03HD087476

Program Abstract #412
DNA methylation independent imprinting during mouse gastrulation
Chelsea Marcho, Gregory Delulio, Dalton Hill, Jesse Mager
University of Massachusetts, USA
Normal development is regulated by the careful coordination of gene expression during both lineage allocation and tissue differentiation, which is accomplished in part through epigenetic mechanisms. One example of epigenetic regulation that occurs at select loci is genomic imprinting, which is defined as parent-of-origin mono-allelic gene expression. Establishment of imprinted expression is largely due to epigenetic differences in sperm and oocyte haploid epigenomes, which are maintained after fertilization and during development. In the mouse, many known imprinted genes are coordinately regulated in large imprinted gene clusters. Differential DNA methylation is thought to be the primary epigenetic mark arriving in the gamete and is responsible for determining mono-allelic expression at these imprinted clusters. Based on previous identification of lineage-specific imprinted expression and epigenetic modifications during gastrulation, we performed allele-specific RNA sequencing on micro-dissected mouse gastrulation stage tissues to identify known and novel imprinted events. We also performed whole genome bisulfite sequencing (WGBS) to attempt to identify corresponding differentially methylated regions with imprinted expression. These experiments have identified a novel set of imprinted genes that exhibit tissue-specific and temporally transient allele-specific expression. Importantly, we do not find any differential DNA methylation at or near these loci, suggesting there must be an alternate epigenetic mechanism responsible for the parent-of-origin expression. Taken together, these data begin to define a novel paradigm of long-lived epigenetic regulation during mammalian gastrulation and provide additional evidence for DNA methylation independent imprinting.

Program Abstract #413
SOX9 binding dynamics during heart valve development
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SOX9 is an important mediator of proliferation, EMT, and ECM deposition during heart valve development. Using ChIP-seq and RNA-seq, we have characterised the transcriptional network regulated by SOX9 and confirmed its control over many genes critical for heart valve development. Epigenetic analysis of SOX9 binding sites in the embryonic day 12.5 mouse heart valves reveals four distinct patterns for SOX9 binding: binding to pan-acetylated, nucleosomal DNA; binding to active promoter regions; binding to active enhancer regions; and binding to unprogrammed enhancer regions. Motif analysis of SOX9 binding sites in active enhancer regions reveals an enrichment of Nuclear Factor I (NFI) consensus sites. Two members of the NFI transcription factor family, Nfia and Nfix, are identified as SOX9 targets and are down-regulated in a Sox9 conditional knockout mouse model. Interestingly, these factors are up-regulated during human heart valve disease and act as cofactors to regulate gene expression during chondrogenesis. We are investigating SOX9 binding dynamics during heart valve development and how SOX9 binding regulates chromatin structures. Within this scope, we want to determine whether molecular interactions exist between SOX9 and NFI transcription factors during heart valve development and how these influence function. Our examination of SOX9 DNA binding dynamics during heart valve development will give insights into its role in chromatin remodeling and tissue specification.
Program Abstract #414
Developmental effects of neonatal anesthesia with sevoflurane are passed to unexposed male, but not female, progeny: Role of epigenetic mechanisms
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Background: Retrospective epidemiological studies of neurocognitive function in children who had general anesthesia early in life found significant deficiencies. The mechanisms of anesthesia-induced developmental abnormalities remain poorly understood even in exposed animals. We have found that rats, primarily males, neonatally exposed to anesthesia exhibit exacerbated stress responses, behavioral abnormalities and reduced hypothalamic K+-2Cl- (KCC2) Cl- exporter expression. The latter forms the basis for impaired inhibitory GABA type A receptor signaling, which is implicated in development of psychiatric disorders, including autism spectrum disorders. We tested whether parental (F0) neonatal exposure to sevoflurane, the most frequently used anesthetic in pediatrics, also affects the next generation (F1) of unexposed rats. Methods: Sprague-Dawley rats, unexposed and exposed to sevoflurane on postnatal day 5, were used to produce offspring. Results: Male, but not female, progeny of sevoflurane-exposed parents were affected. F1 males of both exposed parents exhibited impaired spatial learning and decreased expression of the hippocampal and hypothalamic Kcc2. Offspring of only exposed sires had abnormalities in elevated plus maze and prepulse inhibition of startle, but normal spatial memory, and decreased expression of the hypothalamic, but not hippocampal, Kcc2. In contrast to exposed F0, their progeny exhibited normal corticosterone responses to stress. Bisulfite sequencing revealed increased CpG site methylation in the Kcc2 promoter in F0 sperm and F1 male hippocampus and hypothalamus that was concordant with the changes in Kcc2 expression in specific F1 groups. Conclusions: Our findings provide the first experimental evidence that neonatal exposure to sevoflurane may also affect the next generation of males through epigenetic modification of Kcc2 expression, while F1 females may be at a diminished risk. This work was supported by the NIH, IHAF and NSF of China.

Program Abstract #415
Mixed-lineage leukemia-4, an epigenetic regulator is important for musculoskeletal patterning and development
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Tendons transmit the force generated by muscle contraction to the skeleton and are pivotal to joint movement and overall musculoskeletal function. Only a handful of regulators were so far associated with tenogenic differentiation. Some of these have limited functional roles and fail to account for the major events in tendon development. Epigenetic regulation has emerged as a critical regulator of cell fate differentiation and represents a promising layer of regulation that is yet to be explored in tendons. Here, we have examined the role of MLL4 (mixed-lineage leukemia-4), which was identified as a potential candidate in our RNAseq study to identify genes with distinctive gene expression in tendons. We find that, targeting MLL4 in the limb mesenchyme resulted in complex phenotypes across the musculoskeletal system including substantial tendon patterning defects. A uniform feature in mutant pups was that all tendons appeared smaller which could be reflective of failure in tenocyte recruitment. Indeed, mutant tendons showed reduced expression of Scleraxis (Scx) which is essential for cell recruitment to the growing tendon. Furthermore, high resolution TEM studies suggested an overall disruption in matrix organization. In addition to the tendon phenotypes, skeletal preparations of mutants revealed severe shortening of the long bones. Intriguingly, the deltoid tuberosity, which is normally located at a medial position along the bone shaft, was mis-positioned at the proximal end of the humerus, reflecting a disruption in basic
aspects of skeletal development. These findings reveal significant roles for MLL4 in overall musculoskeletal development and imply the significance of MLL4-dependent cues in early tendon patterning. Funding: Shriners Hospitals for Children (SHC 85410-POR-16) to RS

Program Abstract #416
Inhibition of Epigenetic Remodeling Prevents Tail Regeneration in Xenopus
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Humans have limited ability to regenerate many tissues. In instances such as limb loss or spinal cord injuries there is a dramatic decrease in quality of life and are few therapeutic options to help recover from these serious injuries. My research characterizes how epigenetic changes affect tail regeneration in the frog, Xenopus tropicalis. Xenopus tadpoles have significant regenerative abilities when they are developing but that ability is lost as they age and undergo metamorphosis. Previous work by our group has shown that the accessibility of chromatin undergoes drastic remodeling in which many of the genes are made inaccessible shortly after injury in the Xenopus tail. These genes are then reopened during regeneration. These changes are likely due in large part to histone deacetylases (HDACs) and histone acetyltransferases (HATs). We hypothesize that HDACs are necessary for causing the initial inaccessibility of the chromatin and that HATs are necessary for the reopening of chromatin for regeneration. We used an Assay for Transposase-Accessible Chromatin and sequencing (ATAC-Seq) to show that the chromatin does not undergo its normal shift toward inaccessibility over the course of regeneration when tadpoles are treated with HDAC inhibitors, coupled with a reduction in regenerative ability. This study will help us identify how histone acetylation directs changes in chromatin accessibility. Additionally, with immunohistochemistry we can study specific sites of histone acetylation and validate these antibodies for future epigenetic research. Our study of large-scale epigenetic changes during regeneration will help characterize how epigenetic gene regulation enables regeneration. We would also like to thank the University of Washington for their facilities and the NIH for the funding that made our research possible.

Program Abstract #417
Global genome methylation changes during early embryonic development in medaka, Oryzias latipes
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Studies in mice and zebrafish had demonstrated DNA methylation reprogramming of embryo. In mice, zygotes undergo substantial DNA methylation programming, through global erasure of DNA methylation before blastula followed by de novo methylation at the gastrula stage. To the contrary, zebrafish embryos undergo a different DNA methylation reprogramming event, in which paternal genome maintains hypermethylation, but the maternal genome undergoes remethylation and maintains the same level as paternal genome at blastula and gastrula stages. The mechanism of early embryonic reprogramming in medaka, another biomedical model fish, has not been characterized yet. We examined the genes that are involved in global DNA methylation, and measured global DNA methylation profiles in gametes and the embryo from fertilization until gastrulation in the Hd-rR strain of medaka. Similar to mice, the medaka sperm genome remained hypermethylated and oocyte genome hypomethylated before fertilization. The methylation in sperm was erased within the first cell cycle, resulting hypomethylated genome in embryo from the zygote to 16-cell stage. Global DNA methylation levels gradually increased from 16-cell stage and remained hypermethylated through the gastrula stage. Dnmt1 mRNAs remained high in oocytes and embryos up to late blastula and reduced at gastrula stage. Dnmt3bb.1 and Dnmt3bb.2 mRNA levels increased prior to remethylation suggesting their roles in remethylation. Tet1 and Tet3 mRNAs were maternally expressed, while Tet2 started expression only from gastrula stage. The pattern of genome methylation showed similarity to mammalian genome methylation, not zebrafish. Given Hd-rR strain of medaka has genetic sex determination system and maintains developmental epigenetic programming events similar to mammals, medaka could be a suitable model to study DNA methylation reprogramming and developmental and environmental epigenetics. This work was supported by NIEHS grant ES027123 to RKB.
Program Abstract #418

Somatic inheritance of transgenerational germline epigenetic marks in the medaka fish testis
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Health effects of past exposure to environmental chemicals on current and future generations is currently a public health concern. Studies have demonstrated an epigenetic association with environmentally induced health effects in several model organisms. Stressor-specific differential DNA methylation profiles, also called epimutations, are inherited by offspring via sperm; however, whether germline epimutations can be inherited by somatic tissues (soma) is not clearly understood. To answer this question, we exposed medaka fish embryos to bisphenol A only during embryogenesis and examined germline transmission of epimutations in adults across three generations.

BPA exposure at grandparental generation (F0) caused reduced fertilization success in the offspring two generations later (F2). In the testis, BPA-induced subtle changes in DNA methyltransferase (Dnmt) expression in the primordial germ cells at the parental generation (F0), whereas all Dnmt transcripts (Dnmt1, Dnmt3aa, and Dnmt 3bb) were significantly increased in the offspring at the third generation (F2), followed by 1.5-fold increase in the level of global DNA methylation. A CpG island on androgen receptor alpha (AR alpha) promoter in the germ cells was hyper-methylated in the offspring that belonged to the BPA-exposed family. The epimutation was maintained in the testicular somatic cells, which resulted in significant reduction in AR alpha transcript levels. The data suggest that germ line epimutations can transfer to somatic cells causing relevant alterations in target gene expression. This is the first-time germline to soma transfer of epigenome was observed in any vertebrate species, supporting the concept of transgenerational epigenetic inheritance of environmentally induced phenotypes. Future studies will identify genome-wide epimutations in both germ line and soma.

Program Abstract #419

Chronic developmental glucocorticoid exposure alters long-term glucocorticoid signaling dynamics
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Chronic stress experienced during development can have a life-long impact on disease risk and overall health, but the mechanisms of long-term effects remain unknown. One hypothesis is that early life stress alters a set point for the physiological response to stressful stimuli. Glucocorticoid (GC) signaling plays a critical role in stress physiology, and in both zebrafish and humans is mediated by the hormone cortisol acting through its target, the GC Receptor (GR). The GR is a ubiquitous transcription factor well known for its role as a master regulator of metabolism and inflammation, and our lab has previously demonstrated that developmental exposure to chronically elevated cortisol alters the dynamics of inflammatory gene expression and leads to a pro-inflammatory phenotype in adulthood. Because the dysregulation of inflammation contributes to disease states from cancer to PTSD, we asked how GC signaling dynamics are altered by chronic developmental exposure to cortisol. Fkbp5 is a negative feedback regulator of the GR that acts as a resistor to control cellular sensitivity to GC. In humans, epigenetic modifications to FKBP5 have been observed in survivors of trauma and their progeny, and mutant variants of FKBP5 are associated with altered stress resilience. In adult zebrafish raised from embryos exposed to chronically elevated cortisol, we observe evidence of abnormal cortisol production and tissue transport, suggesting that continuous developmental exposure to elevated cortisol has lifelong epigenetic effects on the neuroendocrine stress system.

Program Abstract #420

Sonic hedgehog is required for the formation of auditory ganglion in the mouse inner ear
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Hearing loss affects over 300 million people worldwide and yet restorative treatment is minimal as we lack understanding on how our hearing organ, cochlea of the inner ear, is normally formed and wired. The auditory
system requires proper connections between sensory hair cells in the cochlea and auditory processing centers in the brain via neurons of the spiral ganglion (SGN). Previously, we have found that sonic hedgehog (Shh), which encodes a secreted signaling molecule, is expressed in the developing SG: first this gene is expressed broadly in a subset of the SG and later on its expression is confined to only SG cells located by the apex of the cochlea. This restricted expression pattern of Shh over time is crucial for regulating the timing of hair cell differentiation within the cochlea; however, the significance of this dynamic Shh expression pattern on SG formation is not known. To address this question, a combination of in situ hybridization, lineage tracing, and cell cycle labeling techniques were used to decipher the spatiotemporal origin of the Shh-positive cells in the SG. My results show that Shh is turned on only in nascent post-mitotic SGNs and is down-regulated within two days as the neurons mature. This transient expression of Shh is important as neuroblasts adjacent to the Shh-positive neurons express the Shh receptor, Patched 1 (Ptc1), suggesting that they are responding to Shh signaling. Without Shh, Ptc1 expression is downregulated and the size of SG is much reduced. These results indicate for the first time that an auto-regulatory loop of Shh signaling controls the developmental timing of SG, as different cohorts of newly generated SG neurons attribute to the dynamic expression pattern of Shh in the developing SG. Understanding how Shh signaling is being regulated in the developing SG will provide insights into how to restore damaged SG. This work was funded by the Division of Intramural Research of NIDCD (1ZIADC000021).

Program Abstract #421
Gap junction mediated glia-glia communication is required for axonal ensheathment and maintenance of glial morphology in the Drosophila peripheral nerve
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To ensure proper neuronal signaling, Drosophila peripheral axons are surrounded by three glial layers; the wrapping glia (WG) that directly contacts axons, the subperineurial glia (SPG) that acts as a barrier through the formation of septate junctions and the perineurial glia (PG) that surrounds the entire nerve. Extensive communication between these layers is required for proper development of peripheral nerves as they undergo extensive growth and differentiation. The mechanisms underlying glia-glia communication in these non-myelinating classes of glia however, remain unknown. Here we show that a gap junction protein, Innexin 2 (Inx2) is present in all three glial layers. Similar to inx2 null mutants, which are embryonic lethal, knockdown of Inx2 in peripheral glia leads to lethality during larval stages. To determine which glial layers require Inx2, we knocked down Inx2 in individual glial layers. Loss of Inx2 in the SPG results in the fragmentation of the neighboring WG, suggesting a role for Inx2 in mediating SPG-WG communication. To determine if the WG defects are due to loss of communication via the Inx2 channel, an Inx2-DN construct that interferes with Inx2 channel function was expressed in the SPG. The WG in these larvae exhibit similar defects to those observed with Inx2 knockdown, suggesting that Inx2 channels mediate SPG-WG communication. To test if Ca2+ mediates SPG-WG communication, we imaged calcium signals in peripheral glia using the GCaMP sensors. Ca2+ pulses are present in both the SPG and the WG of wild type larvae whereas these pulses are absent after Inx2 knockdown in the SPG but not the WG. Altering calcium levels in the larva however, does not affect WG morphology. In summary, we propose that Inx2 channels mediate communication between the SPG and the WG through an unknown mechanism. Moreover, SPG-WG communication ensures that the WG form and maintain processes that can properly ensheath peripheral axons. Funded by CIHR and NSERC.

Program Abstract #422
Regulation of cytoneme-mediated long-range signaling self-generates a tissue-specific FGF morphogen gradient during Drosophila tracheal morphogenesis
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Concentration gradients of morphogens specify positional information for cells to pattern tissue morphologies. How the positional information is established with reproducibility and precision is one of the central questions in
tissue patterning. The traditional view of cell-cell communication considers that the secreted signaling proteins diffuse in the extracellular space to generate concentration gradients. Here, using endogenously expressed FGF:GFP and FGFR:Cherry from genomic knock-in alleles, we uncovered a cytoneme-mediated self-generating mechanism for a long-range receptor-bound FGF gradient in the Drosophila larval air-sac-primordium (ASP), a wing-disc-associated tracheal branch. A restricted group of wing disc cells expresses FGF to induce growth and migration of ASP during 3rd instar larval development. We showed that the ASP cells extend polarized FGFR:Cherry-containing cytonemes to contact the wing-disc fgf-source to directly receive FGF:GFP. FGF:GFP moves together with FGFR:Cherry along the surface of cytonemes that extend from the ASP to the disc and forms a long-range receptor-bound gradient that adopts recipient ASP-specific contours. The number of cytonemes the ASP cells extend is proportional to the FGF:GFP amount they receive, which gradually reduces with increasing distance from the fgf-source, producing a receptor-bound ASP-specific gradient contour. FGF acts as a morphogen, inducing concentration-dependent activation of four genes in different zones of the recipient ASP epithelium. Pointed-P1, a high-threshold target, and Cut, a low-threshold target, antagonize each other and differentially feedback-regulate the number of FGFR-containing cytonemes, thereby creating regions with higher-to-lower numbers of FGF-receiving cytonemes. Therefore, a self-sustaining spatial asymmetry of cytoneme-mediated dispersion through signaling-feedback sculpts gradient contours that adopt and maintain precise tissue-specific morphologies.

**Program Abstract #423**

**Chondroitin sulfate proteoglycan Windpipe modulates Hedgehog signaling in Drosophila**

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Heparan sulfate proteoglycans (HSPGs) are a special type of carbohydrate-modified proteins that are found in the extracellular matrix and on the cell surface. HSPGs are well known to modulate a wide variety of developmental signaling pathways such as Wnt, Hedgehog (Hh), and Bone Morphogenetic Protein (BMP) by serving as co-receptors for their ligands. In addition to HS, Drosophila has chondroitin sulfate (CS), another type of glycosaminoglycan. Besides their role as structural components of the extracellular matrix, CSPGs have more recently been shown to act as signaling modulators. Given the structural similarities between CS and HS, it is not surprising that CSPGs have modulatory, supportive, and/or complementary functions to HSPGs. However, the relationship between CS and HS functions is unknown. Using a glycoproteomic approach, we found that Drosophila Windpipe (Wdp) is a novel CSPG. Wdp encodes a transmembrane protein with leucine-rich repeat motifs. Overexpression of Wdp in the wing disc narrowed the expression domains of both high- and low-threshold targets of Hh signaling. In contrast, knockdown of Wdp by RNAi up-regulated Hh signaling. When specific Ser residues for CS attachment were substituted with Ala, it abolished the ability of Wdp to inhibit Hh signaling, indicating that Wdp regulates Hh signaling via CS chains. Furthermore, we found that Wdp knockdown promotes cell surface accumulation of Smoothened (Smo), suggesting that Wdp negatively regulates Hh signaling by destabilizing Smo protein. Taken together, our findings uncover a novel regulatory mechanism of Hh signaling by a CSPG. This study is supported by a grant from the National Institute of Health (R01 GM115099).

**Program Abstract #424**

**Progress in evaluating an allele that eliminates all BMP R-Smad function in Drosophila melanogaster using CRISPR-Cas9 technology**

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Cell communication via paracrine signaling is a recurring theme that continues throughout development in all organs of metazoans. Patterning of developmental fields heavily relies on competence to respond to paracrine signals for proper regulation of gene expression. Bone morphogenetic protein (BMP) signaling is a conserved cell signaling network composed of ligands, belonging to the TGF-beta superfamily of proteins, which bind to a complex of conserved serine-threonine receptors and directly activate the signal-transducing transcription
factors, Smads. BMP signaling is observed throughout development to direct complex cell behaviors. More specifically, this signaling pathway directs dorsal-ventral patterning of the *Drosophila melanogaster* embryonic ectoderm, and later patterning of the subdivision of the midgut endoderm. In *Drosophila*, the receptor regulated Smad (R-Smad), Mad, is responsible for the transduction of the signal from the membrane into the nucleus. Many developmental studies have employed the best characterized loss-of-function alleles, *Mad[10]* and *Mad[12]*. However, recent evidence raises the possibility that Mad may also be involved in Wnt signaling, in part based on a comparison of *Mad RNAi* phenotypes with *Mad[10]* and *Mad[12]* (Eivers et al., Sci. Signal. (2011), 4(194) ra68). To test for this and other unexplored functions of Mad, we have generated a null allele that lacks the complete coding region of *Mad* using CRISPR-Cas9. Thus far, the deletion of this null allele has been verified. Currently, we are in the process of comparing this null allele with *Mad[10]* and *Mad[12]* to determine any morphological differences in the embryonic phenotype, and assess whether they are independent of BMP ligand signals. Future studies will employ this allele in our ongoing studies of somatic follicle cell patterning during oogenesis, which directs eggshell morphogenesis. Portions of this work are funded by NSF 1355091 (LAR).

**Program Abstract #425**  
The cell-type specific functions of an ER modulating factor, Pecanex in Notch and Wnt signaling pathways  
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Notch signaling is an evolutionarily conserved mechanism that regulates a broad spectrum of cell-specification through local cell-cell interactions. *Drosophila pecanex (pcx)* encodes an evolutionarily conserved multi-pass transmembrane protein. We previously suggested that *pcx* is required for the normal functions of endoplasmic reticulum, which is essential for Notch signaling activation in the central nervous system development in *Drosophila*. However, the functions of *pcx* in *Drosophila* development have not been studied well. Therefore, we examined various cell-fate decisions, which are known to require Notch signaling, in *pcx* mutant embryos. We found that *pcx* mutant embryos showed abnormalities in the tracheal system, which is different from *Notch* mutants, suggesting that *pcx* is required for the tracheal development in parallel to Notch signaling. However, *pcx* was not required for Notch signaling activation in the development of the embryonic hindgut and proventriculus. Furthermore, the visceral mesodermal cells were missing in *pcx* mutant embryos, whereas these cells increased in *Notch* mutant embryos. The phenotypes of the visceral mesodermal cells were reminiscent of the phenotypes associated with the loss of Wnt signaling. Therefore, unexpectedly, *pcx* may be required for Wnt signaling in these cells. This hypothesis is supported by the facts that hyperactivation of Notch failed to rescue the disruption of visceral mesoderm in *pcx* mutant embryos, however overexpressing dTCF, an active form of transcription factor of Wnt signaling rescued. Based on the results, we concluded that *pcx* requirement for Notch signaling is context-dependent. Furthermore, *pcx* is also involved in Wnt signaling pathway in a context-dependent manner.

**Program Abstract #426**  
WNT/β-catenin signaling dynamics are transient in human pluripotent cells, sustained following differentiation, and modulated by TGFβ and BMP signaling  
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Despite its importance for both normal development and cancer progression, little is known about the dynamics of Wnt pathway activity in response to ligand stimulation. Specifically, what is the relationship between the dynamics of signaling input (the Wnt ligand), signal transduction by the transcription factor β-catenin, the resulting gene activation profiles, and cell-fate specification? To test these relationships we used CRISPR-CAS9 genome engineering to create a fusion protein of GFP and β-catenin, at the endogenous locus, in human embryonic stem cells (hESCs). We show that in hESCs the addition of Wnt ligand initially results in a rapid accumulation of both membrane-bound β-catenin and nuclear β-catenin. However, while the membrane-bound β-catenin is sustained when cells are continuously exposed to Wnt ligands, both nuclear β-catenin and Wnt-target gene expression begin to decline after approximately 3 hours of stimulation (i.e., signaling adapts to constant
WNT signals). In contrast to hESCs, many other cell-types, including primitive-streak differentiated hESCs, show non-adaptive dynamics. Finally, we show evidence that both TGFβ and BMP signaling increase WNT/β-catenin signaling throughput by repressing adaptation to WNT signals. These findings reveal that while WNT/β-catenin dynamics are initially adaptive in pluripotent cells, they dramatically shift during and after differentiation, and thus highlight a previously unreported aspect of WNT/β-catenin signaling.

Program Abstract #427
Direct visualization of a native Wnt in vivo reveals that a long-range Wnt gradient forms by extracellular dispersal
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Wnts are evolutionarily conserved signaling proteins with essential roles in development, homeostasis, and disease. In many contexts, Wnts are thought to move between cells and signal at long ranges. However, understanding fundamental aspects of how Wnts move between cells has been limited by challenges with directly observing endogenous ligands in vivo. As a result, the extent to which extracellular Wnt dispersal occurs, and is biologically relevant, is debated. Here, we have generated functional, fluorescently tagged alleles of a C. elegans Wnt homolog that has long-range signaling roles. This has made it possible for the first time to visualize a native Wnt gradient in a living animal. Live imaging of endogenous Wnt in concert with the architectures of source and responding cells provided evidence for extracellular Wnt spreading. Fluorescence recovery after photobleaching experiments showed rapid Wnt movement between cells. By experimentally limiting Wnt transfer between cells using an extracellularly tethered nanobody, we demonstrated that extracellular dispersal generates a long-range gradient along the anteroposterior body axis and is critical for neuroblast migration. Our results demonstrate that native Wnts can spread extracellularly across multiple cells, and that such spreading is important to regulate aspects of long-range signaling in vivo. Funding: NIH R01 GM083071 (B.G.); NIH F32 GM115151 (A.M.P.); American Cancer Society PF-16-030 DDC (A.M.P.).

Program Abstract #428
Retromer-associated proteins and PAD-1 control TAT-5 flippase activity to inhibit extracellular vesicle budding
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Cells from bacteria to man release extracellular vesicles (EV) that carry signaling molecules like morphogens and miRNAs to control intercellular communication and mediate animal development. EV release also sculpts membranes, e.g. repairing damaged membranes to avoid cell death. However, the in vivo functions of EVs are debated because the molecules known to govern their formation are shared with other membrane trafficking pathways. Furthermore, the specific mechanisms of EV release by plasma membrane budding (ectocytosis) are poorly understood. We previously showed that the conserved phospholipid flippase TAT-5 maintains the asymmetric localization of the lipid phosphatidylethanolamine (PE) in the plasma membrane to inhibit ectocytosis in C. elegans. However, no proteins that inhibit ectocytosis upstream of TAT-5 were known. Here, we identify conserved TAT-5 regulators associated with the retromer recycling pathway (PI3Kinase VPS-34, Beclin1 homolog BEC-1, and DnaJ protein RME-8) together with the uncharacterized Dopey homolog PAD-1. PI3Kinase, RME-8, and specific sorting nexins are required for the plasma membrane localization of TAT-5, which is important to maintain PE asymmetry and inhibit ectocytosis. PAD-1 does not directly regulate TAT-5 localization, but is required for the lipid flipping activity of TAT-5. PAD-1 also has roles in endosomal trafficking with the GEF-like protein MON-2, which regulates PE asymmetry and ectocytosis redundantly with sorting nexins. Thus, our work pinpoints TAT-5 and PE as key regulators of plasma membrane budding, further supporting the model that PE externalization drives ectocytosis. In addition, we uncovered redundant intracellular trafficking pathways and revealed new regulators of TAT-5 flippase activity. The new proteins we identified that regulate EV budding by ectocytosis
provide a toolkit to ascertain the in vivo roles of EV-mediated cell-cell signaling. Funded by DFG grant WE5719/2-1.

Program Abstract #429
Distinct Signaling Roles for Type I Receptors within a BMP Heterodimer 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 signal transduction 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 early vertebrate embryonic development. Our previous findings demonstrate that BMP2/7 heterodimers are the only ligands that signal in DV patterning. This sufficiency arises from the the unique ability of the heterodimer to integrate both type I receptor classes into the BMP receptor complex, as Bmpr1 preferentially binds the BMP2 ligand, and Acvr1l binds BMP7. We have also found that kinase dead Acvr1l cannot rescue acvr1l deficiency, while, surprisingly, kinase dead Bmpr1 can rescue bmp1 deficiency. Through truncation experiments we found that, while Bmpr1 kinase function is unnecessary, the Bmpr1 intracellular domain is required for DV patterning. Additional domain swap experiments suggest that the intracellular domains of Bmpr1 and Acvr1l are not interchangeable, and play separate functional roles in BMP signaling. I am now determining the location and nature of the motifs within Bmpr1 and Acvr1l that impart these specific functions through a series of smaller domain swap experiments.

Program Abstract #430
SEMINAL: Behavioral, Biochemical and Genetic Approaches to Identify Male Derived Proteins that Maintain Postmating Responses in Drosophila Females
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The seminal fluid proteins (Sfp) made by male flies are responsible for inducing several post-mating responses in females. One of these Sfps, sex peptide (SP), has been shown to play a major role in several post mating responses, namely — stimulating egg laying and decreasing receptivity (meaning making the female less likely to mate again). However, it has been shown that the long-term functionality of sex peptide is dependent on the protein binding to sperm and that this event requires a complex network of Sfps, female reproductive tract proteins and sperm proteins. This project has served to expand the currently defined SP network and narrow the list of possible candidate genes. Previous computational and proteomics screens by our lab identified ~40 additional candidate Sfp or sperm proteins for involvement in the SP network. To test whether these proteins were part of the network, we knocked them down individually, using RNAi driven by GAL4/UAS in males. We then mated knockdown males to wild-type females, and tested for activity and retention of SP in the females by measuring post-mating responses correlated with successful SP-sperm binding (receptivity and egg laying), and by examining the presence of SP by Western blotting. For knockdowns that exhibited aberrant SP behavior, we tried to identify the role these proteins played in the SP network by testing for their effects on the presence, absence and/or stability of previously identified members of the SP network using western blots. Two proteins affected SP retention: CG15198, whose knockdown males make very few sperm, few to none of which get stored by females, and CG5458, whose knockdown prevents sperm transfer to females. The knockdown phenotypes indicate that the effect of these two genes on SP retention is via their effects on sperm or sperm storage. More research into the role of the other identified proteins is currently under way.
Program Abstract #431
Investigation of eRpL22like function in *Drosophila melanogaster* eye development through consequences on EGF signaling
Caroline Pritchard, Brett W. Gershman, Vassie C. Ware
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The *Drosophila melanogaster* eRpL22 ribosomal protein family contains two structurally similar paralogs: eRpL22 and eRpL22like. eRpL22like exhibits tissue-specific patterns of expression restricted to the eye and testes, when compared to the ubiquitously expressed eRpL22. Co-localization comparison of eRpL22like and core ribosomal components indicate that eRpL22like may have roles both within ribosomes and apart from ribosomal processes. Mosaic disruption of eRpL22like showed 5-8 ectopic cone cells instead of the typical 4 in the midpupal eye. This phenotype is characteristic of epidermal growth factor receptor (EGFR) pathway over-activation. Overexpression of downstream EGFR pathway component Ras results in eye ommatidia fusions. Ras overexpression alone does not result in the ectopic cone cell phenotype, indicating that eRpL22like must act upstream of Ras within the EGFR pathway. Immunohistochemistry analysis of third instar eye discs attempts to characterize etiology of this interaction in the developing eye. Algorithmic-based image analysis revealed a conditionally proportional correlation between eRPL22like-disrupted ommatidia and EGFR expression. These preliminary findings suggest a functionally divergent role of eRpL22like from eRpL22, supporting the idea that ribosomal proteins confer or possess extra-ribosomal functions, or alternatively, indicating ribosomes themselves are more complex than previously thought. Funding: Lehigh University Dept. of Biological Sciences Undergraduate Research Grant

Program Abstract #432
Regulation of adult lipid homeostasis by *Drosophila* Estrogen-Related Receptor.
Katherine Beebe, Michael Horner, Carl Thummel
University of Utah, USA
Nuclear receptors are a large family of evolutionarily conserved transcription factors that play central roles in development, growth and metabolism. Three paralogs make up the Estrogen-Related Receptor (ERR) family in vertebrates: ERRα, ERRβ, and ERRγ. Although ERRα is necessary for lipid homeostasis in mammals, neither the tissue-specific nor mechanistic basis of this phenotype is well understood. Previous work from the Thummel lab has demonstrated that the *Drosophila* member of this family, dERR, establishes a glycolytic metabolic state that supports larval growth. In contrast, adult *Drosophila* physiology does not involve growth or biomass accumulation, but rather requires efficient oxidative metabolism and ATP production to support the demands of flight and reproduction. We thus engineered a conditional allele of dERR to eliminate its function selectively during the adult stage with the goal of identifying possible new functions for this receptor. dERR mutant adults display reduced fertility and motility, mild hyperglycemia, decreased protein, and an almost complete lack of stored triglycerides. Consistent with this, dERR mutants are sensitive to starvation. RNA-seq and ChIP-seq based analyses reveal a central role for dERR in regulating metabolism, including glycolysis and lipid metabolism, as well as distinct profiles of dERR-regulated transcripts between larval and adult stages. Our current studies are focused on determining the molecular mechanisms and tissue-specific functions by which dERR maintains proper lipid stores for adult metabolic homeostasis. This research (KB) is supported by the NIH under Ruth L. Kirschstein NRSA 1F32DK11687-01 from the NIDDK and a grant from the NIH (R01 DK075607).

Program Abstract #433
Intracellular attenuation of BMP signaling via CKIP-1/Smurf1 is essential during neural crest induction
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The neural crest is a multipotent and migratory cell population that contributes to diverse structures including the peripheral nervous system and the craniofacial skeleton. Neural crest induction occurs at the neural plate border downstream of signaling inputs including FGFs, BMPs, and Wnts. While intermediate BMP levels were proposed as critical for neural crest induction in frog and zebrafish, secreted BMP antagonists appear to be dispensable in
chick, raising the question of how the required intermediate BMP levels are generated. Here, we propose a morphogen model where intracellular attenuation of BMP signaling sets the required intermediate levels to maintain neural crest induction. We show that the scaffold protein CKIP-1, and ubiquitin ligase Smurf1, are co-expressed with BMP4 at the chick neural plate border. Knockdown of CKIP-1 during a critical period between gastrulation and neurulation provoked loss of neural crest cells, while Smurf1 knockdown increased neural crest numbers. At a mechanistic level, we find that CKIP-1 and Smurf1 modulate BMP signaling upstream of pSmad1/5/8 accumulation and transcriptional activity, thereby facilitating a dose-dependent response to BMP signals. Through biochemical and epistasis experiments, we discovered that Smurf1 targets receptor Smads for degradation, and CKIP-1 acts to promote Smurf1 autodegradation. Together these results support a model in which CKIP-1 suppresses Smurf1-mediated degradation of Smads, thereby uncovering a novel intracellular mechanism to attenuate BMP signaling to the intermediate levels required to maintain neural crest. Our new model suggests that neural plate border cells “fine-tune” BMP signaling autonomously and reconciles discrepant results on the roles of secreted BMP antagonists from adjacent tissues during neural crest induction. This work was supported by NIH grants F32 HD088022, R01 DE024157 and P01 HD037105.

Program Abstract #434
The Hippo Pathway Prevents YAP/TAZ-Driven Hypertranscription and is Essential for Neural Progenitor Survival
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The Hippo pathway controls the activity of YAP and TAZ transcriptional coactivators through a kinase cascade. Despite the critical role of this pathway in tissue growth and tumorigenesis, it is not fully understood how YAP/TAZ–mediated transcription drives cell proliferation. By analyzing the effects of inactivating LATS1 and LATS2 kinases, the direct upstream inhibitors of YAP/TAZ, on mouse brain development and applying cell-number–normalized transcriptome analysis, we discovered that YAP/TAZ activation causes a global increase in transcription activity, known as hypertranscription, and upregulates many genes associated with increased biosynthetic capacity and proliferation. In contrast, conventional read-depth–normalized RNA sequencing analysis fails to detect the scope of the transcriptome shift and misses most relevant gene ontologies. Hypertranscription in neural progenitor cells triggers DNA replication stress, DNA damage, p53 activation, resulting in massive apoptosis. Our findings reveal the remarkable impact of YAP/TAZ activation on global transcription activity and have important implications for understanding YAP/TAZ function. Funding: R01NS086938 and American Lebanese Syrian Associated Charities (ALSAC).

Program Abstract #435
FGF signaling mechanisms in midface development
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Facial bones are derived primarily from neural crest cells (NCCs), and disruption of their development leads to craniofacial malformations such as cleft lip and cleft palate. We have investigated intracellular signaling mechanisms initiated downstream of FGF-FGFR that play important roles during midface morphogenesis. Our results indicate that in the mouse, conditional deletion of both Fgfr1/2 in NCCs lead to agenesis of multiple midface structures including the mandible, maxilla, frontal bone and nasal cartilage. Interestingly, FGFR1 and FGFR2 primarily regulate localized cell survival in facial prominences that eventually lead to midfacial defects. FGFs are mainly thought to signal through ERK1/2, but the role of other signaling pathways engaged by the receptors have not been well established. To clarify the role of FGF-FGFR downstream signaling pathways, we have introduced point mutations at the Fgfr1 and Fgfr2 loci that prevent interactions of the receptors with the intracellular mediators FRS2, CRK/CRKL, PLCγ/SHB and GRB14, individually or in combination. This gives us an opportunity to interrogate pERK, pAKT or phospholipase signaling in mediating FGF responses. Our analysis of the signaling mutants suggest that while much of the signaling downstream of Fgfr1 and Fgfr2 operates through FRS2
and ERK1/2, context dependent roles for other signaling pathways could also be identified. Taken together, our studies highlight the complexities of FGF signaling and define mechanisms underlying mid-face development.

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Program Abstract #436
RSPO2 potentiates WNT9b-induced WNT/β-catenin signaling by an LGR4 family receptor-independent mechanism during mouse facial development
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The R-spondin (RSPO) family proteins potentiate WNT/β-catenin signaling and may provide a fine-tuning mechanism for controlling the strength of WNT signaling. Although a vast of in vitro studies clearly demonstrate the potentiation of WNT signaling by RSPOs, it still remains unknown whether this regulation indeed acts as a major mechanism for WNT signaling in normal development and tissue function in vivo. Here, we analyzed compound Wnt9b and Rspo2 gene knockout mice and found convincing evidence showing that potentiation of WNT signaling by RSPO2 plays a major role during mouse facial development. Mice lacking both the Wnt9b and Rspo2 genes display more exacerbated deficits in both upper and lower jaws at birth than those observed in either Wnt9b or Rspo2 gene null mutants. These deficits are preceded by synergistic inhibition of WNT/β-catenin signaling and its target gene expression within the facial processes of double mutants at the earlier embryonic stages. Using the facial primordial explant cultures, we further demonstrate that the recombinant RSPO2 protein potentiates WNT9b-induced β-catenin signaling. Interestingly, mice lacking each of the Lgr4 family of the RSPO receptor genes do not show any of the facial defects observed in Rspo2 and Wnt9b mutant mice. The mutant recombinant RSPO2 protein, unable to bind to the LGR4 family receptors, effectively potentiates WNT9b-induced β-catenin signaling in cultured facial explants. This suggests a dispensable role of the LGR4 family receptors in the potentiation of WNT signaling by RSPO2 during facial development. Taken together, our findings provide the first direct evidence that potentiation of WNT/β-catenin signaling by RSPO represents a key regulatory mechanism for WNT/β-catenin signaling in vivo during mouse facial development. This study is supported by 2016R1A2B4012956 (KNRF), 2016K1A4A3914725 (KNRF) and R01AR055278 (NIAMS/NIH).

Program Abstract #437
MicroRNA suppression of Dishevelled results in Wnt pathway associated developmental defects
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MicroRNAs (miRNAs) are highly conserved, small non-coding RNAs that regulate gene expressions by binding to the 3’untranslated region (UTR) of target mRNAs and silence translation. MiRNAs are key regulators of the Wnt signaling pathways which impact cell proliferation, migration, polarity and other developmental processes. This study investigates miRNA regulation of different isoforms of Dishevelled (Dsh/Dvl), an important signaling protein upstream of β-catenin. The sea urchin Dvl mRNA isoforms have similar spatial localization in early development, but one isoform has distinct ciliary staining in the larval stage. Using luciferase assays and site-directed mutagenesis, we demonstrated that the different isoforms of Dvl are directly suppressed by miRNAs. By blocking miRNA suppression of all Dvl isoforms using miRNA morpholino antisense oligonucleotides target protectors (miRNA MASO TPs), we observed dose-dependent defects of spicule length, patterning of the primary mesenchyme cells, gut morphology and aberrant cilia length and arrangement. These defects likely result from increased Dvl protein, leading to perturbation of Wnt dependent signaling pathways and additional Dvl-mediated processes. Overall, our results indicate that miRNA suppression of Dvl isoforms plays an important role in ensuring proper early development. NSF CAREER Award # IOS-1553338 (2016-2021); University of Delaware travel awards (up to $800)
Program Abstract #438
Elucidating the function of RAPGEF5 in beta-catenin nuclear translocation
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Wnt signaling plays critical roles in both embryonic development and adult tissue homeostasis. Wnt activation leads to stabilization of β-catenin in the cytoplasm and enables its subsequent nuclear entry and Wnt target activation. To date, a mechanism by which beta-catenin, a key effector molecule for Wnt signaling, translocates into the nucleus is not clearly defined. Previously, in a patient with heterotaxy, a disorder of right-left patterning that can lead to a severe form of CHD, we identified the guanine nucleotide exchange factor, RAPGEF5 as a candidate gene. Our work demonstrates that RAPGEF5 affects LR patterning via regulating the nuclear entry of β-catenin. Furthermore, our results suggest that Rapgef5 regulates the nuclear localization of β-catenin independently of NLS mediated nuclear transport machinery. Based on these data, we hypothesize that Rapgef5 maintains nuclear Rap protein(s) in an active GTP bound state, which will preferentially import and/or retain β-catenin in the nucleus. The overarching goal of this proposal is to determine if Rapgef5 regulates β-catenin nuclear import and/or export by measuring transport kinetics and to determine specifically which Raps regulate this process. Aberrant activation of Wnt/β-catenin signaling is implicated in multiple congenital anomalies including CHD as well as cancer; therefore, a better understanding of the β-catenin nuclear transport mechanism can be exploited to develop novel therapeutics that acts downstream in the Wnt/β-catenin pathway. Yale Cellular and Molecular Biology Training program (T32GM007223)

Program Abstract #439
Synaptic plasticity is altered in dentate gyrus of hippocampus during postnatal development in AEP knockout mice
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Asparagine endopeptidase (AEP) is a lysosomal cysteine protease that cleaves protein substrates on the C-terminal side of asparagine. It participates in various pathologic processes, such as cancer, immunity and neurological diseases. It has been verified that AEP is activated in an age-dependent manner and involved in process of neurodegeneration. In this study, we investigated the connection between AEP and synaptic plasticity of hippocampus that plays a critical role in mediating several higher brain functions including learning, memory, and spatial coding during development. The levels of N-methyl-D-aspartate receptors (NR2A, NR2B), postsynaptic density protein 95 (PSD 95) and synaptophysin (SYP) in hippocampus of postnatal days (P1, P7, P14, P21, P28 and P56) were examined by Western blot assay. The results indicated that the synapse-associated proteins all increased from P1 to P14, and then decreased and maintained to the adult levels in both wild type and AEP knockout mice. However, levels of the four proteins were increased in AEP knockout mice compared with those in WT mice. Golgi-Cox staining revealed that the densities of apical dendritic spines in dentate gyrus were increased in adult AEP knockout mice compared with those in WT mice. Whole-cell patch-clamp recording displayed that the frequency rather than the amplitude of spontaneous miniature excitatory postsynaptic currents (mEPSCs) was enhanced in dentate gyrus region of adult AEP knockout mice. We propose that synaptic plasticity is altered at the adult age AEP knockout mice, and this phenomenon may relate to the increases of synapse-associated proteins and spine density.

Program Abstract #440
Are all disruptors of neural tube closure created equal? Comparing redox potential changes and induced oxidative stress after Fumonisin B1, Valproic Acid, and Ceramide exposure in chicken embryos
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During the very early stages of human embryonic development, the neural plate bends and folds to create the closed, cylinder-like neural tube. This process of neurulation is necessary for proper brain and spinal cord
development at later stages, while improper closure of the neural tube results in neural tube defects (NTDs) such as anencephaly, spina bifida, and craniorachischisis. More than 300,000 babies worldwide are born with NTDs each year, and while folate supplementation has significantly reduced the NTD rate, NTDs are still a major pediatric health concern. Maternal health, environmental toxicants, and genetic mutations are all likely factors. Our aim is to understand the effect of three developmental toxicants shown to cause NTDs: Fumonisin B1 (FB1), valproic acid (VPA), and ceramide (Cer). Because oxidative stress (OS) has been linked to abnormal neurulation in animal models of diabetes, and cellular redox state is an important regulator of many processes (i.e. gene regulation, protein function, cell viability, proliferation and differentiation), our approach assessed tissue redox state and OS in chick embryos exposed to FB1, VPA or Cer. We employed glutathione (GSH) redox (Eh) potential analysis to evaluate the effect of each toxicant on tissue redox state in developing chick embryos, while also using a P19 neuronal precursor cell line as a tissue culture model. Additionally, we have analyzed reactive oxygen species generation, apoptosis, and protein expression to determine whether these developmental toxicants act through the same, similar, or distinct mechanisms to induce NTDs. This approach allows for the comparison of multiple NTD-inducing toxicants, with the hope of revealing their mechanisms and effective prevention methods. We show unique GSH Eh profiles for embryos exposed to FB1, VPA, and Cer, and report data on other readouts. The authors wish to acknowledge and thank the BYU Undergraduate Mentoring Grant for funding this project.

Program Abstract #441
A population of phox2b-expressing cells arises independently of pre-existing enteric neuronal precursors in the post-colonized intestine, as revealed by in vivo time-lapse imaging of zebrafish embryos
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Background: The enteric nervous system (ENS) derives largely from the vagal neural crest; however, some avian and rodent studies demonstrate contributions from the sacral and thoracic crest. The dynamics of extra-vagal crest contributors are incompletely understood, partly due to limitations performing in vivo analysis of ENS development. Here, we use zebrafish to perform live time-lapse imaging combined with photoconversion technology to assess extra-vagal crest contributors to the ENS. Methods: -8.3phox2b:kaede transgenic zebrafish mark phox2b+ cells with kaede protein, which fluoresces green at baseline but shifts red after exposure to 405nm wavelength light. 4.5 days post fertilization (dpf), all phox2bkaede cells along the entire gut were photoconverted. Then, a 10 hour time-lapse was undertaken with z-stacks collected every 4 minutes. Experiments were performed with the LSM 800 confocal microscope. Results: Pre-conversion, phox2bkaede cells populated the length of the gut and exhibited only green fluorescence. Post-conversion, green fluorescence was weak-to-absent in phox2bkaede cells and was replaced with robust red fluorescence; there were no photobleached cells. After the time-lapse, all converted cells still expressed red fluorescence. Many red phox2bkaede cells were migratory and made contact with neighboring cells. Interestingly, multiple green phox2bkaede cells arose de novo, initially expressing weak green fluorescence that gradually intensified. These de novo cells had no red signal, were not associated with pre-existing red phox2bkaede cells, and were observed mostly in the distal midgut and hindgut. Conclusion: These results suggest de novo appearance of phox2b cells in the zebrafish intestine after vagal crest colonization. As enteric neural precursors express phox2b, we suspect these de novo cells do not originate from resident enteric neural precursors but may represent an extra-vagal crest source of ENS elements. Funding: UCLA STAR Program

Program Abstract #442
Developmental Exposure to Bisphenol-A Causes Axon Outgrowth Defects in Drosophila melanogaster
Brendan Tinsley, Yomira Palacios, Lilly Murphy, Chloe Welch, Roy Mashburn, Kimberly Mulligan
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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that affects 1 in 68 children in the United States. Hundreds of genes have been linked to ASD, but evidence indicates that environmental factors also impact ASD pathophysiology. Identification of environmental factors that confer risk of ASD is critical to both improve our understanding of ASD etiology and to enact necessary preventative measures. Studies have linked
the endocrine-disrupting chemical bisphenol-A (BPA) to impaired neural development. BPA is a high-production chemical (over 6 billion tons are produced each year) commonly used in the production of plastics and epoxy resins; it is found in a vast number of products ranging from baby bottles to thermal receipt paper. The structure of BPA allows it to easily cross the placental barrier and fetal blood brain barrier. Once the scientific community elucidated the dangers of BPA-associated endocrine-disruption, many chemical companies replaced BPA with BPA analogues, including bisphenol-F (BPF) and bisphenol-S (BPS). While there has been less analysis of BPF and BPS, recent work using cultured cells and zebrafish indicate that BPS may impair neurogenesis. The overarching aim of our research is to determine how BPA and its analogues impair neurodevelopment by assessing cellular phenotypes relevant to ASD using Drosophila melanogaster as a model organism. Here, we exposed wild-type (w1118) Drosophila to BPA during embryonic and larval development. We then used immunohistochemistry and confocal microscopy to examine axon outgrowth in the adult brain. We found that millimolar concentrations of BPA cause midline crossing defects in fasciculating axons of the mushroom body, an adult neural structure required for higher sensory integration. We are currently investigating if BPF and BPS phenocopy BPA. Funding: California State University Research and Creative Activities Award and Goethe Research Award.

Program Abstract #443
Exposure to the Environmental Neurotoxicant Polychlorinated Biphenyl-95 Phenocopies a Common Autism Risk Gene in Drosophila melanogaster
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The interaction of environmental chemicals with specific genetic susceptibilities is linked to autism spectrum disorder (ASD). Yet, identification of specific environmental chemicals that interact with genes to cause ASD remains a critical gap in our understanding of ASD etiology. Given that there are over 80,000 chemicals in use with little to no toxicological data, the field urgently needs an efficient method for chemical analysis. This project involves the development of assays using Drosophila melanogaster for identification of chemicals that molecularly converge with fmr1 (fragile X mental retardation 1). Mutations in FMR1 are the most common monogenic cause of ASD, and the role FMR1 plays in neural development is conserved from flies to vertebrates. Data from vertebrate model organisms suggest that gestational exposure to the environmental neurotoxicant, polychlorinated biphenyl 95 (PCB-95), can increase the risk of ASD. Loss of Drosophila fmr1 causes a decreased courtship index (CI; a quantitative measure of innate courtship behavior) and impaired axon guidance in the adult brain. We exposed developing fruit flies to PCB-95 and used the courtship assay to determine that exposure to nanomolar concentrations of PCB-95 significantly decreases the CI in wild-type (wt) flies in a dose-dependent manner, and exhibits a synergistic reduction in the CI of fmr1 flies. We used immunohistochemistry and confocal microscopy to examine adult brains and found a dose-dependent increase in axon-pathfinding defects in the mushroom body (MB) of wt flies, which is the same phenotype caused by loss of fmr1. This finding suggests that PCB-95 may confer increased risk of ASD in individuals with FMR1 mutations—they affect the similar neurodevelopmental processes indicating potential molecular convergence in developing neurons. Funding: California State University Program for Education and Research in Biology (CSUPERB) New Investigator Award.

Program Abstract #444
Transcriptional regulation of intrinsic neuronal polarity and neurite outgrowth by Pax3/7
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Tunicates are the sister group to the vertebrates, meaning they are our closest living invertebrate relatives. The recently mapped connectome of the larva of the tunicate Ciona intestinalis, identified the synaptic connectivity of all 177 neurons in the central nervous system and all 54 peripheral sensory neurons. These 231 total neurons form one of the smallest nervous systems ever described, even smaller than that of C. elegans (302 neurons). Thus, this minimal connectome offers a singular opportunity to understand conserved mechanisms of chordate
nervous system function and development. We are studying neuronal differentiation and morphogenesis in the *Ciona* homolog of Mauthner cells (M-cells), a single pair of contralaterally-projecting hindbrain neurons that are central to conserved escape response circuits in vertebrates. We previously showed that *Ciona* M-cells are specified by the transcription factor Pax3/7, and that ectopic Pax3/7 expression results in supernumerary contralaterally-projecting M-cells. Here we show that this intrinsic, Pax3/7-dependent axon projection is established early in differentiation, when cell polarity inverts 180° and the nascent axon extends medially, towards the neural tube lumen. Transcriptome profiling of differentiating M-cells and neural progenitors expressing ectopic Pax3/7 revealed the upregulation of genes encoding chemoattractant, extracellular matrix, and intracellular adapter proteins. Their expression by the M-cells themselves may underlie a conserved mechanism for self-determination of neuronal polarity and neurite outgrowth, which we have begun to test by knocking out these targets using tissue-specific CRISPR/Cas9. Funding: NIH/NICHD

**Program Abstract #445**

**Regulation of the cell cycle and cell fate by TGFbeta signaling in larval zebrafish retina**

*Jenny Lenkowski, Elizabeth Hannifin, Sarah Vogel, Michael Koropsak, Edna Ferreira, Maeve Downey, Katie Brandt, Husni Alasadi*

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Several studies indicate that the TGFbeta signaling pathway plays an important role in regulating proliferation of progenitors and cell differentiation during mammalian retinal development and zebrafish retinal regeneration. Specifically, TGFbeta signaling has been shown to promote Müller glial cell fate, inhibit proliferation, and increase differentiation into photoreceptors during rodent retinal development. We are using chemical genetics and genetic approaches in zebrafish to manipulate TGFbeta signaling in order to study how the signaling pathway regulates the cell cycle and cell differentiation during retinal development in fish, which has not yet been described. Our preliminary studies of retinal development in zebrafish using fluorescence immunohistochemistry indicate that the ciliary marginal zone where stem cells reside in the mature retina may have fewer proliferating cells and the retina may have fewer Müller glial cells when TGFbeta is upregulated. Ongoing experiments are aimed at generating a new transgenic fish in which to study cell cycle dynamics in vivo and to examine cell-cycle exit in the developing retina when TGFbeta signaling is misregulated. Funding from the NIH (1R15EY027124, Lenkowski) and Goucher College Summer Research Program.

**Program Abstract #446**

**Forebrain control of behaviorally-driven social orienting**

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Deficits in social engagement are diagnostic of multiple neurodevelopmental disorders, including autism and schizophrenia. Genetically tractable animal models like zebrafish (*Danio rerio*) could provide valuable insight into developmental factors underlying these social impairments, but this approach is predicated on the ability to accurately and reliably quantify subtle behavioral changes. Similarly, characterizing local molecular and morphological phenotypes requires knowledge of the neuroanatomical correlates of social behavior. We leveraged behavioral and genetic tools in zebrafish to both refine our understanding of social behavior and identify brain regions important for driving it. We characterized visual social interactions between pairs of adult zebrafish, and discovered that they perform a stereotyped orienting behavior that reflects social attention. Furthermore, in pairs of fish, the orienting behavior of one individual is the primary factor driving the same behavior in the other individual. We used manual and genetic lesions to investigate the forebrain contribution to this behavior and identified a population of neurons in the ventral telencephalon whose ablation suppresses social interactions, while sparing other locomotor and visual behaviors. These neurons are cholinergic and express the transcription factor Lhx8a, which is required for development of cholinergic neurons in the mouse forebrain. The neuronal population identified in zebrafish lies in a region homologous to mammalian forebrain regions.
implicated in social behavior such as the lateral septum. Our data suggest that an evolutionarily conserved population of neurons controls social orienting in zebrafish.

Program Abstract #447
Basigin regulates perineurial glia and nerve morphology in the Drosophila peripheral nervous system
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The perineurial glia (PG) that ensheath the peripheral nervous system are responsible for maintaining the structure and function of the nerves through their interaction with the overlying extracellular matrix (ECM). Basigin is a highly conserved transmembrane protein which is expressed in the PG as well as the axons and neuromuscular junctions, but its role in nervous system function has not been elucidated. Using Drosophila melanogaster as a model system, we assessed the developmental effects of knocking down Basigin in the glia. Basigin was knocked down in the PG using RNA interference, which caused morphological defects in the peripheral nerves. Loss of Basigin causes the PG and overlying ECM to become constricted, and disrupts the actomyosin cytoskeleton of the PG cells. Basigin has been shown to interact with integrins in both vertebrate and invertebrate models, and is found in close proximity to integrin in the PG cells. Our preliminary data shows that knockdown of Basigin in the background of a loss-of-function integrin mutation rescues the glial phenotype. Basigin has also been shown to form a complex with the plasma membrane calcium transporter PMCA in vertebrates and is essential for its membrane localization. We have now shown that Basigin is essential for PMCA localization in the Drosophila nervous system, and that simultaneous loss-of-function mutations in Basigin (preventing PMCA localization) and the endoplasmic reticulum calcium transporter SERCA cause severe defects in nerve morphology. Basigin has been extensively studied with regards to its role in cancer metastasis but its role in the nervous system remains unknown. The glia which surround the peripheral nervous system are essential for upholding the structure and function of the nerves, and Basigin has now been shown to play an essential role in maintaining the integrity of the glial sheath as well as the nerve structure. Funding for this project is provided by NSERC and CIHR.

Program Abstract #448
The Autism-Associated Chromatin Modifier, Chromodomain Helicase DNA Binding Protein 8, Affects Axon Guidance and Behavioral Phenotypes in Drosophila melanogaster
Chloe Welch, Lillian Murphy, Alain Hu, Any Ardon-Castro, Darren Nguyen, Amy Lew, Kimberly Mulligan, PhD
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Autism spectrum disorder (ASD) is a multifactorial neurodevelopmental disorder that afflicts 1 in 68 children in the United States. Recent genomic studies have identified recurrent mutations within specific genes, providing insight into the complex genetic etiologies of this disorder. Mutations in Chromodomain Helicase DNA Binding Protein 8 (CHD8) are the most common de novo mutations associated with ASD. CHD8 is a chromatin modifier that influences the transcription of many other ASD-risk genes—it is a master regulator that defines a common ASD subtype, characterized by macrocephaly and gastrointestinal (GI) problems. Our research is aimed at examining both neural and GI phenotypes in Drosophila to elucidate how mutations in CHD8 impair development. Studies using germ-free mice have begun elucidating mechanisms by which species-specific microbiota metabolites modulate behaviors associated with ASD. Thus, the assays described here will ultimately be used to study connections between gut microbiota and neural phenotypes in fruit flies. Similar to other model organisms, the Drosophila homolog of CHD8, kismet (kis), is expressed during embryonic development and complete loss of kis is embryonic lethal, so we examined heterozygous kis mutants. We used immunohistochemistry and confocal microscopy to show that heterozygous loss of kis causes severe axon guidance defects in adult brains. Disrupted axon guidance is one of the cellular phenotypes common to ASD. We also used the courtship assay—a quantitative measure of an innate behavior—to show that heterozygous kis mutants exhibit significant deficits in courtship behaviors. This result reiterates the important role kis plays during neural development, and it will also
allow us to quantitatively assess how variations in gut microbiota affect the severity of kis phenotypes. FUNDING:
California State University Program for Education and Research in Biology (CSUPERB) New Investigator Award.

Program Abstract #449
Investigation of the Function of ETV1 in Zebrafish Danio rerio
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The interstitial cells of Cajal (ICC), the pacemaker cells of the gut, play a pivotal role in gastrointestinal tract function. Slow waves generated by these cells coupled with neural input from the enteric nervous system (ENS) underlie coordinated intestinal peristalsis. Previous work has implicated ICC in various gastrointestinal diseases with intestinal motility being compromised where ICC are sparse. At present, there are no reliable embryonic markers of ICC in zebrafish. This study investigates whether transcription factor ETV1 is a potential marker for ICC. We utilized in-situ hybridization to characterize this gene’s embryonic expression patterns from 24-120 hours post fertilization (hpf). We then functionally investigated the role of ETV1 in intestinal motility by performing a gene knockdown via splice-blocking morpholino (SBMO) and effective knockdown was confirmed by RT-PCR. We subsequently examined ENS development in morphant embryos. Intestines of ETV1 morphant isolated from transgenic zebrafish expressing GFP in their enteric neurons revealed no significant loss of enteric neurons. We then recorded time-lapse movies of peristalsis in morphant and control intestines. Compared to control embryos, morphant embryos have an uncoordinated peristalsis suggesting that there is a perturbation of the normal development of tissues necessary for normal gut motility. Taken together, these results are consistent with our working hypothesis that ETV1 is essential for normal ICC development and gut motility in zebrafish. To further validate this hypothesis, we are using immunocytochemistry to determine whether the ICC network is perturbed in ETV1 morphant zebrafish. We are utilizing the ANO1 antibody, a known zebrafish ICC marker and an anti-GFP antibody to double stain morphant and control embryos to reveal the ICC and ENS networks. Furthermore, we are also investigating whether enteric neuronal subtype specification is perturbed. This study is funded by the NIH.

Program Abstract #450
Expression of class III Semaphorins and their receptors in the developing chicken inner ear
Mary Scott, Jia Yue, Deborah Biesemeier, Joo Won Lee, Donna Fekete
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Published work from our lab suggests that Sema3D and Nrp2 may play a role in patterning of the auditory organ of the inner ear in the chicken embryo. Class III Semaphorins (Sema) secreted ligands are most commonly known for their repulsive effects on neurites expressing Neuropilin (Nrp) and/or Plexin (Plxn) membrane bound receptors. There is, however, a growing body of literature supporting that Sema signaling also has alternative roles in development such as boundary formation and vasculogenesis. Using in situ hybridization and immunohistochemistry we examined the expression of Sema3D, Sema3F, Nrp1, Nrp2, and PlxnA1 in horizontal cross sections of the auditory and vestibular organs of the inner ear in the chicken from embryonic (E) day 5 to E10. The resulting expression patterns suggest that Sema signaling could be involved in axon repulsion, synaptogenesis, boundary formation, and/or vasculogenesis in the inner ear epithelium and the surrounding mesenchyme. Sema3D expression flanking the sensory tissue in vestibular organs suggests that it repels Nrp2 and PlxnA1 expressing neurites of the vestibular ganglion away from nonsensory epithelia, channeling them into the sensory domains at E5-E8. Expression of Sema signaling genes in the sensory hair cells of both the auditory and vestibular organs on E8 – E10 suggest that these genes could subsequently play a role in synaptogenesis. In the nonsensory tissue of the auditory organ, Sema3D is immediately adjacent to Nrp1 and PlxnA1. This suggests that these genes are involved in forming or maintaining a boundary between two nonsensory domains, the hyaline cells and the tegmentum vasculosum. In the mesenchyme, Nrp1 colocalized with tissue rich in capillaries. Sema3D immediately flanks this Nrp1-expressing tissue, suggesting it may be involved in endothelial cell migration towards the inner ear. These data suggest that Sema signaling may play multiple roles in the developing inner ear. Funding:NIH (R01 to DMF & F31 to MKS)
Program Abstract #451
Spatiotemporal requirements for BMP and WNT signals in an in vitro model of human ectodermal pattern formation
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Early embryonic patterning is a highly coordinated and multistep process that relies on organized signaling to direct position-specific fate choices. The combined activity of WNT and BMP signaling during gastrula and early neurula stages guide the patterned emergence of four distinct ectodermal lineages: neural, neural crest, placode and the future epidermis. Protocols aimed to induce human embryonic stem cells (hESCs) to each fate have revealed the requisite signals to produce enriched populations, however, it remains unclear how signaling pathways are regulated, and their dynamics decoded, to generate patterns within human ectodermal tissue. To bridge this gap in understanding, we developed an in vitro model based on micropatterned colonies of hESCs that recapitulates the spatial patterns of signaling and gene expression found in vivo. We demonstrate that hESCs grown in defined circular geometries, and subjected to a two-step induction generate self-organized patterns consisting of all four ectodermal lineages. Our novel approach consists of an initial phase of restriction to ectodermal lineages through TGFβ inhibition followed by a second phase in which both BMP and Wnt activities are modulated in time. Importantly, live cell reporters of BMP and Wnt signaling demonstrate that the timing and duration of endogenous WNT ligands directs the specification of neural crest and placodal cells downstream of exogenously supplied BMP. This information allows us to rationally design protocols to achieve desired fates and patterns. Our approach introduces a powerful experimental platform to quantitatively dissect the role of multiple signaling pathways and their contributions to human ectodermal pattern emergence. Funding source: CPRIT grant RR140073, NIH R01GM126122

Program Abstract #452
Dissecting the role of astrocyte-derived proteins during synapse formation in Drosophila
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Complex neuronal networks are assembled through the birth of immature synapses between axons and dendrites, followed by synapse maturation and refinement. This three-step process relies heavily on molecular mechanisms from glia to establish functional networks. A subset of glial cells, known as astrocytes, can modify synapse function by secreting extracellular signals in an activity-dependent manner. Astrocytes have also been shown to directly instruct or inhibit the formation of excitatory glutamatergic synapses. In vitro approaches have identified many astrocyte-secreted molecules that modify glutamatergic-synapse dynamics. Presently, little is known of astrocyte-synapse dynamics in vivo, nor the mechanisms by which astrocytes regulate the formation of other types of synapses. We propose Drosophila as a robust system to uncover the astrocyte-expressed factors involved in formation of excitatory cholinergic and inhibitory GABAergic synapses. Using RNAi, we conducted a reverse genetic screen using the astrocyte-specific alrm-Gal4 to knock down genes in astrocytes. Concurrently, using Flippase tools (FLP), we have labeled both neuronal membranes and their pre-synaptic sites (Brp+) using Synaptic Tagging with Recombination (STaR) to assess non-cell autonomous changes in synapse number. We performed two parallel screens, the first labeled individual dorsal bipolar dendritic (Ddb) sensory neurons that form cholinergic synapses. The second targeted neurons that generate both cholinergic and GABAergic synapses localized in the mushroom body. Excitingly, we have determined that the major astrocyte-secreted molecules that induce synapse formation (e.g. TGF-β) or inhibit synapse development (e.g. SPARC) in vertebrates are conserved in fly. More insights on the effects of novel, astrocyte-derived synaptogenetic compounds will be discussed. This research will provide an expanded look at the molecular pathways that glial cells use to inform synaptogenesis in vivo.
Program Abstract #453

Changing roles for BMP signalling across time and space during cerebellar granule cell neurogenesis

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Cerebellar granule cell progenitors (GNP) are the most abundant neuronal progenitor population in the brain, making them a good model for studying principles of neurogenesis. GNPs are born at the rhombic lip and migrate tangentially to form a transiently proliferative layer that covers the cerebellar anlage; the external granule layer (EGL). GNPs undergo massive proliferation in the EGL influenced by Sonic Hedgehog (SHH) secreted by underlying Purkinje neurons. Mutations in this pathway are implicated in medulloblastoma; the most common paediatric brain cancer. However, triggers for termination of GNP proliferation are poorly understood. Whilst BMP signalling has been implicated at various stages, data from in vitro and transgenic mouse studies has been conflicting. In this study we show, in chick, that the role of BMP switches with changes in the signalling environment of the developing cerebellum. In the absence of BMP signalling the EGL fails to form, but GNPs nevertheless migrate away from the rhombic lip and differentiate, still sending axonal projections into the molecular layer of the cerebellum. In contrast, with the misexpression of an activated form of the intracellular mediator of BMP signalling, Smad1, GNPs are recruited to the EGL. However, after the onset of proliferation at embryonic day 8, Smad1 misexpression has the opposite effect by inducing radial migration and premature differentiation. In conclusion, our data suggests that BMP signalling is required initially for proper EGL formation, but that its role in GNP neurogenesis is spatially and temporally variable. These results unify previous conflicting observations surrounding the role of BMP signalling in the granule lineage, and uncover an important function of BMP signalling in late cerebellar development that is developmentally pliable, and may explain variation in foliation between species, as well as identify potential avenues to treat medulloblastoma. Funded by Queen Mary University London

Program Abstract #454

Dendrite branching versus elongation in developing neurons: modulation by a phospho-switch in delta-catenin.

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The formation and function of neurons depends upon the elongation and branching of dendrites, with alterations linked to neurodevelopmental disorders such as autism, schizophrenia and cognitive deficits. We are probing the basis of dendrite morphology via study of two novel protein complexes each involving delta-catenin. In neurons, delta-catenin contributes to cytoskeletal control, cadherin adhesion and disease, and it localizes to dendrites and additional cell compartments. Rat primary hippocampal neurons (P0) are an established developmental model that facilitates the visualization of dendritic morphogenesis. We uncovered two novel interactions of delta-catenin with the PDZ-domain scaffold proteins Magi1 and Pdlim5. Of note, we find that Magi1 promotes dendrite lengthening while Pdlim5 instead enhances branching. This presents us with the puzzle that two proteins with opposing roles each bind delta-catenin. In addressing this point, we discovered a key phosphorylation site within delta’s C-terminus (PDZ-ligand) that determines if delta binds Magi1 versus Pdlim5. Our evidence consistently supports the view that delta’s phospho-switch helps instruct dendrite subregions to elongate or alternatively to branch - a key morphologic decision process that occurs countless times during neuron development. Our work, which will be submitted midyear, points to the upstream kinase(s) controlling the phospho-switch, and potentially, downstream mediators of these two delta-catenin complexes. Such mechanistic insights will point to the functional basis of the delta:Magi1 versus delta:Pdlim5 complexes in neuro-development and possibly disease. Finally, since certain additional catenins possess analogous C-terminal sequences (PDZ-ligands) harboring...
potential phospho-switches, our findings may point to a wider mechanism whereby catenin proteins are able to select between PDZ-domain partners that favor distinct developmental outcomes. (RO1 GM107079 to PDM).

Program Abstract #455
Aberrations in rhombic lip development caused by disrupted mesenchymal signaling is possibly a unifying developmental mechanism for human Dandy-Walker malformation
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Human cerebellar malformations are recognized with relative ease through brain imaging studies. However, the molecular and cellular mechanisms contributing to cerebellar birth defects are poorly understood and their developmental pathology is largely undescribed. We have reported that in rare patients, FOXC1 loss contributes to a posterior fossa phenotypic spectrum that includes Dandy-Walker malformation (DWM), a common human cerebellar malformation. We now demonstrate that the null and hypomorphic Foxc1 mutant mice have early granule and Purkinje cell (PC) abnormalities and subsequent disruptions in cerebellar foliation and lamination. Particularly striking is the presence of a partially formed unpaired posterior vermis lobule which echoes the posterior vermis DW “tail sign” observed in human imaging studies. Lineage tracing experiments in both the null and hypomorphic Foxc1 mouse mutants indicate that the main cause of this feature is the aberrant migration of granule cell progenitors from the rhombic lip that are destined to form the posterior-most lobule. This phenotype is due to loss of required signaling molecules including SDF1 from the mesenchyme surrounding the developing cerebellum. Analyses of rare human DW fetal cerebella with chr 6p25 (FOXC1) heterozygous deletions demonstrate extensive phenotypic overlap with our Foxc1 mutant mouse models, validating our DWM models and demonstrating that many key mechanisms controlling cerebellar development are conserved between mouse and human. Ongoing analysis of additional DWM fetal samples of unknown genotypes demonstrates remarkably similar features, suggesting that we have identified a unifying developmental mechanism for DWM.

Program Abstract #456
Epigenetic factors in enteric nervous system development and disease
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Epigenetic modifications play an important role during development, but very little is known about their function in development of the enteric nervous system (ENS), which innervates the gut. One prominent type of epigenetic modification connected with ENS development and disease is methylation. Mutations in the cellular histone methylation machinery reduce ENS neuron density in mice and have been linked to Hirschsprung disease (HSCR), in which the distal gut is uninnervated. Decreased expression and potentially pathogenic missense mutations of the de novo DNA methyltransferase (Dnmt) have been found in HSCR patients. Expression changes in main ENS development regulators – genes that when mutated cause an HSCR phenotype, such as RET – are thought to be under control of DNA methylation. However, how epigenetic modifications such as DNA methylation affect ENS development and disease remains poorly understood. DNMT1 works together with Ubiquitin-like protein containing PHD and RING finger domains 1 (UHRF1) in maintaining DNA methylation. In a forward genetic screen, we isolated a zebrafish mutant with strong, variable reduction in ENS neuron numbers and showed that it results from a mutation in uhrf1. Mutants exhibit severe disruption of intestinal smooth muscle and epithelial morphology. Genetic chimeras reveal that during ENS development, Uhrf1 functions both cell-autonomously, in ENS progenitor cells, and non-cell-autonomously, in as yet unknown intestinal cells. Consistent with known
interactions between UHRF1, DMNTs, and Histone deacetylases (HDACs), we found that dmnt1 and hdac1 mutants exhibit similar ENS phenotypes to uhrf1 mutants. Double mutant analysis demonstrated that uhrf1 and dnmt1 function together in regulating ENS neuron development. Our work demonstrates that DNA methylation has a key function in establishing the ENS and provides further support that epigenetic modifiers play important roles in ENS development and disease. PPG - P01HD022486

Program Abstract #457
Taking a Roundabout Investigation of the Forebrain: Testing the Requirements of robo1 and robo4 in Zebrafish Forebrain Development
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Many signals and receptors coordinate their functions to facilitate the building of a brain. A critical step in brain development is the connection of the two halves of the nervous system through midline crossing axons forming structures called commissures. The accessibility, transparency, fast development, and genetic and molecular techniques of the zebrafish model organism provides a tractable system to study the first commissure to form in the brain called the post optic commissure (POC). POC formation relies on Slit repellent guidance cues, Slit1a/2/3 and Roundabout receptors (Robos), Robo1-4, and current studies show that slit2/3 and robo2/3 are involved in axon repulsion. We seek to decipher the distinct ways in which the different robos may be mediating differential slit signaling. Although robo2 and robo3 have been previously reported to impact optic chiasm formation and hindbrain development respectively, little is known about their role in POC formation and nothing is understood about robo1 or robo4. Morpholino knockdown studies suggest that Robo1 may serve as the receptor for Slit1a signaling. In addition, we have been interested in how intimately POC axons associate with astroglial cells at the midline during crossing. Interacting astroglial cells exclusively express robo4, unlike the POC neurons. Robo4 has been implicated in angiogenesis which suggests it may play a role in the formation of the blood brain barrier (BBB). To characterize the role of robo1 and robo4 we have created knockouts in these receptors. robo4 mutants show subtle phenotypes in POC formation and glial bridge condensation. We will also implement a newly developed computational method, DeltaSCOPE, to quantify both robo1 and robo4 mutant POC phenotypes as well as extend this analysis to the BBB. We intend to utilize these techniques to fully investigate the Slit-Robo code of axon guidance and elucidate an important mechanism required for brain development. NSF 1656310

Program Abstract #458
Identifying protein interactions required for correct specification of dI2 spinal interneuron neurotransmitter fates
William Haws, Samantha England, Ginny Grieb, Gabriela Susana, Katharine Lewis
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One of the most important properties that neurons acquire during development is their neurotransmitter phenotype. In all cases examined so far, this is, at least initially, specified by transcription factors. Our lab has identified a transcription factor that is required in zebrafish for the glutamatergic (excitatory) neurotransmitter phenotype of at least some dI2 spinal neurons. Given that this protein is expressed in an equivalent region of mouse spinal cord, this function is probably conserved in mammals. We have created several zebrafish mutant lines that should produce truncated versions of this transcription factor, but only some of these mutant alleles cause a neurotransmitter phenotype. We hypothesize that interactions with other proteins may be required for this transcription factor’s function in dI2 neurons, and that these interactions might be lost in mutants with a phenotype, but remain in mutants without a phenotype. Therefore, we performed a yeast two-hybrid (Y2H) screen followed by next generation sequencing (NGS) of positive colonies to identify proteins that interact with this transcription factor. We are currently performing bio-layer interferometry (BLI) to independently confirm interactions identified in the Y2H screen, and to investigate whether confirmed positives can bind the different truncated proteins encoded by our mutant alleles. So far, we have confirmed one of these binding partners and
shown that it binds both wild-type protein and a truncated protein that does not result in dI2 neurotransmitter phenotypes but does not bind a truncated protein that causes dI2 phenotypes. This suggests that this protein interaction may be important for specifying the glutamatergic fates of dI2 cells. This Y2H-NGS-BLI pipeline is a novel high-throughput way to not only confirm protein-protein interactions, but also investigate phenotypic differences between mutant alleles. This work is funded by NINDS R01NS077947 and NSF IGERT DMR-DGE1068780.

Program Abstract #459
Transcription Factors Required for Specification of Spinal dI2 Interneuron Neurotransmitter Fates
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In the spinal cord, distinct excitatory and inhibitory interneurons must form at the correct times, in the right locations and in the appropriate numbers to properly generate somatosensory and locomotor circuitry. Deficits in interneuron development and function underlie pathologies as diverse as central touch disorders and Amyotrophic Lateral Sclerosis. However, there are still fundamental gaps in our knowledge of how interneurons become specified during development and this lack of understanding impedes our ability to treat debilitating spinal cord disorders, injuries and developmental defects. Using a combination of techniques, including fluorescence-activated cell-sorting, microarray analysis and in situ hybridization, we have identified two homeodomain transcription factors that are co-expressed in dI2 interneurons in zebrafish spinal cord. Excitingly, analysis of zebrafish embryos injected with translation-blocking morpholinos has shown that these genes function partially redundantly to specify the excitatory (glutamatergic) fates of these neurons. In double knockdown embryos, dI2 interneurons change their neurotransmitter fates to become inhibitory. To perform a more detailed analysis of dI2 neurotransmitter specification we have generated single and double CRISPR mutants for these genes. Furthermore, we have established a stable transgenic line to assess dI2 cell morphology and connectivity in our mutant embryos. Interestingly, the phenotypes of some of our mutants suggest that the only known protein domain in these transcription factors, the DNA-binding homeodomain, may not be required for these proteins to specify the dI2 neuron excitatory phenotype. This research should significantly increase our understanding of neuronal neurotransmitter specification, not only in zebrafish, but also in mammals including humans, given the high degree of conservation of spinal interneuron function and gene expression in vertebrates. Funding: NIH NINDS R01 NS077947

Program Abstract #460
SOX4A and SOX4B mutants display neural crest and ocular defects
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SoxC transcription factors are highly conserved among vertebrates and are characterized by the presence of a Sry-related high mobility group (HMG) DNA-binding domain, and a C-terminal transactivation domain (TAD). Our lab has previously demonstrated that SoxC factors regulate choroid fissure closure and photoreceptor development in the zebrafish retina. Zebrafish contain two copies of the SoxC transcription factor sox4, sox4a and sox4b. To further explore the role of sox4a/b during development, we generated zebrafish sox4a and sox4b mutants. Methods: All animal procedures were performed in accordance with guidelines established by the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The sox4a and sox4b genetic mutant lines were generated by CRISPR/Cas9 using two single strand guide RNAs targeting before and after the HMG domain for each gene. Amplification of the coding sequence by PCR was used to identify organisms with a large deletion in either sox4a or sox4b. Retinal cell type differentiation in wild-type and sox4 mutants was analyzed through use of immunohistochemistry and fluorescent reporter transgenic lines. Alcian blue staining was used to analyze craniofacial development. qPCR was used to examine mRNA levels of sox4a/b and other genes of interest. Results: We generated both a sox4a and a sox4b mutant, each with the loss of both its HMG and TAD. Maternal zygotic mutants for both sox4a and sox4b displayed smaller than normal eyes and fewer rod photoreceptors.
Additionally, sox4b maternal zygotic mutants displayed fewer retinal ganglion cells, were hypopigmented and had craniofacial defects. Conclusions: These data suggest that sox4a/b are required for proper ocular morphogenesis and retinal cell type differentiation in the zebrafish. The pigment and craniofacial defects in sox4b mutants also suggest a role for SoxC factors in neural crest lineages. **Financial support:** NIH grant 1RO1EY02176

**Program Abstract #461**

**Loss of function of Her9 causes retinal, craniofacial, and digestive system abnormalities**

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Her9 is a bHLH-O transcriptional repressor that has been shown to promote floor plate and inner ear development. Her9 is the homologue of human HES4, which is absent from the mouse genome. In previous work, our lab demonstrated an upregulation of Her9 during chronic rod degeneration and showed that Her9 is regulated by RA signaling. To further investigate the role of Her9 during development, we generated her9 mutants using CRISPR technology and characterized their phenotypes. Two mutant alleles of her9 were isolated – a 1bp deletion and a 1bp insertion that both produce early stop codons resulting in a truncated Her9 protein. Using RT-PCR and qPCR we show a decrease in her9 mRNA expression in the homozygous mutants compared to their wild type and heterozygous siblings, suggesting the mutations result in nonsense-mediated decay. In the retinas of her9 homozygous mutants, we observed a reduced number of rod photoreceptors, cone photoreceptors with an abnormal morphology, reduced ciliary marginal zone, and a reduced number of Müller glia. Her9 homozygous mutants also demonstrated an abnormal visual background adaptation (VBA) response, suggesting possible visual system impairment. In addition to retinal defects, her9 mutants also have an enlarged liver, abnormal digestive tract, and abnormal or missing pharyngeal arches. The her9 homozygous mutants also lack a swim bladder and die between 10-13 dpf, possibly due to abnormal feeding behavior. Taken together, our results indicate that Her9 has an important role in retinal development and may regulate neural crest cell lineages that contribute to craniofacial structures and parts of the developing digestive system. This project is supported by funding from a NIH grant EY021769 and LTJ (C.E.C)

**Program Abstract #462**

**Shaping the vertebrate head: a role for Zic2**

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The forebrain, neural retina and neural crest-derived facial cartilages form in close proximity in the developing vertebrate head. The importance of cross-lineage interactions during cranial morphogenesis is suggested by the frequent association between congenital malformations of forebrain (holoprosencephaly, HPE), the retina (e.g. coloboma), and craniofacial skeleton (frontonasal dysplasia, FND) in human. Nonetheless, the cellular and genetic bases of these interactions are poorly understood. We are modeling cranial morphogenesis in zebrafish by investigating the functions of ZIC2, a zinc-finger transcription factor causally linked to HPE in humans. We have demonstrated a role for zebrafish zic2 in the developing forebrain, facial cartilages, and neural retina. We have identified alx1, a homeobox transcription factor, as a putative target of zic2. Alx genes (Alx1, Alx3, and Alx4) are essential for craniofacial and ocular morphogenesis, since mutations in each of these genes are associated with FND with ophthalmic malformations in humans. Notably, Alx genes are expressed in overlapping domains that are restricted to neural crest-derived periocular mesenchyme and frontonasal cartilage precursors. These expression patterns predict that Alx genes have overlapping (redundant), cell-autonomous functions in the neural crest-derived lineages, and that they function non-cell-autonomously to direct retinal morphogenesis. We have mutagenized zebrafish alx1 and found it to be dispensable for normal development, consistent with the predicted redundancy of alx functions. We are generating double knockouts of alx1 with alx3, alx4a or alx4b and will report their phenotypic characterization. These mutant lines represent important new models in which to address the mechanism of neural crest/brain/retina interactions during cranial morphogenesis, and to elucidate the genetic underpinnings of FND and retinal malformations.
**Program Abstract #463**  
Hierarchical organization of the electrical synapse  
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The 86 billion neurons of the human brain form functional circuits via connections called synapses. Electrical synapses, composed of plaques of Connexin (Cx) channels, allow for the direct movement of ions and other small molecules resulting in a fast, often bidirectional, mode of communication. The channels are formed by the apposition of membrane-localized hemichannels between pre- and postsynaptic neurons, with each hemichannel composed of a Cx hexamer. While we know that Cxs are critical to synapse function, the molecular mechanisms by which these structures are formed are not well understood. Using zebrafish spinal cord neurons as a model system, we found that cytosolic MAGUK scaffolding protein Tjp1b/ZO-1 is localized to and required for electrical synapse formation and function. We hypothesize that there is a direct interaction between Tjp1b and the electrical synapse Cxs and that this interaction is required for proper synapse development. To test this model, we performed *in vitro* binding assays and demonstrated a direct interaction between the predicted binding domains of Tjp1b and the Cxs. We hypothesize that the same domains are required for interaction *in vivo* and are testing this both in cell culture and in zebrafish. To examine the relationship between Tjp1b and the Cxs *in vivo*, we used null mutations in each of the genes and found that Tjp1b is required for Cx localization. This suggests that their direct interaction facilitates electrical synapse formation. By contrast, the Cxs were not required for Tjp1b localization, suggesting hierarchical organization at the synapse. This suggests a model in which electrical synapse formation is specified upstream of a direct Tjp1b/Cx interaction that is required for the trafficking and/or stabilization of Cxs at the synapse. Funding was provided by the University of Oregon Developmental Biology Training Program (2T32HD007348-26) and the NINDS Pathway to Independence Award (R00NS085035).

**Program Abstract #464**  
The genetic basis for the first connections in the brain  
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The function of a neural network is the result of the patterns and properties of the connections between the neurons. Neuronal connections, or synapses, are either chemical, where neurotransmitters are released and received, or electrical, where Connexin (Cx)-based channels mediate direct ionic and small molecule communication. Electrical synapses have been found to extensively connect neurons together during development throughout the animal kingdom, leading to a model in which Cxs form the first synapses of the nervous system. Yet the Cxs that form these early electrical synapses are not known. To address this question, we have utilized the zebrafish spinal cord and the first behavior elicited by developing fish, coiling. This system provides an ideal model as the circuits are simple and accessible and work in electrophysiology has demonstrated that electrical synapses drive network function and behavior. In zebrafish, there are 42 genes encoding Cx proteins. To identify Cxs that might form the early electrical synapses, we used FACs-sorting and RNA-seq and found that Cx46.8 was expressed in coiling-circuit neurons. We knocked out Cx46.8 using CRISPR/Cas9 and found that mutant animals had defects in the initiation, strength, and symmetry of coiling behavior. These data suggest that Cx46.8 is contributing to the electrical synapses that mediate the first behavior of the fish. Identifying the temporal and spatial dynamics of Cx46.8 expression and examining its role in coiling circuit function and maturation will provide insight into the first electrical synapses within the nervous system. Funding: NIH Pathway to Independence Award R00NS085035 and the University of Oregon to A.C.M. VPRI Undergraduate Fellowship and UROP Mini-grant from the University of Oregon to A.P.A.

**Program Abstract #465**  
Developmental and Molecular Determinants of Synaptic Connectivity in *Drosophila melanogaster*  
Claire Williams  
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With billions of neurons and trillions of synapses, elucidating connectivity in the human brain has proven to be an extraordinarily difficult problem. Proper connectivity throughout the nervous system is critically important to evoke expected behaviors in response to various stimuli, and disrupted connectivity has been associated with a number of neurodevelopmental disorders, including autism spectrum disorder, epilepsy, and schizophrenia. Given this importance, one key outstanding question is what determines patterns of neuronal connectivity. To identify determinants of connectivity, we are focusing on a well-characterized pair of synaptic partners in the *Drosophila melanogaster* larval ventral nerve cord: the nociceptive sensory neurons, class IV dendritic arborization neurons (c4da), and one of their interneuron targets, A08n. We first explored developmental determinants of synaptic connectivity by asking why c3da sensory neurons, which are closely related to c4da neurons in lineage, function, and axonal projection position, are unable to synapse with A08n, using a variety of genetic and imaging tools to assay synaptic presence, number, and function. We next sought molecular determinants of synaptic connectivity by defining the transcriptome of the two partner neurons, generating a candidate list of known adhesion molecule binding partners across the neurons, and establishing an optogenetic behavioral screen to test for the functional importance of each of these candidate genes in synaptic partner matching. Following this study, we will have a model for how specificity is achieved in one synaptic pair, and will be able to test this model in multiple other known synaptic pairs in the *Drosophila* nervous system, leading to a broader understanding of how synaptic partners are chosen across circuits. This work was supported by an NSF fellowship (DGE1256032) and an NIH fellowship (F31-NS106775-01) to CRW and funds from the NIH (R01-NS076614) to JZP.

Program Abstract #466
**Conserved and novel role for the Planar Cell Polarity component Vangl2 in shaping the zebrafish anterior neural tube**

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Neurulation is the process by which the neural tube, the precursor of the brain and the spinal cord, forms during early development. In the prospective forebrain (FB), neurulation is coupled with eye formation. Fate maps of vertebrate embryos reveal that the prospective FB occupies the lateral edges of the eye field. The optic vesicles evaginate as neurulation proceeds and the neural tube is positioned in between the developing eyes. Despite the FB regulating many essential physiological functions, little is understood about the cellular and molecular events that shape this brain region. Our laboratory is interested in understanding the cellular dynamics that underlie FB morphogenesis, using zebrafish as a model organism. The optical clarity of the zebrafish embryo and its amenability to genetics make it uniquely well suited for studying tissue dynamics. In contrast to more posterior regions of the neural tube, the events that shape the FB in this teleost are mostly unknown. Preliminary data from our laboratory indicate that FB morphogenesis in zebrafish presents hallmarks of primary neurulation in amniotes, namely medial and lateral hinge point-like structures and neural folds that converge and fuse at the dorsal midline. The medial hinge point (MHP) forms in the superficial layer of the bilayered eye field, which resolves into a single layer via radial intercalation (RI). We report here that disruption of the planar cell polarity component Vangl2, previously implicated in MHP formation in amniotes, is required for the formation of its analogous structure in zebrafish. Lastly, we show that RI of MHP cells is impaired in *vangl2* mutants. Together, these findings highlight conservation of central aspects of primary neurulation in zebrafish and reveal a novel role for the PCP pathway in driving RI in the developing anterior neural tube. Funding: IMSD Meyerhoff Graduate Program (2 R25-GM55036), National Science Foundation, Chemistry/Biology Interface (T32 GM066706)

Program Abstract #467
**Domineering Non-Autonomy in Vangl1;Vangl2 Double Mutants Demonstrates Intercellular PCP Signaling in the Vertebrate Inner Ear**

**Michelle Stoller**, Orvelin Roman, Michael Deans

*University of Utah, USA*

Planar Cell Polarity (PCP) is the coordinated orientation of polarized structures within the plane of an epithelium
that underlies dynamic cellular movements during development, and cellular and tissue function in the mature organism. A striking example of PCP in mammals is inherent in the organization of polarized stereociliary bundles critical for the function of the vestibular hair cells of the inner ear that detect sound and motion. However, the establishment of PCP is better understood in Drosophila wing epithelial cells where the essential genes regulating PCP were first discovered, and where the function of PCP proteins encoded by these genes have been deciphered through the phenotypic analysis of mutant clones. An illuminating outcome is the domineering non-autonomy phenotype observed where abrupt disruptions in PCP signaling impacts the orientation of neighboring wild type cells, because this demonstrates the intercellular propagation of PCP signals. We have used Emx2-Cre transgenic mice to generate analogous mutant boundaries in the mouse inner ear, and thereby disrupted vertebrate PCP signaling in Vangl1;Vangl2 conditional knockouts. Due to unique aspects of vestibular anatomy in the mouse, core PCP protein distribution along the Emx2-Cre boundary generated in the utricle resembles the proximal side of vang mutant clones in the Drosophila wing. In contrast protein distribution along the boundary generated in the saccule resembles and the distal side of a vang clone. Consistent with these complementary protein distributions, domineering non-autonomy phenotypes occur along the Emx2-Cre boundary in the mutant utricle that do not occur in the saccule. These results demonstrate that intercellular PCP signaling is propagated across the sensory epithelia of the mouse ear, and further support the hypothesis that core PCP function is conserved between vertebrate and invertebrate systems.

Program Abstract #468
Differential Activation of Dopaminergic Addiction Circuits by Drugs of Abuse
Brad Serpa
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The disease of addiction hijacks the brain's reward system, a vital evolutionary system that drives the motivation and actions necessary for our survival and success. The reward system includes a circuit of nuclei extending from the forebrain to the midbrain that is connected by dopaminergic neurons. The communication of dopamine (DA) among these circuits underlies feelings of pleasure, reward, and the motivation for seeking rewarding stimuli. Drugs of abuse, like amphetamines and cannabinoids, can trigger the reward system and perpetuate addiction behaviors for which the motivation to seek and take drugs overrides personal safety. The zebrafish serves as an ideal model for addiction study, because of the homology to the human brain among elements and signaling in the reward pathway. Conditioned place preference behavioral assays indicate that both stimulants (amphetamines; AMPH) and depressants (cannabinoids; THC) activate the zebrafish reward system, leading to addictive behaviors. Because these oft-prescribed and widely used drugs have vastly different mechanisms of action in the body, the goal of my research seeks to determine where and how these drugs are active in the fish reward system. After drug exposure and measurement of behavioral changes, zebrafish brains were dissected and cryosectioned. Fluorescence immunohistochemical staining against tyrosine hydroxylase was done to map the dopaminergic reward pathway in the zebrafish brain, while antibodies against the dopamine receptor type 1 (DRD1) and dopamine receptor type 2 (DRD2) reveal differential signaling mechanisms and areas within the reward system that are triggered by AMPH versus THC. To understand how potential drugs of abuse activate the reward circuit, will help inform on potential drug abuse therapies and abuse mitigating treatments.

Program Abstract #469
Let-7 regulates cell cycle dynamics in the embryonic retina and cortex
Corinne Fairchild, Simranjeet Cheema, Anna La Torre
University of California - Davis, USA
In many regions of the developing central nervous system (CNS), progenitors generate specific neuronal cell populations in a sequential, conserved order. This is true in the cerebral cortex and retina, where multipotent progenitors change their competence over time. The correct proportion of neurons and glia ensures synaptic function; however, we do not fully understand how progenitors change their competence and generate the required cell fates. Competence transitions in the retina and cortex are controlled by miRNAs; short, single-
stranded nucleotides that bind mRNAs to negatively impact expression. Specifically, three miRNAs are involved, including let-7. Using miRNA in situ hybridization in mouse embryos, we show that let-7d levels increase over time in the progenitors of many CNS regions. Furthermore, let-7d levels fluctuate spatially within progenitors at E13.5, correlating with the location of mitosis. Using a GFP-based miRNA sensor in immortalized human retinoblast (HER10) cells, combined with visual analysis and flow cytometry, we find that let-7 activity varies at different phases of the cell cycle. Importantly, following sensor GFP expression in live HER10 cells also confirmed that, in an individual cell, let-7 activity fluctuates during cell division. Additionally, we performed sequential EdU/Ki67 staining in HER10 cells and mouse E11.5 cortex primary cultures and found that manipulating let-7 levels alters cell cycle kinetics, with overexpression reducing cell cycle entry and knockdown resulting in a faster re-entry. Together these results suggest that let-7 regulates cell cycle dynamics and increase our understanding of how miRNAs control progenitor competence. This will aide in the development of stem cell-based transplant therapies and provide novel insight into the development of the fovea, a high acuity area in the primate retina that provides our sharp sense of daytime vision. Study funded by R01:EY026942 to A.L.T and T32:EY015387 to C.L.F.

Program Abstract #470
Characterizing the role of histidyl-tRNA Synthetase in neurodevelopment
Ashley Waldron, Claire Wilcox, Graham Wright, Christopher Francklyn, Alicia Ebert
University of Vermont, USA

Vision and hearing are two of our most valued senses, and are critical to the survival of a myriad of animals. These senses are possible thanks to specialized neurons found in the retina and inner ear. Loss of retinal neurons or mechanosensory hair cells results in blindness and deafness, respectively. Recently, a human genetic disorder characterized by both vision and hearing loss during early childhood was associated with a recessive mutation in the HARS gene, which encodes the Histidyl-tRNA Synthetase protein (HARS). HARS is responsible for attaching the amino acid histidine to its cognate tRNA molecules, and is therefore required for protein synthesis. Given its essential function, it is perplexing that a mutation in this protein would be associated with such a specific phenotype. Our goal is to better characterize the function(s) of HARS in retinal and auditory tissues using the zebrafish as a model organism. Using gene knock-down techniques we have found that zebrafish retinal and mechanosensory hair cells are particularly sensitive to hars expression. Specifically, hars appears to be important for the proliferation and survival of the progenitors that give rise to these neurons. We are currently investigating the link between HARS and cell cycle regulation in order to glean insight into both HARS-related disorders and HARS' influence on neurodevelopment in general.

Program Abstract #471
DDX3 induces neural crest through activation of an Akt-Wnt signaling axis
Mark Perfetto1,2
1West Virginia University, USA; 2University of Delaware, USA

DDX3 is a DEAD-box RNA helicase critical for normal development. Recently, mutations in DDX3 were found to be associated with multiple abnormalities including cleft lip/palate, phenotypes that are often caused by aberrant neural crest development. Here we show that knockdown of DDX3 inhibits neural crest induction in Xenopus embryos. This is accompanied by reduction in Akt activity and canonical Wnt signaling. Blocking Akt activity phenocopies DDX3 loss of function in inhibiting Wnt signaling and neural crest induction, and a constitutively active mutant of Akt rescues the neural crest induction phenotypes caused by DDX3 knockdown. These results show for the first time that a DDX3-Akt-Wnt axis is required for neural crest induction.

Program Abstract #472
Developmental Specification of Neural Circuit Connectivity
Austin Seroka, Chris Doe
University of Oregon, USA

During Drosophila development neural progenitor cells, known as neuroblasts, form at different times and then
undergo asymmetric division to produce a series of neuronal progeny. These progenitor cells are patterned by the sequential expression of temporal transcription factors to produce a diverse array of neurons with distinct molecular and morphological identities. As each neuron is born from its parental neuroblast, it retains the temporal transcription factor identity of its progenitor and encounters a unique extra-cellular environment. How does this neuron know which synaptic partners to target? I address this question in the well-characterized NB 7-1 lineage of the Drosophila ventral nerve cord, which produces five morphologically and molecularly distinct motor neurons within its first five divisions. I utilize stochastic cell labeling, optogenetic tools and the manipulation of temporal transcription factors in this lineage to identify developmental correlates of synaptic partner specification. This allows me to determine whether a neuron's connective preferences are specified by its intrinsic temporal identity, or the extrinsic environmental cues it sees upon differentiation from the neuroblast.

Program Abstract #473
Ret receptor signaling and retrograde transport is required for sensory pioneer axon outgrowth
Adam Tuttle1, Catherine Drerup2, Molly Harding1, Alex Nechiporuk1
1Oregon Health & Science University, USA; 2National Institutes of Health, USA
During development or following injury, axon terminals must navigate a complex environment to form functional connections. Often, axon growth and guidance are driven by pioneer axons, the first axons to grow in a region. One regulator of this process is neurotrophin signaling, where target-derived neurotrophic factors bind to specific receptors at the tips of growing axons. Activation of neurotrophin receptors induces their internalization and retrograde transport to the cell body, triggering a transcriptional response which in turn promotes axon outgrowth. Ret is a neurotrophin receptor with two distinct isoforms (Ret9/51) found on growth cones of extending axons where it is activated by its ligand, GDNF. Ret signaling is required for axon growth and guidance in sensory and motor neurons, but specifically how it promotes axon and growth cone advancement is largely unknown. Furthermore, while axonal trafficking of some neurotrophin receptors, such as TrkB, is well-studied, much remains unknown about activated Ret receptor trafficking in growing axons. We find the Ret51 isoform is specifically expressed in pioneer neurons of the zebrafish posterior lateral line ganglion (pLLG), and is required for pioneer axon outgrowth. ret mutants have fewer pioneer growth cones and/or significantly altered growth cone morphology. JNK-interacting protein 3 (Jip3) has known roles in retrograde transport in pLLG axons and jip3 mutants display a failure of pLL axon outgrowth similar to ret mutants. We found that Jip3 mediates retrograde transport of Ret51 and we identified a domain in Jip3 that putatively interacts with Ret51. Injection of a jip3 construct lacking this domain in jip3 mutants rescues all aspects of the mutant phenotype except the failure of axon outgrowth. These data suggest retrograde transport of Ret51 from the growth cone via Jip3 is required for proper sensory pioneer axon outgrowth and regulates cell biological behavior of the advancing growth cone.
Funding: OHSU
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