Society for Developmental Biology 83rd Annual Meeting
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ABSTRACT BOOK

Program Abstract #1
Exploring the Functional Significance of Shadow Enhancer Architecture
Jillian Ness, Yu Wang, Christian Mei, Renata Serio, Zeba Wunderlich
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Many developmental genes are regulated by a set of seemingly redundant enhancers that drive overlapping spatiotemporal patterns. These multi-enhancer systems are termed shadow enhancers. Shadow enhancers can buffer genetic and environmental stress to drive normal gene expression patterns. Using the Drosophila embryo as a model, we showed that shadow enhancers for anterior-posterior patterning genes drive consistent gene expression patterns by binding distinct sets of input transcription factors. Nevertheless, it remains unclear why shadow enhancer transcription factor binding sites are distributed across multiple enhancers rather than within a single enhancer. The mechanisms shadow enhancers employ could conceivably be encoded into a single enhancer. We have generated enhancer reporters in which the endogenous DNA between shadow enhancers is eliminated to make a 'squish' configuration. Surprisingly, we found little difference in RNA patterns, levels, and dynamics between the squish and endogenously spaced enhancers, suggesting the endogenous spacing is not needed for shadow function. We are building synthetic squish enhancers of varying lengths to test the lower size-limit at which the squish enhancer retains function. To understand the evolutionary dynamics that give rise to shadow enhancers arise in animal genomes, we have used bioinformatics to identify the origins of developmental shadow enhancer sets. Thurs far, we find duplication events and transposable elements appear to be a relatively small (<10%) source of developmental shadow enhancer birth in *Drosophila* compared to our findings in mouse genomes (~30%). These data indicate that combined, single enhancers can function comparably to distinct shadow enhancers, without compromising fidelity under stress. We will probe the evolutionary dynamics that create and maintain shadow enhancers to rationalize their pervasive role in animal development. Work funded by NIH grant R01HD095246.

Program Abstract #2
Genomic Regulatory Loops in the Drosophila Embryo and Brain
Michael Levine
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Long-range enhancer-promoter interactions depend on two classes of architectural elements in the Drosophila genome, boundaries and tethering elements. These serve to produce precise and timely activation of segmentation genes and Hox genes in the Drosophila embryo. Micro-C XL assays identified hundreds of regulatory loops in the Drosophila brain. A subset of loop anchor sequences is engaged in ultra-long-range interactions spanning 2-20 megabases across chromosome arms. Most of the genes associated with meta-loops encode IgSF (immunoglobulin superfamily) proteins, which mediate the formation of specific neural synapses in the optic lobe and other regions of the brain. I will discuss the possibility that they arise from chromosomal inversions and produce novel combinations of IgSF associations in different dipteran species such as mosquitoes. I will also discuss the possibility that variable folding of meta-loops could be a source of stochastic gene expression in the CNS. This study was funded by the NIGMS.

Program Abstract #3
Development and Aging, Rejuvenation and Disease
Juan Carlos Izpisua Belmonte
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George Williams once wrote, "It is remarkable that after a seemingly miraculous feat of morphogenesis a complex metazoan should be unable to perform the much simpler task of merely maintaining what is

already formed". Nonetheless, it is also true that the progress made in the last decades in unraveling the cellular and molecular processes that underlie development is already having and will certainly have major clinical implications for human disease and aging. Amongst the major advances is our enhanced understanding on the role played by the epigenome during embryogenes. Disease and aging are associated with altered epigenetic mechanisms of gene regulation. I will discuss some of the epigenetic changes that occur during development disease and aging, and how partial reprogramming and epigenetic changes elicited by the Yamanaka factors might be a potential avenue to restore cell health and resilience through cellular rejuvenation programming to reverse disease, injury, and the disabilities that can occur throughout life.

Program Abstract #4

Regeneration leads to global tissue rejuvenation in aging sexual planarians

Xiaoting Dai[1], Xinghua Li[1], Alexander Tyshkovskiy[2], Cassandra Zuckerman[1], Nan Cheng[1], Peter Lin[1], David Paris[1], Saad Qureshi[1], Xiaoming Mao[1], Jayakrishnan Nandakumar[1], Vadim Gladyshev[2,3], Scott Pletcher[1], Leonid Kruglyak[4], Jacob Sobota[1], Longhua Guo[1]

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The possibility of reversing the adverse impacts of aging could significantly reduce age-related diseases and improve the quality of life in the older population. Here, we report that the sexual lineage of the "immortal" planarian, *Schmidtea mediterranea*, exhibits physiological decline within 18 months of birth, including altered tissue composition, impaired fertility and motility, and increased oxidative stress. Single cell profiling in young and old planarian heads identified new adult stem cells with embryonic-like gene expression, es-neo-1/2, uncovered loss of neurons and muscle but not stem cells in older age and revealed generally slow aging rate in somatic stem cells. Comparative analysis of age-altered gene expression revealed shared signatures with aging in rodents and humans and planarian specific longevity signatures, comparable to lifespan extension interventions in mice. Remarkably, amputation followed by regeneration of lost tissues in older planarian led to a global reversal of these age-associated changes in tissues both proximal and distal to the injury. Our work comprehensively characterized aging signatures of the extremely long-lived planarians and suggested multiple mechanisms mediating global rejuvenation, which may provide valuable insights for anti-aging medicine in humans. This work is funded by grant number 0423 from the Global Consortium for Reproductive Longevity & Equality (GCRLE) and R21AG084959 (NIH).

Program Abstract #5

Members of the claudin family of tight junction proteins uniquely regulate distinct steps in neural tube morphogenesis via different molecular mechanisms.

Aimée Ryan[1,2,3], Tabitha DiCintio[1,3], Enrique Gamero-Estevez[1,3], Elizabeth-Ann Legere[1,3], Zafina Budhwani[1,3]

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Neural tube closure is a well-orchestrated morphogenetic process that requires the integrity of the neural epithelium and adjacent non-neural ectoderm to be maintained via intercellular junctions. Tight junctions are the most apical complex in eukaryotic epithelia, where claudins control paracellular permeability, apical-basal cell polarity, and link the tight junction to the actin cytoskeleton via their C-terminal cytoplasmic domains. Our work supports an evolutionarily conserved role for claudins in vertebrate neural tube closure. By individually depleting specific claudins, we discovered that each has a critical role in regulating distinct steps in neural tube closure. Moreover, overexpression of human CLDN variants identified in a spina bifida patient cohort affects neural tube closure in chick embryos. CLDN8 is required for convergent extension and apical constriction of cells in the neural plate, while CLDN3 is required in the non-neural ectoderm for neural fold fusion, the final epithelial remodelling event that gives rise to a closed neural tube and an overlying layer of ectoderm. Distinct changes in cell morphology and patterns of apical protein localization were associated with removal of different claudins. We hypothesize that claudin cytoplasmic C-terminal domains act as hubs for the network of apical proteins that direct specific events in neural tube closure. To test our hypothesis we identified claudin-specific interaction partners for CLDN1, 3, 4, 8 and 14 and are validating their roles in neural tube closure. Indeed analysis of candidate claudin-interacting proteins for the CLDN3 and CLDN8 C-terminal cytoplasmic domains

revealed distinct sets of proteins relative to those that were pulled-down by other claudins. Our goal in dissecting the claudin-specific mechanisms in neural tube closure is to identify new molecular pathways that can be targeted in future preclinical trials to prevent NTDs. This work has been funded by an NSERC Discovery Grant.

Program Abstract #6

Zebrafish models of idiopathic scoliosis link oxidative stress with intervertebral ECM defects, identifying first-in-kind prognostic biomarkers and possible therapeutic targets

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Pathomechanisms underlying adolescent idiopathic scoliosis (AIS), a pervasive pediatric disorder characterized by rotational spine deformity, are poorly understood. Patient exome/genome sequencing and mouse functional studies have associated genetic variants in musculoskeletal collagen and cartilaginous extracellular matrix (ECM) defects with a fraction of AIS cases. However, GWAS meta-analyses estimate that >95% of total genetic variance underlying AIS remains to be determined. Using defined zebrafish AIS models, we discovered that *oxidative stress* and *pro-inflammatory signals* in the spinal cord, which develop because of cerebrospinal fluid homeostasis defects, are necessary and sufficient to drive spine curvature. Indeed, immunomodulating drugs can efficiently block scoliosis onset and severe curve progression in fish models. Although this provides proof-of-principle that AIS might be managed therapeutically, uncertainties regarding downstream mechanism and their link to human disease pose barriers to clinical translation. Here, we demonstrate that oxidative stress in fish AIS models induces ER stress and ECM remodeling defects within intervertebral segments of the spine. Using shear wave elastography (SWE), we show that zebrafish scoliotic spines are consequently stiffer than healthy controls - a property also reported for intervertebral discs in human AIS patients. Remarkably, both elevated stiffness and collagen ECM phenotypes are detectable *prior* to scoliosis onset (suggesting a causal role) and are suppressed by antioxidant drugs known to suppress scoliosis in zebrafish. Together, our results identify direct mechanistic links between oxidative stress and connective tissue/intervertebral defects identified in both fish and human AIS studies. As SWE is non-invasive and widely applied in the clinic, tissue stiffness may translate into a valuable prognostic biomarker and therapeutic target for AIS. [Supported by CIHR FDN-167285 and CRC Program awards to B.C]

Program Abstract #7

Tracing the Origins of Structural Birth Defects in a Mouse Model of Cornelia de Lange Syndrome

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Cornelia de Lange Syndrome (CdLS) is a multisystem birth defects disorder caused most frequently by haploinsufficiency for *NIPBL*. *NIPBL* encodes a cohesin-interacting protein with broad effects on chromatin topology and gene expression. Nipbl-deficient individuals and animals exhibit many structural birth defects, including heart defects, and show numerous, small changes in gene expression in all tissues. Using *Nipbl*$^{+/-}$ mice as a model, we previously found evidence suggesting that such defects develop as early as gastrulation in CdLS. To study this, we performed scRNAseq on wildtype (WT) and *Nipbl*$^{+/-}$ mouse embryos at 2 stages spanning early and late gastrulation. *Nipbl*$^{+/-}$ embryos at both stages showed many small but significant changes in gene expression in every cell type, as expected. Surprisingly, they exhibited significant overexpression of *Nanog* in every germ layer. *Nanog* encodes a transcriptional repressor that is normally expressed transiently, first prior to implantation, and then during gastrulation. In *Nipbl*$^{+/-}$ embryos, however, *Nanog* expression remains significantly elevated post-gastrulation. Moreover, the majority of gene expression changes we observed in *Nipbl*$^{+/-}$ embryos were related to *Nanog* overexpression. Cell allocation analysis showed that *Nipbl*$^{+/-}$ embryos have fewer mesoderm cells than WT, as well as altered proportions of mesodermal cell subpopulations. These cell allocation differences

were associated with under-expression of genes implicated in driving specific mesodermal lineages, and these driver genes are also Nanog targets. Altogether, these findings establish a link between Nipbl-deficiency, Nanog overexpression, and dysregulation of gene expression leading to lineage misallocation, which ultimately manifest as birth defects in *Nipbl*$^{+/-}$ animals and in CdLS. *Supported by CCBS, NSF-Simons Ctr for Multiscale Cell Fate Research, NINDST32NS082174 (SC), R01HL138659 (ALC, ADL), R01DE019638 (ADL).*

Program Abstract #9

Germ cell to maternal transition during Drosophila oogenesis

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Our research program is focused on unraveling the mechanisms underlying the remarkable immortality of germ cells. Unlike somatic cells, germ cells, responsible for producing gametes, do not undergo aging. Instead, they pass on cellular components to the next generation without aging effects. The mature oocyte, in particular, plays a crucial role by contributing a haploid genome along with maternal RNAs, proteins, and organelles, which are essential for initiating the next generation and resetting cellular age. We are elucidating the quality control mechanisms that ensure the selection of appropriate components from maternal contributions while screening out damaged/inappropriate ones. One significant discovery we have made is the coordinated process of screening maternal contributions is linked to establishing proper oocyte identity. We refer to this process as the germ cell-to-maternal transition (GMT). I will discuss unpublished aspects of our findings during GMT where we find a dynamic regulation of the nuclear pore complex during oocyte specification.

Program Abstract #10

Physical mechanisms of primordial germ cell migration through the developing mouse embryo

Katharine Goodwin

MRC Laboratory of Molecular Biology, GB

Primordial germ cells (PGCs) eventually give rise to sperm and eggs, and are the only cells of the embryo that produce future generations. In many species, including fly, mouse, and human, PGCs are specified far from their final destination (the future gonads) and must travel through a diverse range of developing tissues in the embryo. Successful completion of this journey is required for fertility, and yet little is known about how PGCs migrate in mammals. Here, we use adaptive light-sheet microscopy, time-lapse imaging, and transgenic reporter mice to image PGC migration during mouse embryogenesis. We show that PGC migratory behaviour is influenced by the developing tissues around them. They form actin-rich protrusions during migration, suggesting different migration modes than those described in other model organisms. As they traverse ECM barriers and tight intercellular spaces, PGCs and their nuclei undergo significant deformation, sometimes leading to nuclear rupture and/or cell death. Later stages of migration are associated with an increase in DNA damage markers, which may result from the effects of confined migration on the nucleus. The nuclear lamina changes drastically over the course of migration, leading to wrinkled nuclear morphology and potentially helping PGCs squeeze through confined spaces without damage. Our high-resolution and dynamic imaging approaches provide new insights into the expansive and historically inaccessible journey of PGCs during mouse embryogenesis. Funding: Medical Research Council, as part of UK Research and Innovation [MCUP1201/23]

Program Abstract #11

The dynamics of parental allelic imbalance at single-cell resolution in hybrid prote-vertebrate lineages

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The inheritance of both maternal and paternal copies of the zygotic genome is essential for normal animal development. Yet, we are not simply the sum of our parents' genes. Classical studies have identified a handful of examples of allelic imbalanced events including imprinted genetic loci, olfactory receptors, and X-inactivation. Allelic imbalance has been suggested to influence key developmental processes during embryogenesis, and also influence genetic predilections to disease. Emerging RNA sequencing technologies raise the possibility that conditional allelic imbalance may be prevalent in

animal genomes, but the underlying mechanisms are poorly understood. Here we obtained the first high-resolution map of allelic imbalance for a complete animal embryo during development, using hybrid embryos of two divergent species of the ascidian, Ciona: *C. intestinalis* and *C. savignyi*. The resulting comprehensive single cell transcriptome lineages of hybrid embryos identified global allelic heterogeneity in single cells, which is masked by conventional bulk measurement of average gene expression across cells. Notably, we observed allelic imbalance in the migrating trunk ventral cells, which may contribute to heart cell migration. This finding provides insights into how transient regulatory states interface with the dynamic cellular processes underlying morphogenesis. Furthermore, we found that the allelic imbalanced expression of the Hox gene family is associated with cell positions along A-P axis, which sheds light on the role of Hox genes in establishing the A-P axis in Chordates. Considering the close relationship between ascidians and vertebrates, uncovering the regulatory patterns of allelic imbalance in Ciona could provide valuable insights into how such imbalances contribute to inherited diseases in humans. This study is supported by NICHD (R00HD102584).

Program Abstract #12

The identification of type I MADS box genes as the upstream activators of an endosperm-specific invertase inhibitor in Arabidopsis

Dongfang Wang

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Nuclear endosperm development is a common mechanism among Angiosperms, including Arabidopsis. During nuclear development, the endosperm nuclei divide rapidly after fertilization without cytokinesis to enter the syncytial phase, which is then followed by the cellularized phase. The endosperm can be divided into three spatial domains with distinct functions: the micropylar, peripheral, and chalazal domains. Previously, we identified two putative small invertase inhibitors, *InvINH1* and *InvINH2*, that are specifically expressed in the micropylar region of the syncytial endosperm. In addition, ectopically expressing *InvINH1* in the cellularized endosperm led to a reduction in embryo growth rate. However, it is not clear what are the upstream regulators responsible for the specific expression of *InvINHs* in the syncytial endosperm. Using protoplast transient expression system, we discovered that a group of type I MADS box transcription factors can form dimers to activate *InvINH1* promoter. Promoter deletion assays carried out in the protoplast system revealed the presence of an enhancer region in *InvINH1* promoter, which contains several consensus cis-elements for the MADS box proteins. Using promoter deletion assay in planta, we further demonstrated that this enhancer region is required for *InvINH1* expression in the syncytial endosperm. One of the MADS box genes, *AGL62*, is a key transcription factor required for syncytial endosperm development. Using promoter-GFP reporter assay, we demonstrated that *InvINH1* and *InvINH2* are not expressed in *agl62* mutant seeds. Collectively, our data supports the role of *AGL62* and other type I MADS box genes as the upstream activators of *InvINHs* expression in the syncytial endosperm. Our findings revealed several type I MADS box genes that are responsible for activating *InvINH1* in the syncytial endosperm, which in turn regulates embryo growth rate during early stage of seed development. Funding source: NSF-BIO-IOS, RUI Grant #1656556

Program Abstract #13

The first two blastomeres contribute unequally to the human embryo

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Retrospective lineage reconstruction of humans predicts that dramatic clonal imbalances in the composition of the body can be traced to the 2-cell stage embryo. However, whether and how such clonal asymmetries arise in the pre-implantation embryo is unclear. Exploration of these questions in human embryos remained challenging due to extremely limited human zygote access and lack of appropriate techniques. We have performed the first prospective lineage tracing of human embryos, from the zygote to the blastocyst stage, using live imaging, non-invasive cell labelling and computational predictions to determine the contribution of each 2-cell blastomere to the epiblast (body), hypoblast (yolk sac) and trophectoderm (placenta). We discover that most epiblast cells originate from only one 2-

cell stage blastomere in most embryos. Mechanistically, we observe that the first wave of inner cell allocation by asymmetric cell division at the 8- to 16-cell stage transition underpins this clonal imbalance. Only one to three cells become internalized at this stage, unbalancing the clonal makeup of the inner cell founding population. Moreover, these internalized cells are more frequently derived from the first blastomere to divide at the 2-cell stage. We propose that cell division dynamics and a cell internalization bottleneck in the early embryo establish asymmetry in the clonal composition of the pre-implantation epiblast, the source of the future human body. This work was supported by Human Science Frontiers Program, NOMIS Foundation, Wellcome Trust, Open Philanthropy Project, and the Curci and Weston Heavens Foundations.

Program Abstract #14

Decoding FGF, BMP, and Nodal signaling using molecular optogenetics in zebrafish

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Embryonic cell fate decisions are influenced by signaling levels, dynamics, and combinations. To experimentally manipulate these signaling features and determine how they are decoded by embryonic cells, we are characterizing a suite of optogenetic tools that reversibly activate FGF, BMP, and Nodal signaling in zebrafish embryos in response to blue light. We demonstrate that these blue light-responsive optogenetic tools activate specific pathways with 450 nm light exposure, but not with wavelengths above 495 nm. In addition, we are quantifying the tools' on/off kinetics (minutes), light dosage dependence, and ability to locally activate signaling. To facilitate future experiments, we are generating transgenic zebrafish containing maternally deposited, ubiquitously expressed optogenetic signaling activators. Finally, to explore how pathway target genes respond to signaling levels and dynamics, we are using RNAseq to identify transcriptome-wide gene expression responses to optogenetically delivered signaling pulses with different amplitudes and durations. These tools provide a powerful platform to investigate how embryonic cells decode signaling levels, dynamics, and combinations. Funding: NIH Intramural ZIAHD009002-01 to KWR.

Program Abstract #15

Sea urchin larvae utilize light for regulating the activity of the gut

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Light is essential for the development and functioning of life on Earth. For example, it is integral to the development of visual systems through reflection and plays a pivotal role in setting the circadian rhythms of many organisms according to sunlight cycles. The role of light in the evolution of deuterostomes, particularly within the context of Ambulacraria—a major group of deuterostomes and the sister group to chordates—has been less explored, especially concerning the signaling pathways involved in light response. In our study, we focus on sea urchin larvae, demonstrating how light regulates activity in the digestive tract. Our experiments show that light exposure can trigger pyloric and anal openings in the absence of food. Through our investigations, we have identified a light>Go-Opsin>serotonin>nitric oxide pathway responsible for regulating pyloric opening, while different opsins and neurotransmitters are involved in anal opening. Our findings shed light on the evolution of light-dependent systems related to digestive tract activities and neurotransmitter function, providing valuable insights into their establishment during the animal evolution. Drawing from previous research on brain-gut interactions in vertebrates, we speculate that one primitive function of anterior neuroectodermal neurons (brain neurons) might have been to regulate digestive tract function in the common ancestor of deuterostomes. Considering the fundamental importance of food consumption and nutrient absorption for animals, the acquisition and development of a sophisticated gut regulatory system driven by the brain could have played a significant role in deuterostome evolution. This work is supported by JST JPMJPR194C and MEXT KAKENHI 22H02670.

Program Abstract #16

Alternate pathways for enhancer activation during the maternal-to-zygotic transition

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Our lab aims to discover conserved, convergent, and divergent mechanisms regulating the maternal-to-zygotic transition (MZT) across diverse animals, during which an initially silent egg genome becomes transcriptionally active in the developing embryo. Here, we present the unexpected confluence of two lines of inquiry in different taxa -- the vertebrate zebrafish and the cnidarian *Hydractinia symbiolongicarpus* -- that further elucidates how regulatory DNA is activated during the MZT. First, in zebrafish we and others previously described essential roles for pluripotency factors Nanog, Pou5f3 (Oct4 homolog), and Sox19b (Sox2 homolog) (NPS) in embryonic genome activation, which were later found to function as "pioneers" that facilitate open chromatin and activating histone modifications. However, some gene activation still occurs in the absence of these factors. By profiling the embryonic histone modification landscape using CUT&RUN, we discovered a subclass of enhancers that uniquely bear H3K4me2 that do not require NPS pluripotency factors for activation. Rather, they seem to be epigenetically bookmarked to attain chromatin accessibility, thus implicating a parallel pathway in zebrafish for embryonic genome activation. Second, in Hydractinia, we discovered an expanded family of novel Kmt5a-like methyltransferases coordinately expressed at genome activation, suggesting a role for H4K20 methylation during the MZT. Although initial comparison with other cnidarians suggested these Kmt5a-like genes were specific to hydrozoans, we have now found likely orthologs in a small selection of phylogenetically diverse animals, including sea urchin and bony fish, that are similarly activated in early embryos. Thus, we are currently investigating the roles of the Kmt5a-like genes and H4K20 methylation in zebrafish embryos and how they may contribute to enhancer regulation and transcriptional reprogramming during the MZT. This work was funded by NIH grant R35GM137973.

Program Abstract #17

Evolutionary origin of the outer ear through co-option of an ancestral gill program

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The outer ear is a mammalian innovation whose evolutionary origins have remained a mystery. It is formed through outgrowth of neural crest-derived cells and contains a unique type of elastic cartilage. Here we find that the gill filaments of diverse fishes are supported by a similar type of elastic cartilage to that of the outer ear. Comparative single-nuclei multiomic analyses of the human fetal outer ear and zebrafish gill cartilages reveals common gene expression and enrichment of transcription factor binding motifs in differentially accessible regions (DARs) of chromatin. This conserved gene regulatory architecture is reflected by specific transgenic activity of human outer ear enhancers in zebrafish gills, and reciprocal activity of zebrafish gill enhancers in the mouse outer ear. Despite loss of gills in early tetrapods, we identify multiple gill-specific DARs that are sequence conserved and differentially accessible in the human fetal outer ear. These include a Dlx5/6 enhancer that drives specific expression in fish gills and the mouse outer ear, consistent with enrichment of a conserved DLX-binding motif in gill and outer ear DARs. Remarkably, we find that this DLX-mediated gill program may be conserved in the book gills of the Atlantic horseshoe crab, which are supported by tissue resembling elastic cartilage. Single-cell multiomics of horseshoe crabs reveals a cartilage-associated mesenchyme population expressing DLX homolog *distalless* and with DARs enriched for a similar DLX motif to that of zebrafish gill and human outer ear DARs. Further supporting homology with vertebrate gills, a horseshoe crab *distalless* enhancer drives specific expression in zebrafish gills. These findings suggest the mammalian outer ear arose from co-option of an ancestral gill program and show the power of cross-species enhancer testing to reveal cryptic conservation across vast evolutionarily timescales. Funding: NIDCR training grant F31DE030706, USC Stem Cell Challenge Grant.

Program Abstract #18

On the origins of novelty and diversity in development and evolution: insights through the study of horned beetles

Armin Moczek

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The initiation, individuation, and diversification of evolutionary novelties are key foci of evolutionary developmental biology. Beetle horns and horned beetles have emerged as a powerful model system

with which to investigate the nature of - and mechanisms underlying - these phenomena. In this talk I synthesize recent advances in our understanding of the origins of beetle horns. Even though with respect to their function as weapons in male combat *a horn is a horn is a horn* regardless of where it develops, this is not the case with respect to their genetic and developmental underpinnings, nor their evolutionary histories. Specifically, our work shows that horns developing from the first thoracic segment (T1) derive from partial wing serial homologs found bilaterally in every thoracic and abdominal segment. Horn initiation and positioning are thus under the control of a dedicated, conserved, and ancient gene regulatory network (GRN). In contrast, horns developing from the posterior head appear to derive via localized over-proliferation of head sclerites and without the governance of a dedicated head horn GRN. Intriguingly, despite their disparate evolutionary origins, initiation mechanisms, and degree of individuation, the developmental genetic means that facilitate diversity in horn formation appear to be widely shared across both head and thoracic horns, such as the regulation of sex- or nutrition-responsive horn growth. I discuss the implications of these results for our understanding of the genesis of novelty in development and evolution in insects and beyond. This work was made possible through generous financial support from the U.S. National Science Foundation, the John Templeton Foundation, the John Simon Guggenheim Foundation, and the Australian American Fulbright Foundation.

Program Abstract #19

Sensory receptor expansion and neural accommodation between flies and butterflies

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The evolution of larger and more complex brains required existing neurons and neural circuits to accommodate new inputs. The genetic and developmental basis of how neural accommodation occurs is largely unknown. Relative to other insects such as *Drosophila*, butterflies have evolved more complex retinal mosaics and expanded color vision through the addition of a second R7-type photoreceptor per ommatidium (unit eye). We investigated the developmental basis of this change in the retina and how butterfly brains accommodate an expansion in sensory receptor input. We identified four differences in transcription factor expression during retina development between flies and butterflies and tested the genetic relationships between each. Modifying *Drosophila* retinas to have butterfly-like expression produces flies that recruit the "mystery cell" as a second R7, in the same position as in butterflies. These ommatidia have two R7s and each makes an independent stochastic choice, like butterflies, leading to three stochastically distributed ommatidial types instead of two. In *Drosophila*, the main target of each R7 is a single Dm8 neuron per retinotopic medulla column, and we asked how additional R7s impact Dm8 number and position. We show that in the presence of extra R7s, additional Dm8 cells are retained during development and that two Dm8s can now target the same medulla column. We propose a model in which an excess of Dm8 cells provided developmental flexibility that helped immediately accommodate an increase in sensory receptor input. Neurons like Dm8, which are made in excess during development to help accommodate population-level variability in input types, could provide increased developmental flexibility and enable broader neural evolution. Funding for this work was provided by the NIH and AFOSR.

Program Abstract #20

Mechanism of Fruit Initiation and Diversity in Rosaceae

Zhongchi Liu

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Rosaceaeis an economically important family with species prized for high value fruits and ornamentals. The family also exhibits diversefruit types, including drupe (peach), pome (apple), drupetum (raspberry), achenetum (strawberry), and others. The distinct fruit-forming ability of homologous floral organs in these four species allows us to investigate the mechanism, under which different fruit types are derived. Through developmental, morphological, physiological, and transcriptomic analysis of each of the four fruit types and subsequent comparative analyses, we identified B and E class MADS box genes and lignification as some of the determining or contributing factors for the evolution of different fruit types. At the same time, we selected *AGL62,* a type I MADS box transcription factor gene, for further functional studies. Through CRISPR gene editing, we found that *AGL62* plays a critical role in initiating fruit development by inducing auxin biosynthesis in the seed. Our work lays the foundation for future engineering of different fruit types,

including parthenocarpic fruits, andpromotes both basic understanding and application toward increasing fruit quality and yield. This work has been supportedby a grant from the National Science Foundation (IOS 1444987).

Program Abstract #21

Zebrafish *her3* knockout impacts developmental and cancer-related gene signatures

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Rhabdomyosarcoma is an aggressive pediatric muscle-like cancer, accounting for approximately 5-8% of all pediatric cancers. Fusion-positive rhabdomyosarcoma (FP-RMS) is driven by one of several chromosomal translocation mutations, the most common and lethal of which is PAX3::FOXO1. Previously, we found that the neural transcription factor, HES3, is overexpressed in FP-RMS tumors and predicts reduced overall survival. HES3 is a basic helix-loop-helix transcription factor involved in neural stem cell renewal during early development. However, its mechanism of action in FP-RMS is still unknown. To investigate the role of HES3 in both development and FP-RMS, we used CRISPR/Cas9 to generate a knockout in zebrafish of the *HES3* ortholog, *her3*. Consistent with previous morpholino studies, we found that Her3 negatively regulates its own transcription, leading to an increase in *her3* mRNA in the knockout. *her3* knockout mutants displayed early developmental delays, and a subset of adults developed severe eye defects. Differentially expressed genes in the *her3* knockout mutants compared to wildtype controls were significantly enriched for motifs such as OLIG2 and SIX1, as well as HOX and SOX10, suggesting these genes play a key role in Her3/HES3 dysregulation. Further, pathway analysis of transcriptional signatures showed significant suppression of the matrix metalloprotease and tumor microenvironment pathways, indicating that Her3/HES3 dysregulation is linked to tumorigenic processes. Using ChIP, we identified a consensus binding motif for zebrafish Her3 and found it binds mostly to promoter regions of developmental genes. Interestingly, we found that PAX3::FOXO1 directly binds to the promoter region of *her3* to regulate its expression. Altogether, this data is suggestive of a direct regulatory relationship between Her3/HES3 and PAX3::FOXO1 to target various neuronal and developmental pathways in FP-RMS. Funding: T32CA269052, R01CA272872, ALSF A Award, V Foundation V Scholar Grant

Program Abstract #22

Mad/Medea and Zen Dynamics are Predictive of BMP Target Gene Transcription

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Embryonic development in numerous species hinges on the dynamics and regulation of the BMP signaling pathway. However, we have limited understanding as to how cells produce diverse but specific responses to similar signaling inputs *in vivo*. In the *Drosophila* embryo, a steep gradient of BMP signaling is dynamically established prior to gastrulation. This gradient is interpreted by different populations of cells to establish the dorsal-ventral axis of the embryo, with cells at the dorsal midline turning on a unique set of transcripts compared to more lateral cells. We do not know how the dynamics of gradient formation and the final gradient pattern are interpreted by cohorts of cells in the embryo to produce the correct spatiotemporal transcriptional response. We have developed an endogenous reporter of BMP signaling, allowing us to measure signaling dynamics in live embryos for the first time. Using MS2 reporters of live transcription, we show that BMP responsive promoters become active in response to small, but rapidly changing levels of BMP activity. By modeling the input-output relationship between BMP activity and transcription, we predict the kinetics of BMP transcription factor activity. In *sog* mutant embryos, which have altered BMP dynamics, we can still predict the transcriptional output of target genes based on the history of BMP signaling in each nucleus. However, in *zen* mutant embryos, the input-output relationship between BMP and transcription is altered. Zen is a transcription factor that we hypothesize promotes transcriptional activity at BMP target genes, lowering the predictive Kd for BMP-responsive transcription

factors in our model. Ongoing ATAC-seq experiments from *zen* embryos will allow us to test if Zen opens chromatin at BMP responsive promoters, perhaps allowing them to quickly respond to a dynamic signaling environment. Funding: NIH F32, NIH R01

Program Abstract #23
The role of FGF/ERK signaling in 2D human gastrulation model
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During gastrulation the body plan is laid out and the three germ layers are established. An important role in gastrulation is played by secreted cell signaling molecules called morphogens. Studies in the mouse have shown four different families of morphogens are required for mammalian gastrulation: BMP, Wnt, Nodal, and FGF. While much has been learned about the first three, the role of FGF is less understood. Moreover, there may be significant interspecies differences. For example, FGF8 is the only FGF which has been shown to be required for gastrulation in the mouse, yet FGF8 is conspicuously not expressed in the human embryo. Here, we explore the role of FGF/ERK signaling in the cell fate patterning of micropatterned human pluripotent stem cells. We show that a spatial ring of ERK activity forms in an FGF-dependent manner and expands inward largely in lockstep with the formation of primitive-streak like cells but extending further inward. We find that this pattern of ERK activity is due to localized FGF Receptor activation that requires basal FGFR polarization. We show that exogenous FGF in the media is required for pattern formation to occur but can be removed well before the phosphorylated ERK and BRA rings appear, suggesting the ERK activity instead depends on endogenous FGF gradients. Using single cell RNA-sequencing, we determine the expression of FGF pathway components and identify FGFR1 as the main receptor and FGF2, FGF4, and FGF17 as the main ligands. We find that FGF4 expression profile closely matches expression of BRA and ERK activity and that knockout and knockdown of FGF4 severely diminish BRA expression, in contrast to FGF17 knockout which leads to a smaller reduction in BRA. In summary, we have gained significant insight into how FGFs control formation of a primitive streak-like ring in a model for human gastrulation. This work is supported by NSF RECODE 2033654, the NIGMS312 R35GM138346, and the Branco Weiss Fellowship–Society in Science.

Program Abstract #24
Regulation of the temporal variations of limb development during tetrapod evolution
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Alterations in tissue growth timing, known as heterochrony, is thought as a driving force for evolution, but has been poorly addressed mechanistically. A prominent example is the limb heterochrony, where different tetrapod clades exhibit distinct temporal patterns for forelimb and hindlimb growth. In general, mammalian species display delayed hindlimb development compared to the forelimb. However, this feature is absent in avian species, where forelimbs and hindlimbs grow simultaneously. Such a distinction has been hypothesised as an adaptation to the energy supplies. Yet, the regulatory mechanisms of limb heterochrony remain elusive. To comprehend limb heterochrony regulation in mammals, I compared the timing of key limb developmental events in chicken to that in mouse and opossum embryos. I found that the mammalian limb heterochrony starts at the limb initiation, characterised by the Epithelial-to-Mesenchymal Transition (EMT). The varying timing of EMT is in turn associated with the timing of limb outgrowth regulators. Unexpectedly, I found that this heterochronic expression is not due to changes in cis-regulatory elements or the timing of growth factor induction but is linked to the physiological conditions experienced by the growing embryo. Functional navigation revealed that, the differential oxygen levels to which avian and mammalian embryos are exposed mediate the heterochronic expression of limb outgrowth factor. Specifically, hypoxic environment in early mammalian embryogenesis suppress hindlimb initiation. By integrating RNA-sequencing analyses with gain- and loss-of-function assays, I determined that hypoxia's impact on hindlimb development is at least partially implemented through the expression of NFKB transcription factors. Taken together, these results provide a

comprehensive mechanistic account of developmental heterochrony, and exemplifies how tissue growth alters the timing during evolution. Funding: HFSP Long-term fellowship (LT000676/2020-L).

Program Abstract #25
Specification and survival of motor neurons in a non-vertebrate chordate by conserved transcription factors Pax2/5/8 and Phox2
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Tunicates are the sister group to the vertebrates, yet most species have a life cycle split between swimming larva and sedentary adult phases. During metamorphosis, the transition between larval and adult phases, larval neurons are largely replaced by adult-specific ones. This is thought to require the establishment of quiescent neural progenitors during the larval phase. The regulatory mechanisms underlying this neural replacement remain largely unknown. Using tissue-specific CRISPR/Cas9-mediated mutagenesis in the tunicate Ciona, we show that orthologs of conserved hindbrain and branchiomeric neuron regulatory factors Pax2/5/8 and Phox2 are required to specify the "neck", a compartment of cells set aside in the larva to give rise to cranial motor neuron-like neurons in the adult. Surprisingly, we find that neck-derived adult ciliomotor neurons begin to differentiate in the larva, contrary to the long-held assumption that the adult nervous system is formed only after settlement and the death of larval neurons during metamorphosis. Moreover, we find evidence for complex functions for Phox2-mediated regulation of cell proliferation and neuronal differentiation. Finally, we show that manipulating FGF signaling during the larval phase alters the patterning of the neck and its derivatives. Suppression of FGF converts Neck cells into larval neurons that fail to survive metamorphosis, while prolonged FGF signaling promotes an adult neural stem cell-like fate instead. Taken together, we provide the first insight into Neck-specific gene regulatory networks and unique cell behaviors not yet characterized. This work is supported by NIH grants R01GM143326 and F32GM150234.

Program Abstract #26
Cytoneme signaling provides essential contributions to mammalian tissue patterning
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During development, morphogens instruct cell fate to pattern tissues by inducing distinct transcriptional programs. This is achieved through graded concentrations and durations of signal exposure across tissues. Visualizing morphogen transport in situ has been unfeasible, so the molecular mechanisms controlling morphogen delivery remain unclear. To tackle this longstanding problem, we developed a mouse model for compromised sonic hedgehog (SHH) morphogen delivery and discovered that endocytic recycling promotes SHH loading into signaling filopodia called cytonemes. We developed methods to preserve in vivo cytonemes and show endogenously expressed SHH localized to cytonemes in developing mouse neural tubes. Depletion of SHH from neural tube cytonemes alters neuronal cell fates and compromises neurodevelopment. Mutation of the filopodial motor myosin 10 (MYO10) reduces cytoneme length and density, which corrupts neuronal signaling activity of both SHH and WNT. Combined, these results demonstrate that cytoneme-based signal transport provides essential contributions to morphogen dispersion during mammalian tissue development and suggest MYO10 is a key regulator of cytoneme function. This work was supported by NIH R35GM122546, NCI P30CA021765 (SJCRH Cancer Center Support Grant), and ALSAC of St. Jude Children's Research Hospital.

Program Abstract #27
Novel roles for centriolar protein WDR90 in endothelial cells and cardiac tissue
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Congenital heart disease (CHD) is the most common type of birth defect and the leading cause of birth defect-associated infant mortality. Interestingly, genetic studies from patient cohorts with CHD reveal an unexpectedly high association with cilia-related gene variants. Through collaborative access to whole-

exome sequencing, whole-genome sequencing, and RNA-sequencing datasets from parent-offspring trios enrolled in the Pediatric Cardiac Genomics Consortium, we have been able to identify de novo and recessive cilia-related variants that associate with diagnosed, symptomatic CHD. From this, we have uncovered WDR90 as a novel driver of heart malformations. WDR90 has been previously identified as a centriolar wall protein, but no connection has been made between WDR90 function and vascular defects. We have found that while *wdr90* mutant embryos survive through the embryonic stages, adult *wdr90* mutant zebrafish experience partial lethality, stenosis, and an appreciably decreased heart size, consistent with CHD defects. Using cell culture, we find that WDR90 is highly expressed in endothelial cells and co-localizes with VE-cadherin at adherens junctions, suggesting a role for WDR90 in the function or stability of EC junctions. Furthermore, WDR90 colocalizes with CAMSAP3, a microtubule minus-end marker, at adherens junctions. Interestingly, the C-terminal domain of WDR90 can act as a weak nucleator of actin filaments, which may indicate a role for WDR90 in nucleating and bridging cytoskeletal structures. Consequently, uncovering the mechanisms behind novel cilia-related CHD driver genes such as WDR90 will have a meaningful impact on understanding and treating CHD. **AHA Postdoctoral Fellowship #906436 1R35GM137976**

Program Abstract #28
Ancestral modules of appendage patterning
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Modularity is key to the evolution of novel forms and function. Modules of integrated developmental signaling, or regulatory modules, are engrained into the logic of development and underlie the generation of diversity in evolution. It is not entirely clear how such modules became established, or how they vary. Here, I detail work on the origin and evolution of dorsal-ventral (DV) patterning in vertebrate fins and limbs. Tetrapod limbs require DV polarity for articulation and locomotion. Interactions between the genes En1, Wnt7a, and Lmx1b are essential to establish DV polarity in the limb. However, it is not known how this patterning module arose in evolution. Using high-resolution *in situs,* we show that *en1*, *wnt7a*, and *lmx1bb* are expressed in non-overlapping, DV-resolved domains of the developing zebrafish pectoral fin as seen in limbs. While somatic deletion of *lmx1bb* is lethal, mosaic loss of function in crispant fish results in loss of dorsal identity, causing a "double ventral" phenotype of the pectoral fin. This phenotype closely resembles that observed in mouse Lmx1b loss of function mutants. Next, we identified *Lmx1b* limb regulatory elements conserved across gnathostome evolution. As in mouse, targeted removal of a master regulatory hub from zebrafish recapitulated the double ventral phenotype while retaining viability. These results indicate that the regulation of Lmx1b and its function to specify dorsal identity was present in the bony fish ancestor, conserved in both tetrapods and teleost fishes. Surprising, although many regulatory mechanisms of paired appendages are modular and can be traced to their action in unpaired fins, we find that these fins are unaffected in our *lmx1bb* mutants. These data suggest that Lmx1b may have evolved divergent functions in paired fins, reflecting the establishment of a module of axial patterning in the evolution of paired appendages. This work was supported in part through NICHD 5R01HD112906

Program Abstract #29
Enhancer Modularity in Long-Range Developmental Gene Regulation
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Transcriptional enhancers are non-coding DNA elements that are typically 200–2,000 bp in length and drive gene expression patterns in space and time. Developmental gene regulation by enhancers exhibits several levels of modularity. On a scale of an individual regulatory element, each enhancer typically contains many modular binding sites for sequence-specific transcription factors. On a scale of a genetic locus, a typical metazoan gene contains multiple modular cell type-specific enhancers spread across large genomic distances, which collectively produce a complex gene expression pattern during development. I will discuss a potentially new level of modularity important for long-range enhancer action. Through in vivo whole mouse enhancer replacement experiments, we uncovered a novel conserved cis-acting element, Range EXtender (REX), that acts in a modular fashion and confers extreme-distance regulatory activity. This discovery arose from the observation that short- and medium-

range limb enhancers cannot initiate gene expression and support limb development when transplanted in place of the Shh ZRS, a benchmark long-range enhancer. The addition of the REX element, which itself shows no enhancer activity, enables a limb enhancer with a native range of 71 kb to act over 840 kb of genomic space. The REX element contains two highly conserved [C/T]AATTA homeodomain-like motifs. A genome-wide examination across thousands of other limb enhancers revealed that the presence and density of [C/T]AATTA homeodomain-like motifs correlates with enhancer-promoter distance. In summary, we identify a sequence signature associated with long-range enhancer-promoter interactions and describe a prototypical REX element that is necessary and sufficient to confer extreme-distance gene activation by remote enhancers.

Program Abstract #30

A matter of time: Sulfatase activity governs the onset of gastrulation morphogenesis

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To shape the emerging body plan, morphogenetic cell movements must be precisely coordinated not only in space, but also in time. In zebrafish, the convergence & extension (C&E) cell movements that shape the anteroposterior axis are triggered at mid-gastrulation by an unknown signal. Zebrafish embryonic explants faithfully recapitulate not only these cell movements, but also their precise timing in culture, providing an ideal system to identify the C&E timing signal in the absence of other confounding cell movements. We determined that new gene expression at gastrulation onset is required to 'kick start' C&E in explants, and that *sulfatase modifying factor 2* (*sumf2*) is first expressed at this time. *Sumf1* and *sumf2* encode Formylglycine Generating Enzyme (FGE) and its antagonist and paralog pFGE, respectively, a pair whose balance determines the activity of every sulfatase enzyme in the body. In both embryos and explants, the abundance of *sumf1* transcripts drops just as *sumf2* is expressed, leading to an inversion of *sumf1/sumf2* levels that we hypothesize triggers C&E cell movements through a decrease in sulfatase activity. We found that shifting the balance between *sumf1* and *sumf2* disrupts C&E in zebrafish gastrulae, as does overexpression of two sulfatases targeting heparan sulfate proteoglycans (HSPGs): *sulf1* and *sulf2a*. Incredibly, increasing sulfatase activity through overexpression of *sumf1* or *sulf1* delays C&E onset within explants, while increasing *sumf2* (and thus decreasing sulfatase activity) drives precocious C&E *ex vivo*. These manipulations similarly delayed or advanced the onset of C&E cell behaviors within intact gastrulae. These results support a model in which a switch in HSPG sulfation patterns triggers the onset of morphogenetic cell movements, thus elucidating the molecular basis of a critical embryonic timing system. Supported by: NIH/NICHD R00 HD091386 and R01 HD104784.

Program Abstract #31

Cadherin switching and mesoderm differentiation trajectories in human embryonic stem cells

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The process of mesoderm differentiation involves the formation of the primitive streak and an epithelial to mesenchymal transition (EMT), marked by a switch from E- to N-cadherin expression (E-Cad and N-Cad, respectively). Protocols to differentiate human embryonic stem cells (hESCs) to subtypes, such as paraxial and lateral mesoderm (PM and LM, respectively) have been developed, but it is unclear if cadherin switching dynamics differ between subtypes and if switching and differentiation are intrinsically linked. We addressed these questions in mesoderm differentiation of hESCs. We found that signaling pathways regulated cadherin switching independently from the final induction outcome. Modulating WNT, BMP, or Activin/Nodal signaling during PS induction on day1 had little effect on the fate outcome, but a strong effect on cadherin switching. BMP inhibited cadherin switching, while ACTIVIN and WNT promoted it. Consistently, we found that previously described protocols to generate anterior, mid, or posterior PS progenitors on day1, were all capable of efficiently inducing PM or LM on day2, however, the final degree of cadherin switching was strongly affected by the day 1 protocol. Moreover, the plasticity of PS progenitors was dependent on E-Cad, as in E-Cad knockout cells, the fate outcome also depended on the day1 signaling protocol. To visualize and quantify cadherin dynamics in real-time, we built a dual-reporter hESC line with fluorescently-tagged E-Cad and N-Cad at the endogenous loci. Live-cell imaging revealed that E-Cad downregulation preceded the start of N-Cad upregulation by ~4 hrs. Real-time analysis confirmed that ACTIVIN promotes EMT, but revealed nuances in the relationship between BMP

and EMT. Together, our work shows that cadherin switching and cell fate decisions can be separately modulated, but that cadherins preserve the plasticity of cells during differentiation. The research is funded by NIH R01GM126122 and R35GM149328, NSF MCB-2135296

Program Abstract #32

Tissue Geometry Affects Distribution of Chemical Cues that Guide Collective Cell Migration

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Graded concentrations of diffusible signals, such as morphogens and chemoattractants, instruct important developmental processes, like cell specification and cell migration. However, signal distribution in complex tissue geometries *in vivo* is not well characterized, which has implications in understanding concentration-dependent signaling effects. Using the border cells, which migrate collectively in the Drosophila egg chamber during oogenesis, we can study these important issues *in vivo*. We hypothesize that distinct features in the tissue geometry along the migration route, specifically acellular gaps at cell-cell intersections, disrupt local concentrations of secreted chemoattractants to affect directed border cell migration. In live-imaged wild-type egg chambers, we observed declines in posterior speed that occur specifically at intersections, suggesting that migratory cues differ in these regions. We developed a mathematical model, informed by this data, to predict the migration changes in response to both chemical and architectural inputs. *In silico*, border cells slowed down in intersections due to local changes in the chemoattractant gradient. Similarly, *in vivo*, border cells slowed and behaved differently in response to elevated levels of one known chemoattractant within specific architecture types over the course of migration. Importantly, slow border cell migration due to high chemoattractant levels could be rescued by genetically manipulating the tissue geometry, which strongly suggests that chemoattractant concentrations can be buffered by tissue architecture. To understand these phenotypes better, we expressed tagged chemoattractant and visualized its distribution live, which was irregular and correlated with features of the tissue geometry. Our results show that *in vivo* tissue geometry affects the distribution of important signaling molecules, with consequences for essential developmental processes. Funding: NSF-IOS 2303857 & NSF-NIGMS 1953423.

Program Abstract #33

Molecular and Cellular Evidence for Lateralized Asymmetries of Anterior Somites

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The vertebrate body exhibits left-right (LR) asymmetry in visceral organs while maintaining apparent bilateral symmetry in musculoskeletal system. LR patterning during the early stages of embryogenesis has long been studied, which involves signaling cascades spanning from the initial symmetry breaking at the embryonic organizer to the transfer and amplification of LR signal to lateral plate mesoderm (LPM). Paraxial mesoderm, on the other hand, has been largely overlooked in our understanding of LR patterning, as paraxial mesoderm and later musculoskeletal system have been assumed to be symmetric without thorough characterization. Here we describe extensive morphological and molecular asymmetries in paraxial mesoderm of chick embryo. The left anterior somites are more anteriorly positioned and larger than the right ones. The experiments on segmentation clock gene oscillation suggest that key signaling pathways in LR patterning modulate the period of oscillation and underlie the somite asymmetry. Moreover, single cell RNA sequencing of chick embryo reveals novel molecular asymmetry in paraxial mesoderm, that may hint a potential asymmetry in developmental trajectories of left and right paraxial mesoderm. This projected is funded by NIH R01HD097068, awarded to Olivier Pourquie.

Program Abstract #34

Body shape control by the cuticle in *Drosophila*

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The animal body shapes are defined by rigid extracellular matrices (ECMs), such as the skeleton, the skin and the shell. While morphogenesis is typically understood by reducing it to the dynamics of cells, mechanical roles of the ECMs in body shaping are less appreciated. The insect cuticle is ECM that covers

the entire body surface, produced by the epidermis through its apical surface. It is a composite of a single polysaccharide called chitin and a large number of cuticular proteins. We identified mutants of cuticular protein genes that exhibit abnormal body shapes in *Drosophila*. Mutations in *obstructor-E* (*obst-E*), encoding a cuticular protein with three chitin-binding domains, result in a thin and elongated body shape. In contrast, mutations in *Cuticular protein 11A* (*Cpr11A*), another cuticular protein, result in a fat and short body shape. The latter resembles the phenotype of *Tubby* (*Tb*), a classically known mutation in yet another cuticular protein. I will present our works on how self-organizing capacities of the cuticular proteins Obst-E, Cpr11A and Tb contributes to mechanical properties of the cuticle, which in turn affect the whole body shape in a physical manner. Funding: KAKENHI 20K06666, 20H05945, Takeda Science Foundation 2022034459, Suntory Rising Stars Encouragement Program in Life Sciences (SunRiSE), Toray Science and Technology Grant 22-6305 and JST FOREST Program JPMJFR224W.

Program Abstract #35

Investigating neuropeptide-based protection to anoxia in *C. elegans*

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Most multicellular animals require oxygen for metabolism, development, and essential physiological processes. In mammals, hypoxia (0.5 to 1% oxygen), or anoxia (0% oxygen) causes cellular dysfunction and is the foremost cause of adverse health outcomes. When animals are exposed to prolonged hypoxic or anoxic environments, they respond by decreasing their overall activity, which decreases demands for cellular respiration. The cellular and molecular pathways that allow animals to mount a coordinated response to low oxygen conditions is not fully understood, but regulation is likely multifactorial. We are using *Caenorhabditis elegans*, a soil nematode, to investigate the cellular, molecular, and neural mechanisms underlying response to anoxia. Upon exposure to anoxia, *C. elegans* become hypometabolic, suspending development, and reproduction. We find that *C. elegans* developmental stage L4 animals, the last larval stage before adulthood, are sensitive to 48-hours of anoxia. Recent studies have identified a role for neuropeptides, small peptides that can be synthesized and secreted from neurons, in the mediating response to anoxia. Loss in *C. elegans* neuropeptide processing and secretion genes, *egl-3*, and *unc-31* respectively, increases survival to 48 hours of anoxia in L4 stage animals. Restoring neuropeptide gene function broadly in neurons restores vulnerability and decreases survival to anoxia, suggesting a role for neuropeptide signaling in the nervous system in mediating response to anoxia. The biological relevant cells where neuropeptides function for the response to anoxia remain elusive. Moreover, the mechanisms by which loss of function in neuropeptide signaling confers protection and increases survival is unknown. Ongoing studies in my lab aim to elucidate the cellular, molecular, and neural mechanisms underlying the neuropeptide response to anoxia.

Program Abstract #36

Metabolic reprogramming in microglia and macrophages during spinal cord regeneration

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Following spinal cord injury (SCI), activation of the immune system is required to provide a permissive enviornment for the survival and regrowth of spared neurons. However, chronic immune activation contributes to secondary damage as well as inflammatory and fibrotic complications. It is unclear what specific cell identities and molecular pathways drive beneficial immune activation following neural injury. Here, we utilize the innate regenerative capacity and evolutionarily conserved immune system of the adult zebrafish to identify key immune pathways necessary for spinal cord (SC) regeneration. After SCI, we found that activation of the zebrafish immune system is transient and comprised of predominantly microglia and macrophages. Depleting these cells in specifically the acute phase of injury response abrogates functional and anatomical recovery. Using cross-species comparisons, we identify *transcription and immune response regulator* (*tcim*) as a microglia/macrophage-enriched gene that is necessary for SC regeneration and the clearance of immune activation following SCI. Using immune chimeric zebrafish, we identify the processing of phagocytic cargo as a key step during SC regeneration that is regulated by *tcim*. Additionally, overexpression of human *TCIM* in the zebrafish is sufficient to enhance regeneration

and reverse immune defects, suggesting *TCIM* function is conserved across species. Together, our studies identify a previously unknown pro-regenerative factor that represents a novel therapeutic target to alleviate the detrimental effects of SCI in human patients. This work was supported by the W.M. Keck Postdoctoral Fellowship (D.K.S.), Center of Regenerative Medicine Postdoctoral Fellowship (D.K.S.), Training in Regenerative Medicine T32 EB028092 (D.K.S.), NIH Ruth L. Kirschstein NRSA F32 HD107935 (D.K.S.), NINDS R01 NS113915 and R01 NS123708 (M.H.M.), and the Hope Center for Neurological Disorders.

Program Abstract #37
Fish(ing) in the Hi(gh)-C(sea)
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Adapting to changing environment is central to survival, and the fish *Astyanax mexicanus* shows a unique example of adaptation to extreme environment. Cave dwelling morphs of *Astyanax* have evolved to thrive in the dark, food-scarce caves. Compared to their river-dwelling surface counterpart, multiple cave morphs show repeated evolution of cave traits, which include loss of eye, pigmentation, and robust metabolic adaptations to food scarcity. This altered metabolic profile in cavefish is also coupled with an altered transcriptomic profile. Physical interactions between enhancers and promoters of genes, associated DNA looping, and the overall 3-dimensional (3D) genome organization are among the regulators of transcription. Previous studies have shown that genome organization is associated with DNA repair efficiency and fragility, factors contributing to mutational hotspots, driving repeated evolution of specific traits. In this study, we investigate the 3D genome architecture of the surface and two cave morphs, to understand the evolution of cave-specific 3D genome features and their role in metabolic adaptation. We performed Hi-C to map genome-wide 3D chromatin contacts of the metabolically active tissue – liver and identified cave-specific active/in-active genome compartments, TADs, DNA loops, as well as chromosomal inversions. Aligning the cave-specific 3D genome features, with available liver RNAseq dataset, we identified candidate genes whose expression was affected by altered 3D genome architecture. Additionally, comparing ATACseq and histone ChIP-seq datasets of liver, we identified putative cave-specific enhancers that were differentially looped near the candidate genes in cavefish. Finally, we have also developed *in vitro* models of *Astyanax* liver spheroids and organoids to facilitate functional experiments aimed at understanding the metabolic roles of candidate genes, enhancers, and 3D genomic features. Funded by Stowers Institute for Medical Research.

Program Abstract #38
Metabolic switches modulate retinal pigment epithelium cell fate
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Metabolism is a network of biochemical reactions necessary to produce energy and metabolites for all biological processes. However, the role of metabolism in cell reprogramming and fate is not entirely understood. Here, we characterized the metabolic requirements of retinal pigment epithelium (RPE) reprogramming into neural retina in the embryonic chicken. Embryonic RPE is a plastic tissue capable of reprogramming into neural retina in the presence of FGF2. Using RNA-seq, we found that glycolysis, cell proliferation, and neural genes were upregulated during RPE reprogramming. By manipulating the cell media composition, we found that glucose, glutamine, and pyruvate can be used as carbon sources by the RPE for reprogramming. Glycolysis-inhibition blocks RPE reprogramming independently of the carbon source, indicating that this pathway is required for RPE reprogramming. Interestingly, induction of oxidative metabolism (OXPHOX) by the activation of pyruvate dehydrogenase promotes an epithelial-mesenchymal transition (EMT) program that is FGF2 dependent. Supplementation of the medium with the antioxidant N-acetylcysteine (NAC) partially redirects the RPE to a neural fate when OXPHOX is activated, suggesting that OXPHOX might produce an oxidative environment that stimulates EMT. These findings provide evidence of how metabolism modulates cellular fate and could contribute to understanding the role of metabolism in RPE-related diseases, such as age-related macular

degeneration. This work was supported by NEI R01 EY026816 and EY034980, Miami University Rapid Investment Program, and the John W. Steube Endowed Professorship to KDRT; NINDS F99 NS129167 to JAT.

Program Abstract #39

Maternal obesity alters development of liver progenitors: An indirect effect of adipocyte-derived small extracellular vesicles

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Maternal obesity increases the risk of metabolic disease in the offspring. Babies born to mothers with obesity have a 70% increase in liver fat and an increased risk of developing metabolic-associated steatotic liver disease (MASLD). We previously linked adipocyte-derived extracellular vesicles (ad-EVs) to obesity-related complications. In this project, we investigated if ad-EVs mediate transgenerational inheritance of metabolic dysfunction. We hypothesize that ad-EVs from patients with metabolic dysfunction will alter liver development during early gestation (E8.5-9.5). To test this idea, we utilized a mouse embryonic culture system where E8.5 murine embryos are cultured in DMEM/rat serum(1:1) supplemented with ad-EVs from patients with obesity and insulin resistance (metabolically healthy; MH;n=5) or obesity and diabetes (metabolically unhealthy; MUH;n=5). In a parallel experiment, embryos were cultured with serum from high-fat-diet-fed rats (HFD; 60% fat). After 24 hours, embryo growth parameters were recorded. RNA from embryos, YS, and ad-EVs were sequenced. Differentially expressed genes (DEGs) and ad-sEVs miRNAs were identified using DESeq2. Our data show that exposure of embryos to HFD or ad-EVs does not alter embryo morphology or growth, but MUH ad-EVs and HFD induce transcriptomic changes in E9.5 embryos and YS compared to MH ad-EVs and control diet, respectively (p-value<0.05;FC<|1.5|). Notably, DEGs in embryos exposed to HFD or MUH ad-EVs are expressed in liver progenitors (*e.g.*,*Ttr, Apob*, *Afp*, and *Rbp4*), suggesting an increase in liver size. Interestingly, *Asct2*, a glutamine transporter upregulated in the YS of embryos cultured with MUH-ad-sEVs, is associated with liver fibrosis in mice and is a target of ad-sEVs microRNAs (*i.e.*,hsa-miR-199b, and hsa-miR-324). Our data suggest a model where MUH ad-sEVs increase the expression of Asct2 and liver progenitors, possibly contributing to MASLD development. Funding: The Frank and Nancy Parsons Foundation

Program Abstract #40

Cellular responses to nutritional environment during thyroid morphogenesis

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During organogenesis, the thyroid plays a pivotal role by secreting essential hormones from its follicular structures, vital for development of multiple organs. Despite the well-established significance of thyroid hormones during organogenesis, the intricacies of thyroid follicle morphogenesis remain largely unknown. Using *Xenopus laevis* tadpole as a model, we discovered that thyroid follicle formation starts in response to nutritional intake. We have found that feeding led to a decrease in the expression of cell adhesion molecules and an increase in collagen level. Conversely, a lack of feeding resulted in an increased expression of cell adhesion molecules, which in turn, impeded follicle morphogenesis. Moreover, we observed that the thyroid cells of tadpoles that were fed exhibited unclear apical-basal cell polarity, suggesting that these cells might temporarily adopt a partially mesenchymal character during follicle formation. Live imaging of thyroid tissues from the fed tadpoles revealed distinct cell movements that contribute to the expansion of thyroid follicles. Remarkably, in the later stages of thyroid follicle formation, thyroid cells showed definitive epithelial characteristics, including apical-basal polarity, indicating a transition from their earlier, partial mesenchymal state. This finding highlights the cellular plasticity in response to nutritional cues and underlines the critical role of feeding in regulating the balance between cell adhesion and ECM dynamics for thyroid morphogenesis. Funding: Japan Society for the Promotion of Science, Fusion Oriented Research for Disruptive Science and Technology

Program Abstract #41

States of longevity in the eusocial mammal, the Naked mole-rat

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Comparative studies of diverse mammals reveal that lifespan inversely correlates with metabolism and fecundity, but the naked mole-rat (NMR) uniquely decouples this relationship. Similar to honeybees, NMRs live in colonies with one reproductive queen while other females remain prepubertal by social suppression. Queens live substantially longer than subordinates in captivity as well as in the wild. This, together with the potential for virtually all subordinates to undergo reproductive activation, argues for an epigenetic regulation of lifespan in NMRs that is coupled to puberty. We found that primary dermal fibroblasts from queens had discrete phenotypes compared to subordinates: slower growth kinetics, dampened migratory behavior and decreased senescence. We devised a novel reprogramming platform for NMRs and found, surprisingly, that queen fibroblasts reprogram more slowly. Resulting queen and subordinate NMR induced pluripotent stem cells (iPSCs) retained phenotypic differences, even through subsequent differentiation. This suggests increased epigenetic buffering and resistance to cellular reprogramming in queen NMR cells. Profiling of gene expression in queen fibroblasts and their derivative iPSCs revealed elevated levels of the quiescence regulator HES1 and altered expression of mitochondrial genes. Our ongoing efforts are testing the hypothesis that increased quiescence and epigenetic resilience promote longevity in queen NMRs through maintenance of a discrete epigenetic state.
Funding from this work comes from the Keck Foundation, Weston-Havens Foundation and EMBO

Program Abstract #42
Exploring mechanisms of developmental robustness by understanding anoxia induced diapause
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Despite the stochastic nature of biochemical reactions, spontaneous mutations, and fluctuations in the external environment, development remains canalized and produces consistent phenotypes. A striking example of developmental canalization is the ability of the zebrafish embryo to undergo developmental pausing when exposed to low levels of oxygen. Remarkably, embryos have the ability to resume development upon reentry into normoxia without severe consequences. Although this phenomenon has been noted in teleost embryos since 1966, a detailed molecular understanding of how development can stop and start without defects remains elusive. To determine a molecular mechanism of developmental canalization, I characterized how zebrafish embryos undergo the transition from hypoxia-induced developmental pausing to recovery by single-cell RNAseq and ATACseq. I found that hypoxia induces a dramatic and reversible change in the embryo's transcriptome and epigenome. In particular, several critical signaling pathways were aberrant during developmental arrest including increases in Wnt and changes in NFkB signaling. However, despite these major assaults to the gastrulating embryo, development remains canalized and resists defects. To identify critical factors that allow the embryo to undergo pausing and recovery, I performed a small CRISPR screen. I identified both H1.10 linker histone (h1.10) and terminal nucleotidyltransferase 5ba (tent5ba) as necessary for canalization during hypoxia induced diapause, as their removal during arrest causes embryo dorsalization/anteriorization. I hypothesize that during arrest, tent5ba and h1.10 are acting to stabilize the transcriptome and epigenome, respectively. Together my research suggests that hypoxia causes disruptions in signaling that should disrupt embryonic axis patterning, however tent5ba and h1.10 functionally canalize the embryo by storing cell state information and serve to protect development. NSFGRFP, NIHDP2, NIGMSR00, CZI

Program Abstract #43
Generation of rat forebrain tissues in mice
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Interspecies blastocyst complementation (IBC) provides a unique platform to study development and holds the potential to overcome worldwide organ shortages. Despite recent successes, brain tissue has not been achieved through IBC. Here, we developed an optimized IBC strategy based on C-CRISPR, which facilitated rapid screening of candidate genes and identified that *Hesx1* deficiency supported the generation of rat forebrain tissue in mice via IBC. Xenogeneic rat forebrain tissues in adult mice were structurally and functionally intact. Cross-species comparative analyses revealed that rat forebrain tissues developed at the same pace as the mouse host but maintained rat-like transcriptome profiles. The chimeric rate of rat cells gradually decreased as development progressed, suggesting xenogeneic

barriers during mid-to-late pre-natal development. Interspecies forebrain complementation opens the door for studying evolutionarily conserved and divergent mechanisms underlying brain development and cognitive function. The C-CRISPR-based IBC strategy holds great potential to broaden the study and application of interspecies organogenesis.This work was supported by the New York Stem Cell Foundation (NYSCF), the NIH (HD103627-01A1), Welch (854671), the R&D Program of China (2017YFC1001300 and 2018YFC2000100) and the CAS Strategic Priority Research Program (XDB32060000). J.W. is an NYSCF-Robertson Investigator and Virginia Murchison Linthicum Q18 Scholar in Medical Research.

Program Abstract #44
Organismal strategies for stem cell resilience in *S. mediterranea*
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The planarian flatworm *Schmidtea mediterranea* has fascinated scientists for over a century due to its' remarkable capacity for whole body regeneration. Here, we focus on identifying planarian cell-cell signaling mechanisms that support stem cell recovery and re-establishment of regenerative capacity after sublethal doses of ionizing radiation. After 1250Rads of ionizing radiation, stem cells are significantly depleted and will divide and expand to re-establish a healthy stem cell compartment over 2-3 weeks. During this period, stem cells proliferate, but do not replace missing tissue following amputation. Single cell sequencing of planarian tissues after ionizing radiation identified distinct irradiation responsive stem cell states, as well post-mitotic tissues that respond to DNA damage and support stem cell recovery. We are now testing the hypothesis that these DNA-damage induced cell states inhibit regeneration of missing tissues while promoting the repair of the regeneration competent stem cell compartment. This work was supported by funding from Baylor College of Medicine and the Cancer Prevention Research Institute of Texas.

Program Abstract #45
Sea robins as a model for dramatic evolutionary innovations in vertebrates
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Although studies in evolutionary biology have been revolutionized by the ability to test hypotheses using modern genetics and genomics, many examples of evolutionary change in wild organisms have focused on traits that have been reduced or lost. Less understood are the molecular mechanisms involved in the evolution of novel or gained traits in wild species. To address this, we are developing sea robins as a new model for studying dramatic skeletal, sensory, and nervous system innovations in vertebrate evolution. Sea robins are an abundant, bottom-dwelling ocean fish that exhibit 1) expanded, wing-like pectoral fins, 2) free fin rays (called "legs") that are separated from the pectoral fin, 3) the ability to walk and probe the substrate with the legs, and 4) novel lobes in the spinal cord that support unique sensory capabilities. We have set up successful *in vitro* fertilization and culture conditions for two sea robin species (*Prionotus carolinus* and *Prionotus evolans*), and established methods for efficient microinjection and CRISPR-Cas9 editing. Using transcriptomics, we have identified key genes that play a role in leg formation, including genes classically involved in limb development such as *hoxd12a* and *tbx3a*. Targeting of these genes with CRISPR-Cas9 editing results in major alterations to leg and lobe structures. We have also found that the two species exhibit several fascinating trait differences, including alterations to leg structure and sensory function. We have successfully crossed the two sea robin species to produce viable hybrid animals and are currently exploring the genetic basis of species-specific differences using the F1 hybrid system and genomic tools we have established. These studies provide a novel system for revealing the molecular basis of remarkable evolutionary innovations in both the skeletal and neural systems of vertebrates.
Funded by: Howard Hughes Medical Institute, Marine Biological Laboratory, Helen Hay Whitney Foundation.

Program Abstract #46

Functional heterogeneity in tendons and ligaments reveals force-dependent feedback regulation of gene expression

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Structural requirements generate functional diversity in the same cell types exposed to different environments during development. Tenocytes that secrete the extracellular matrix (ECM) of tendons and ligaments form and adapt to mechanical forces depending on the structural demands of the muscles and bones to which they attach. Everyone deals with tendon/ligament injuries as well as loss of strength with age, yet little is known about the genetic mechanisms that control their development or maintenance. Transcription factors such as Scleraxis (Scx) and Sox9 directly regulate expression of collagens and other ECM components to achieve appropriate tendon stiffness depending on force demands. Here we investigate heterogeneity in developing tenocytes and how altering muscle contraction affects their underlying gene regulatory network. We have isolated and performed scRNA-seq with scxa+ cranial tenocytes from wild-type and paralyzed larvae. We show that tenocytes normally come in different transcriptional flavors depending on tendon type (load-bearing versus soft) as well as their locations within individual tendons (skeletal attachments known as entheses versus myotendinous junctions, MTJ) and that the flavor they adopt depends on the tensile force of muscle contraction. We also show that both TGFb and canonical Wnt signaling play critical roles in establishing these force-dependent transcriptional signatures. TGFb balances the ratio of Scx and Sox9 and the collagens that they regulate in tenocytes at entheses. In contrast, canonical Wnt signaling regulates tenocytes at MTJs. In situ analyses confirm distinct transcriptional signatures of functionally distinct tendons/ligaments, as well as several uniquely force-responsive genes. These findings are among the first to profile gene expression broadly across embryonic tendon/ligament lineages and to show that force-dependent feedback regulates their functional heterogeneity. This work is supported by NIAMS [R01AR67797].

Program Abstract #47

A 36-hour trajectory of fate transition in plant regeneration

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Plant cells are remarkably plastic in their ability to change their fate during regeneration. In order to reprogram, cells must respond to injury, lose their prior identity, and adopt a new cell identity. It is clear that cell division and chromatin remodeling play key roles in this trajectory, but exactly how they mediate the key steps is not well described. Here, we dissect one case of plant regeneration among reprogramming cells in the Arabidopsis root, with a combination of tools to track single cells over 36 hrs. We model a path to reprogramming in which the intermediate state does not resemble dedifferentiation but rather a novel state of stress. Histone deacetylation activity is required within 1 hr to suppress the stress response and shut down remnant cell identities allowing regeneration to proceed. Loss of old identity is at least partially independent of gain of identity, with transient intermediate chimeric cell identities. Surprisingly, neither the loss of prior identity nor gain of new identity is dependent on cell division to initiate, but division is necessary to complete both processes and enable complete regeneration. We show that fast divisions, mediated by truncated G1 phase that is dependent on the rapid import of glutathione into the nucleus at the wound site, increase the efficiency of cellular reprogramming. Specific tissue layers appear to be the source of glutathione, which spreads through gap junction-like structures in the plant cell wall called plasmodesmata. The results show, in one *in vivo* system, how plant cells rapidly transdifferentiate without the need to return to a dedifferentiated state. This work was funded by the National Institutes of Health Grant R35GM136362 to K.D.B. and an NSF Fellowship to L.L.

Program Abstract #48

Reengineering somite segmentation without the vertebrate segmentation clock

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Somitogenesis establishes the segmental pattern of the vertebrate body axis. A molecular segmentation

clock in the presomitic mesoderm sets the pace of somite formation. However, how cells are primed to form a segment boundary at discrete locations remains unclear. Here we developed precise reporters for the clock and double phosphorylated ERK (ppERK) gradient in zebrafish. We show that the Her1–Her7 clock proteins periodically inhibit ppERK, therefore projecting their oscillation onto the ppERK gradient. Pulsatile inhibition of the ppERK gradient can fully substitute for the role of the clock, and kinematic clock waves are dispensable for sequential segmentation. The clock functions upstream of ppERK, which in turn enables neighboring cells to discretely establish somite boundaries in zebrafish. We here propose a "Clock-dependent Oscillatory Gradient (COG)" model in which the clock periodically triggers discrete shifts of the positional information. The COG model explains all experimental observations and effectively replaces the long-standing clock-and-wavefront textbook model. This work was funded by a US NIH (Eunice Kennedy Shriver National Institute of Child Health and Human Development) grant (R01HD103623) to E.M.Ö.

Program Abstract #49

Myocardin condensation drives cardiovascular cell lineage specification

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Abstract: During development, cells make switch-like decisions to activate new gene programs specifying cell lineage. The mechanisms underlying these decisive choices remain unclear. Here, we show that the cardiovascular transcriptional coactivator myocardin (MYOCD) activates cardiac and smooth muscle genes by concentration-dependent and switch-like formation of transcriptional condensates. MYOCD forms such condensates and activates cell identity genes at critical concentration thresholds achieved during smooth muscle cell and cardiomyocyte differentiation. Mechanistically, MYOCD condensates selectively aggregate active transcriptional components to promote gene expression. The carboxyl-terminal disordered region of MYOCD is necessary and sufficient for condensate formation. Disrupting this region's ability to form condensates prevents gene activation and smooth muscle cell reprogramming. Rescuing condensate formation by replacing this region with disordered regions from functionally unrelated proteins rescues gene activation and smooth muscle cell reprogramming. These results demonstrate that MYOCD condensate formation is required for gene activation during cardiovascular lineage specification. Our findings provide a new perspective that the formation of transcriptional condensates at critical concentrations of cell type–specific regulators provides a molecular switch underlying the activation of key cell identity genes during development.
Funding sources: Cancer Prevention and Research Institute of Texas grant RR190090; NIH grants AR071980, HL130253, HL157281, and GM147583; Robert A. Welch Foundation grants 1-0025 and 1062707; and American Heart Association postdoctoral fellowship 825635.

Program Abstract #50

Deciphering the Role of Epigenetic Reprogramming in Zebrafish Fin Regeneration

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While is it known that epigenetic reprogramming is pivotal for kickstarting dormant developmental programs during regeneration, the regulatory mechanisms orchestrating this phenomenon remain elusive. The zebrafish, recognized for its regenerative capabilities, serves as an ideal model to explore this at the molecular level. We recently developed a novel zebrafish "EpiTag" transgenic reporter line that permits real-time visualization of tissue-specific epigenetic silencing or activation in living animals with cellular resolution. We observe early and striking activation of the EpiTag reporter in regenerating cells, including during fin regeneration after amputation, suggesting the efficacy of this line as a highly specific marker for cells undergoing epigenetic reprogramming at extremely early, otherwise inaccessible stages of regeneration. By selectively enriching early-stage regenerating cells from EpiTag fish using FACS sorting, we employ a multi-omics approach, encompassing ATAC-seq, bisulfite-seq, bulk RNA-seq, CUT&Tag, and single-cell RNA-seq analyses, to comprehensively profile chromatin accessibility, DNA methylation patterns, gene expression profiles, and single-cell transcriptomes within these regenerative cells. Our preliminary scRNA-seq analysis reveals specific cell types undergoing epigenetic changes during regeneration, notably mesenchymal and proliferating cells, while RNA-seq demonstrates the enrichment

of genes crucial for cell cycle regulation and chromatin dynamics and ATAC-seq highlights regulatory motifs linked to chromatin accessibility and cell proliferation. These compelling findings are paving the way for the identification of candidate genes for further analysis of their role in regeneration. Moving forward, the potential to manipulate the expression of these genes also holds the potential to provide potential targets for therapeutic interventions in regenerative medicine. This work is supported by the NICHD Intramural Program (to BMW).

Program Abstract #51

H3K27me3 resolves unique, spatially distributed patterning states in *Drosophila* embryogenesis

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The creation of discrete structures that define the body plan during development requires the establishment of positional identity. Maternal factors establish long-term, heritable patterns of hox gene expression through transient gap gene regulatory networks. Gap genes direct Polycomb group proteins to establish trimethylation of Histone H3 lysine 27 (H3K27me3), a repressive chromatin modification which is epigenetically inherited. This activity prevents the ectopic expression of hox genes outside of their normal expression domains over the lifespan of the fly. Although there are clear requirements for H3K27me3 in long-term maintenance of hox gene patterns, how gap genes direct H3K27me3 into spatially restricted patterns is unclear. A challenge in studying this problem is that embryos represent a mixture of regulatory states. To deconvolve the role of H3K27me3 in spatially organized patterning, we have studied mutant embryos that develop uniformly with a single-segment, neuroectodermal identity. These mutant embryos allow us to resolve the unique regulatory state of the hox cluster at the level of H3K27me3 as well as within the neurogenic program. We observe that H3K27me3 reflects "off" states of patterning genes from the beginning of zygotic genome activity. Gain and loss of H3K27me3 is surprisingly rapid as cells activate developmental repertoires. We have identified that patterns of H3K27me3 are dynamic; when neurogenesis begins in these mutants, H3K27me3 is rapidly lost at neural genes. This study provides a basis to determine the regulatory logic for the establishment and maintenance of H3K27me3 as a function of developmental patterning cues. This research was funded by the NIH/NICHD, Pew Charitable Trusts, NU CMBD T32, and Startup Funds.

Program Abstract #52

CRISPy Chickens: An in vivo CRISPR screen reveals role of MLLT3 in the regulation of neural tube fate acquisition

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Development relies on the coordinated differentiation of stem cell populations in dynamically changing environments. Vertebrate neural tube formation is a well characterised example as it is fuelled by stem cells in the caudal epiblast transitioning through a changing signaling landscape to form neural tissue. Despite an overall understanding of the signaling pathways and cellular processes involved, the detailed gene regulatory mechanisms controlling formation of neural cells remains ill-defined. To address this, we developed an in vivo CRISPR screening approach in chicken embryos. We then performed a multiplexed in vivo single-cell perturbation screen of genes that change in their expression between the caudal epiblast and the neural tube lineage. This revealed a role for the gene MLLT3, a component of the super elongation complex, in specification of subsequent neural identity. Perturbation of MLLT3 disrupted the caudal epiblast and resulted in misregulation of genes involved in Wnt and Retinoic Acid (RA) signaling, key regulatory pathways of epiblast and neural fate, respectively. Since, MLLT3 reportedly interacts with Retinoic Acid Receptor A (RARa), we compared the effect of MLLT3 loss to the forced expression of mutant RARa, either lacking the MLLT3 binding domain or consisting of only the MLLT3 binding region. In all cases, neural tube progenitors were depleted, resulting in smaller neural tubes. However, only MLLT3 loss caused disruption of epiblast fate, indicating a dual role of stem cell maintenance coupled with RA driven neural fate acquisition. Together this data demonstrates a system appropriate for performing in vivo CRISPR screens in chick embryos and identifies a previous unanticipated role for MLLT3 in the specification of neural tube lineages. More broadly, it highlights a mechanistic gene regulatory strategy of lineage emergence in a dynamic signaling landscape. Work funded by a European Molecular Biology Organization Postdoctoral Fellowship.

Program Abstract #53

Lineage recording in monoclonal gastruloids reveals heritable modes of early development

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Mammalian stem cells possess remarkable self-organizing abilities, generating diverse in vitro models such as organoids, embryoids, and gastruloids that mimic aspects of in vivo tissue biology. However, even in carefully controlled experiments, most stem cell-derived models often exhibit significant heterogeneity, raising questions about their origins. Is this interindividual heterogeneity a consequence of stochastic events that occur after induction, or is there some contribution from heritable variation in the intrinsic states of founder stem cells prior to induction? As a first step towards answering this question, we developed a protocol for generating monoclonal gastruloids from single mouse embryonic stem cells (mESCs), eliminating founder cell differences as a source of downstream heterogeneity. Single-cell transcriptomic analysis of 144 monoclonal gastruloids revealed unexpected diversity, with some individuals hardly progressing and others with cell types more advanced than those observed in conventional polyclonal gastruloids. We then engineered mESCs with the DNA Typewriter lineage recording system to infer the hierarchy of cell type relationships from the reconstructed cell lineage trees of monoclonal gastruloids. Finally, we performed a two-stage experiment, where in the first epoch, a single ancestor cell was expanded to a small population of founders, and then in the second epoch, these founders seeded the induction of 108 monoclonal gastruloids. Remarkably, although interindividual heterogeneity was again observed, closely related founders were much more likely to give rise to gastruloids with similar morphologies and cell-type compositions. This result suggests that spontaneous yet heritable fluctuations in the intrinsic states of mammalian stem cells shape their responses to external stimuli, and shed light on the origins of interindividual heterogeneity observed in *in vitro* mammalian stem cell models. [Funding: NIH/HHMI/Allen Institute]

Program Abstract #54

Novel 3D explant culture to study mechanical, genetic, and signaling contributions to kidney branching morphogenesis

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Complex tissue-level organization established during development is critical for adult kidney function. Despite ongoing interest in genetic and molecular factors shaping the urinary drainage network and nephrons, their interplay with tissue-level organization, geometry, and mechanics remains unclear. Understanding these factors is pivotal for generating kidney replacement tissues through developmental mimicry. Tissue-level dynamics are often modeled in air-liquid interface (ALI) culture, which flattens the explant and renders it unfit for live imaging of tissue geometry and mechanics. We introduce a 3D kidney culture method compatible with live imaging by suspending explants in hydrogel droplets. In 3D culture, explants closely resemble the ureteric bud (UB) tubule tip:tip distance of freshly dissected kidneys compared to ALI culture. UB tips in 3D also dynamically rotate and reposition as they branch, unlike the persistent radial expansion in ALI culture, suggesting in vivo-like competition among UB tips for organ surface area. We assess how surface confinement, matrix stiffness, and adhesive ligand density affect development in 3D culture using acrylated hyaluronic acid (AHA) hydrogels. Preliminary data finds that kidneys cultured in soft (~1.5 kPa) AHAs phenocopy extracellular matrix-mimetic matrigel/collagen 1 gels, and those in stiff (6 kPa) AHA adopt irregular morphologies, highlighting the importance of mechanical constraint on overall organ morphogenesis. Our ongoing work is investigating how matrix stiffness and adhesion impact UB branching, genetic mutation penetrance, and nephron:UB tip balance. This work presents a novel tool for recapitulating 3D kidney morphogenesis with live imaging and investigating the effect of genetic and mechanical perturbations on ex vivo development. This work was supported by NIH NIGMS MIRA R35GM133380, NIH NIDDK R01DK132296, NSF CAREER award 2047271, and Penn Center for Precision Engineering for Health (CPE4H) pilot grant.

Program Abstract #55

Decoding a dynamic cellular algorithm for the emergence of limb morphology using organoid model

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The emergence of organ morphology transcends a mere heap of genes and molecules governing cellular behaviors such as proliferation, polarity, and survival. The convergence of organoid technology with in silico modeling has been pivotal, enabling the isolation of morphogenetic events in vitro and the synthesis of dynamic cellular algorithms integrating essential genes, molecules, and cell behaviors. This synergy has proven particularly transformative in the study of epithelial tissues such as eye and gut, where it has enhanced our capacity to manipulate these tissues in vitro. Yet, the morphological emergence of mesenchymal tissue remains underexplored. Here, we present an integrative study that employs limb organoids derived from embryonic mouse hand to model the morphogenesis of mesenchymal tissues. By uniformly delivering Fgf and Wnt—signals typically provided from the overlying ectoderm during in vivo development— these organoids undergo symmetry breaking, culminating in the outgrowth of digit-likestructures and joint segmentation. We employed lineage tracing to monitor the fate of the proximal (palm) and distal (digit) cells, revealing the role of Fgf/Wnt in mediating cell sorting and the establishment of a self-organized proximal-distal axis. To delineate the cellular and molecular underpinnings during the emergence of digit-like structures, we performed live-imaging for cell tracking, analyses of cell adhesion, proliferation, chemotaxis, and scRNA-seq. By weaving this empirically grounded data into agent-based models, we anticipate unveiling a dynamic cellular algorithm that aptly explains limb morphological emergence. Capitalizing on these insights, our objective extends to reconstructing limb morphogenesis using limb mesenchyme derived from human ES cells. Our research aspires to augment our understanding of tissue morphogenesis and enhance our capacity to manipulate cellular architectures through bioengineering. Funded by JSPS and ASHBi.

Program Abstract #56

Decoupling principles of mammalian body plan development

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The foundation of the human body plan is laid during early embryogenesis. However, our understanding of early human development is significantly constrained by current ethical and technical limitations. In recent years, considerable effort has been devoted to overcoming these barriers through the development of advanced in vitro engineering approaches, leveraging the self-organizing properties of stem cells derived from embryos. Our study introduces a new strategy that illustrates how human pluripotent stem cells can be prompted to autonomously organize into three-dimensional structures. These structures recapitulate key spatiotemporal events of early human post-implantation embryonic development. This system consistently captures the spontaneous differentiation and co-development of embryonic epiblast-like and extra-embryonic hypoblast-like lineages, establishes crucial signaling hubs with secreted modulators, and undergoes events reminiscent of symmetry breaking. A comprehensive understanding of the interconnected cellular events in this system is essential for advancing our knowledge of developmental and reproductive health, offering a unique opportunity to unravel the cellular and molecular mechanisms underlying early miscarriage and congenital pathologies. The Sozen lab is funded by NIH Early Innovators Award, the Richard and Susan Smith Family Foundation, the Yale Chen Innovation Award and Reprograms.

Program Abstract #57

A novel gene expression system Gal4ADλFi/TrpRλM/tUAS confers ability to restrict exogenous gene expression to specific populations in overlapping-expression areas of any two different promoters

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Brigham Young Unversity, USA

Expression of exogenous genes in selective tissues or areas can be challenging as it greatly relies on enhancer/promoter availability. We developed a novel system that restricts gene expression to overlapping expression-areas of any two distinct enhancer/promoters. We fused the tryptophan receptor DNA binding domain (TrpR) to the bacteriophage λ M protein (λM), and the Gal4 activation domain

(Gal4AD) to the bacteriophage λ Fi protein (λFi), which binds to λM. For proof of concept, we placed both fusion proteins (TrpRλM and Gal4ADλFi) under the *neuroD* promoter (CNS and spinal cord) and injected them into one-cell stage zebrafish embryos, along with *tUAS*:nlsEos. Thus, the fluorescent protein Eos should express only when TrpR binds to its operon binding sequence (*tUAS*), subsequently recruiting Gal4AD through the interaction between TrpRλM and Gal4ADλFi. Injection of all three plasmids showed consistent Eos expression in spinal cords of 1dpf zebrafish embryos. We next generated the stable transgenic lines Tg(*neuroD*:nlsTrpRλM; *tUAS*:nlsEos) and Tg(*neuroD*:nlsλFiGal4AD); which, when crossed, recapitulated earlier proof of concept results. To further test our system we established an additional line Tg(*ribA*:nlsλFiGal4AD), which should share an expression domain within the pineal gland when crossed with Tg(*neuroD*:nlsTrpRλM; *tUAS*:nlsEos). At 3dpf offspring from these lines confirmed Eos expression exclusively in pinealocytes. This demonstrates the efficacy of our system with distinct promoters, and confirms its ability to restrict gene expression based on unique expression domains, mitigating the need to identify a specific promoter for each area of interest. Finally the *tUAS* sequence, which lacks CpG islands, resists silencing via methylation and provides a more faithful and consistent exogenous gene expression over subsequent generations compared to the Gal4/*UAS* system. *Internal funds.*

Program Abstract #58

Trimology integration – CRISPR based targeted knock-in of large cargo in *Xenopus* and mammals.

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The precise and targeted integration of transgenes can be highly useful in understanding protein function during development and beyond. It also holds great promise for gene therapy approaches, where it is paramount that genomic integrity is maintained. Typically, CRISPR/Cas9-mediated integration relies on homology-directed repair (HDR), which is most active during the G2 phase of the cell cycle. Non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ), however may result in larger unintended genomic alterations at transgene-genome borders. We established a new integration strategy that relies on tandem trinucleotide repeats of small homologies (so called Trimologies), placed at the edges of transgene cassettes to facilitate on-target integration via MMEJ of large cargo (2.5kb). Extensive *in vitro* analysis determined the conditions and contextual rules with successful integration. *In vivo* experiments showed successful integration of reporter constructs into a novel safe harbor locus (h11) in *X. tropicalis* at frequencies of 2-5% of injected F0 embryos. Germline transmission was confirmed for several transgenic lines. Analysis of boarder regions confirmed that Trimology repair arms safeguarded both the genome and transgene cassette during DNA integration, precluding extensive trimming. Trimology integration was also effective to endogenously tag tubulin (*Tubb2a*) in neuronal cells of adult mouse brains. Thus, Trimology integration is an effective method to insert large cargo at desired loci in tissues where HDR is largely ineffective, such as rapidly cycling early vertebrate embryos or fully differentiated, post-mitotic cells such as neurons. This method will enhance the genetic engineering toolkit for disease modelling and research on fundamental questions of vertebrate development. This work was funded by the Swiss National Science Foundation and the European Commission (ERC, Horizon 2020).

Program Abstract #59

The choreography of brain development: Lessons from RNA

Debra Silver
Duke University, United States

The cerebral cortex controls our higher cognitive capacities and helps define us as humans. Cortical development is orchestrated by neural progenitors which give rise to neurons and glia. Aberrant cortical development can result in devastating neurodevelopmental diseases. Our lab aims to elucidate genetic and cellular mechanisms controlling cortical development and contributing to neurodevelopmental pathologies and brain evolution. This talk will highlight some of our recent discoveries including how spatial and temporal control of RNA impacts neural progenitor morphology and function.This research is funded by NINDS, NIMH, and NICHD.

Program Abstract #60

Blood flow directs Yap/Taz-mediated transcriptional regulation of self-renewal programs to control developmental HSPC expansion by mechanical stimulation of Piezo1

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Hematopoietic stem/progenitor cells (HSPCs) emerge from artery-derived hemogenic endothelium (HE) in vertebrate embryos, driven by the Runx1 transcription factor (TF). Physical forces of wall shear stress (WSS) and cyclic stretch (CS) produced by blood flow are required to generate HSPCs from HE, but mechanisms by which these forces are sensed and converted into a "stemness" regulatory module remain incompletely understood. Using scRNA-sequencing of zebrafish trunk endothelial cells, we find via gain- and loss-of-function that the Hippo pathway TF YAP drives lipid metabolism, cell cycling and propagation of a hematopoietic gene regulatory network in the earliest specified HE cells. By employing a heat shock-inducible dominant negative YAP zebrafish line, we reveal an unanticipated role for YAP paralogue TAZ in hematopoiesis, which can promote CD41+ and Flk+/Myb+ HSPC production upon reduced YAP function. YAP and TAZ initiate transcriptional responses downstream of mechanical stimuli and require DNA binding cofactors to regulate target genes. Surprisingly, luciferase assays in HEK293 cells demonstrate a potent synergistic effect of TAZ/RUNX1, but not YAP/RUNX1, in transcriptional regulation at RUNX enhancers. Finally, by pharmacologic and genetic manipulation, we identify the stretch-gated membrane ion channel Piezo1 as a regulator of CS-induced YAP/TAZ mechanotransduction in HE. Stimulation of zebrafish embryos with the Piezo1 small molecule agonist Yoda1 increases HSPC number and YAP target gene expression in a YAP-dependent fashion. A similar modulation of blood and YAP target genes in human iPSC-derived CD34+ HE cells is seen with Yoda1, suggesting that this stretch-Piezo1-YAP axis can be chemically tuned in vitro to enhance HSPC differentiation. These results have broader implications for alternate regulatory effects of mechanically-stimulated Hippo TFs depending on the transcriptional milieu in cell-type specific contexts. Funded by K01DK129409-WWS

Program Abstract #61

Probing Conserved Regenerative Pathways in Embryonic Eye Regrowth

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Regeneration research has identified a myriad of genes and pathways in diverse models that regulate regrowth. However, a key question remains unanswered: why regenerative ability varies amongst species. Confounding the issue is the spectrum of different tissues that can regenerate. One approach to identifying mechanisms to stimulate regeneration in multiple tissue types is to look for shared molecular pathways between different organs in the same animal. Our previous research has investigated the signaling that regulates tail (and spinal cord) regrowth in the African clawed frog, *Xenopus laevis*. This model system has high regenerative ability and well-characterized developmental pathways, especially for the eye and neurulation. Our recent efforts demonstrate that tailbud embryos can also readily regrow eyes after ablation. These regrown eyes are functional and contain the normal morphology, complement of cell types, and innervation to the brain. During regrowth, retinal progenitor cell proliferation was extended by one day, with a concomitant delay in retinal layer formation, which was largely restored by three days after injury. Loss-of-function studies showed that both apoptosis and V-ATPase were required for embryonic eye regrowth. These results are similar to our previous studies showing that both V-ATPase and apoptotic signaling are also required for tail regrowth. Interestingly, during eye regrowth Notch1 loss-of-function phenocopied the effects of V-ATPase inhibition, where retinal proliferation but not differentiation was blocked during eye regrowth. Furthermore, overexpression of the Notch intracellular domain (NICD) rescued the loss of eye regrowth due to V-ATPase inhibition. Together, these data suggest that apoptosis, V-ATPase, and Notch might represent conserved pathways for initiating regrowth. This work was supported by NIH GM146672 (KT), NASA 80NSSC20M0043 (DG), and NSF 1757316 and IIA1301726 (BG).

Program Abstract #62

A Gold(en Apple Snail) mine for studying visual system development and regeneration

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Although vertebrate eyes sensitivity and image-resolution are astonishing, their regenerative capacities are either limited or absent. Their repair is difficult to study due to the lack of models for the *de novo* camera-type eye regeneration. We discovered that the invertebrate golden apple snail (*Pomacea canaliculata*) has camera-type eyes that can fully regenerate, even in adults. We characterized apple snail eyes through light and electron microscopy and transcriptome analyses and found several morphological and molecular distinct cell types in their retina. We showed that regeneration occurs through the formation of a blastema with intense cell proliferation, and that most morphological and transcriptomic features are recovered one month after amputation. To mechanistically investigate gene function, we developed protocols to microinject and culture zygotes, and used CRISPR/Cas9 to establish – for the first time – stable mutant lines in *P. canaliculata*. After we gained a detailed understanding of *P. canaliculata* embryogenesis through bulk RNA-seq at various stages of its direct development and HCR *in situ* hybridization, we targeted *pax6*, a transcription factor necessary for eye development in vertebrates and flies. Excitingly, *pax6* mutant snails completely lacked eyes, revealing a conserved role for *pax6* in apple snail eye ontogeny. This work establishes *P. canaliculata* as a genetically tractable organism for mechanistically exploring the *de novo* regeneration of complex sensory organs and the evolutionary history of eye development.

The work was funded by Howard Hughes Medical Institute (HHMI), SDB Emerging Research Organisms Grant, American Association for Anatomy Postdoctoral Fellowship and by institutional supports from the Stowers Institute for Medical Research and UC Davis.

Program Abstract #63

Planarians regulate stem cell activity in ways that defy the classic structure of a niche

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Stem cells enable regeneration by self-renewing and differentiating as instructed by a local microenvironment called a niche. The two classic features of a niche are: direct contact to the stem cell and regulation of its activity over a very limited spatial field. However, in organisms that regenerate using abundant adult pluripotent stem cells, niches that support tissue repair have not been found. Since these adult pluripotent stem cells are often more widely distributed and plentiful than lineage-restricted stem cells of other organisms, defining their microenvironments may uncover alternative forms of stem cell regulation. Here we used unbiased spatial transcriptomics to define the cellular and molecular environments that support pluripotency in the highly regenerative freshwater planarian *Schmidtea mediterranea*. Stem cells associate with a diverse collection of differentiated cells, but only one cell population is enriched in the regions immediately adjacent to them: *matrix metalloproteinase 1*$^{+}$ secretory cells, which we characterize and name 'hecatonoblasts.' Reconstructing the stem cell microenvironment using light and electron microscopy revealed that stem cells reside in close proximity to hecatonoblasts, and are enriched near the intestine, but not immediately adjacent to it. We developed a new FACS-based method to sort and sequence *mmp1*$^{+}$ cells, which identified *ets-2*, a transcription factor required for the hecatonoblast lineage. In contrast to their physical associations with stem cells, hecatonoblasts do not regulate stem cell number or activity, while intestinal cells do. Thus, planarian stem cells are maintained not by interactions with their immediate neighbors, but instead at a distance by the intestine, defying the conventional concept of a niche. This work was funded by the Stowers Institute for Medical Research, HHMI, the Jane Coffin Childs Memorial Fund, NIH Grant R37GM057260, and Baylor College of Medicine.

Program Abstract #64
Identification of a localized stem cell niche in regenerating adults of the segmented worm *Capitella teleta*
Elaine Seaver
University of Florida, USA
Annelids are a large group of segmented worms that display a wide range of adult regeneration abilities. Regeneration of somatic and reproductive tissues require birth and patterning of new cells from resident adult stem cells, which reside either in specific locations within the body or are more broadly distributed. *Capitella teleta* is an annelid that exhibits posterior regeneration of both somatic and reproductive tissues and has differing regenerative abilities depending on the site of amputation. Furthermore, *Capitella* has several favorable characteristics for in vivo studies of regeneration and stem cell biology, including availability of functional genomic tools, a stereotypic cleavage program and associated fate map, along with a sequenced genome. In this study, we identified a cluster of undifferentiated cells suspended by mesenteries in the ventral coelomic cavity of thoracic segments 4 - 6 in *C. teleta*. Cells in this cluster are derived from a single cell in the early embryo (called 4d) and express genes of the highly conserved multipotency cell program such as *vasa*, *nanos*, *piwi*, *PL10* and *PCNA*. Characterization of cell division profiles and gene expression patterns by in situ hybridization reveals heterogeneity among cells within this cluster. The number of cells in the cluster gradually increases between early juvenile and adult stages, although the number of cells in the cluster remains stable following a challenge of repeated amputations. Cells in the cluster in occasionally divide, indicating a self-renewing mechanism to maintain homeostasis. Transverse amputations at multiple axial positions demonstrate that the difference between successful regeneration of somatic and gonadal tissue and regeneration failure maps to a single segment in adults. We suggest that this cluster is a stem cell niche and hypothesize that cells within the cluster can generate both somatic and germline descendants. Funded by NSF (IOS2316882)

Program Abstract #65
Designing Graphical Abstracts – Big Science in Small Spaces
Martin Krzywinski
Canada's Michael Smith Genome Sciences Centre, Canada
This workshop will distill the core concepts of information design into practical guidelines for creating graphical abstracts. My focus will be on clarity and concision and on the idea that form follows function. To illustrate these guidelines concretely, I will walk you through my redesigns of graphical abstracts (see past case studies) — submitted by you or from past workshops. You'll learn visual strategies for organization, emphasis and theme. By submitting your work (it doesn't have to be recent), you'll see how design principles can be applied to graphics that are directly relevant to you. Submit vector art (EPS, PDF or AI preferred). Make sure that your submission doesn't have any linked files. Submission deadline Friday 5th July 2024.

Program Abstract #66
Bringing biochemistry to the embryo to unravel developmental signaling pathways
Daniel Dickinson
University of Texas at Austin, USA
To enable tissue formation and function, cells must coordinate their behavior in response to signals from surrounding cells and the environment. This coordination is thought to be achieved by developmental signaling pathways, which transmit and process information by altering the abundance and composition of signaling protein complexes. Although the roles of key signaling pathways *in vivo* are well established, much of what we know about the molecular interactions that build these pathways comes from *in vitro* biochemistry and tissue culture studies, which necessarily lack physiological context. Our group has worked to build and apply experimental tools that can bridge the gap between biochemistry and developmental biology, by allowing us to observe and measure signaling complexes in their native context *in vivo*. We label endogenous proteins using CRISPR knock-in; observe the localization and diffusion of proteins in living cells; and extract and count native protein complexes from single cells and embryos. I will describe the path that led us to these approaches, and I will share an example of how we have applied this toolkit to understand how cooperative assembly of the PAR complex enables polarity establishment during asymmetric cell division. Our work is supported by the National Institutes of Health

(R01 GM138443 and R21 GM144817); the National Science Foundation (MCB 2237451); the Welch Foundation (F-2138-20230405); and the Cancer Prevention and Research Institute of Texas (RR170054).

Program Abstract #67

Regulation of epithelial organ morphogenesis: From transcription factors to molecular effectors

Deborah Andrew, Ji Hoon Kim
Johns Hopkins Univ Sch of Med, USA

How is the unique architecture, size and position of organs achieved? Over the past many years, our lab has taken a top-down approach to answering this question using the simple model system of the Drosophila salivary gland. We have discovered the genes that specify this organ and that determine the number of cells that form the different salivary gland cell types. We have identified and characterized early expressed transcription factors that control the conversion from the two dimensional primordial placodes into elongated fully internalized epithelial tubes and that control the specialization of the salivary gland into a dedicated secretory organ. Using genome-wide approaches, we are now discovering and characterizing the molecular effectors that function downstream of the transcription factors that control the changes in cell shape and arrangement to drive tube morphogenesis. Perhaps surprisingly, many of the downstream effectors impact two major molecules – the cytoskeletal protein Myosin II and the apical membrane determinant Crumbs. This presentation will focus on the identity, regulation and function of these morphogenetic effectors. This work was funded by NIH grants 5RO1DE013899 and 5RO1GM145873.

Program Abstract #68

The Developmental Studies Hybridoma Bank: sharing monoclonal antibodies through open science

Douglas Houston, Gemma Kerr, Karla Daniels, Yang-Nim Park
The University of Iowa, USA

The Developmental Studies Hybridoma Bank (DSHB; https://dshb.biology.uiowa.edu) is an international resource dedicated to the sharing of monoclonal antibodies (mAbs) for research. Started in 1986 and housed at the University of Iowa since 1998, the DSHB's mission is threefold: 1) to bank and maintain the availability of important research monoclonal antibodies and hybridomas - without regard to commercial profitability; 2) to distribute these reagents at the lowest possible cost for non-profit research and teaching; and 3) to disseminate information on the 'art' of hybridoma technology and on the use and applicability of these antibodies. In addition to its growing collection of over 600 recombinant mAbs, the DSHB maintains over 5000 different hybridoma cell lines, many of which have been faithfully propagated and shared for over 40 years. Many of these lines produce mAbs of critical importance for developmental biology, including hundreds against Drosophila embryo targets (which cross-react across many invertebrate species) as well as those against neural development, muscle development and stem cells. The fruit of many large antibody generation initiatives have been entrusted to the DSHB, including the NCI's Clinical Proteomic Technologies for Cancer Program (CPTC, 800+ mAbs), the NIH Director's Common Fund Protein Capture Reagent Program (PCRP, 700+ transcription factor mAbs), the NeuroMab collection of neuroscience and neurodevelopment antibodies (500+ mAbs), the BRAIN Initiative Cell Census Network (BICCN; 300+ mAbs), and over 20 useful Xenopus embryo mAbs. Data on the use of these mAbs and on their target proteins are available through our website, which also facilitates easy ordering and mAb deposition. This presentation is an overview of DSHB, our monoclonal antibody and web resources, and some research activities of DSHB. DSHB is funded entirely by user fees.

Program Abstract #69

BioME, A Good StartEfficacy, Appreciation and Utilization of an Open Access Program of Animated Lessons

Peyton Kiser, Jack Thatcher
West Virginia School of Osteopathic Medicine, USA

Biology from Molecules to Embryos (https://BioME.wvsom.edu), an open access program consisting of animated lessons for Genetics and Developmental Biology, was posted online on September 9, 2022. After over a year and a half, it was important to conduct a quality analysis to confirm that it is performing as a valuable educational resource. This analysis entailed assimilating evidence to determine its educational efficacy, user opinion of its value, and user utilization. The results are encouraging for all

three criteria. For instance, from its posting to the submission of this abstract (4/10/24), the program was accessed 2,351 times by 487 different users. This poster will present the data supporting these conclusions. Moreover, the animated lessons themselves will be demonstrated during the poster session. This project was generously funded by the West Virginia Higher Education Policy Commission and WVSOM Academic Development Committee.

Program Abstract #70

Partnerships between tribal colleges and Montana State University to create STEM CUREs

Christa Merzdorf[1], Jennifer Forecki[1], Chelsea Morales[2]

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We developed the Trails to Research program to increase research capacity at Tribal Colleges and to ease the transition of students from Tribal Colleges (which are often very small 2-year institutions) to 4-year universities. This program is based on strong relationships that we built with instructors and administrators at Montana's Tribal Colleges. In phase one, we developed and taught introduction to research courses that are based on zebrafish embryonic development. We have been teaching these courses at Montana State University and at Montana's Tribal Colleges for the last 9 years and they are continuing strong into the future. A goal for this summer was to enlarge our program beyond Montana. We succeeded in recruiting students from 3 Tribal Colleges in Oklahoma and Arizona to our course at MSU, in addition to a large cohort of Montana Tribal College students. Over the years, multiple instructors from Tribal Colleges have participated in our courses to experience the CURE model. In phase 2 we have begun to work with Tribal College faculty from Aaniiih Nakoda College (Chelsea Morales) and Little Bighorn College (Sara Plaggemeyer) to develop their own CUREs. We started with an intensive week-long retreat to work out learning goals, research goals, experimental approaches, syllabi, protocols, lists of items to order. The goal was to have Chelsea and Sara outfitted with courses that are nearly ready to go. Chelsea decided on a project examining the diet of bison on the Ft. Belknap Reservation by DNA isolation and genomic sequencing. Sara decided to examine the difference in the microbiome in healthy vs disturbed soils on the Crow Reservation. At this point, Chelsea's course has been taught once and Sara's course will hopefully be taught during the coming academic year. This program is funded by the NSF.

Program Abstract #71

Developing Scientists: Results from an Interinstitutional Developmental Biology CURE and Regional DB Symposium

Adriana Visbal[1], Mardelle Atkins[2], Elda Rueda[1], Sharmin Hassan[2]

[1]University of Houston-Downtown, USA; [2]Sam Houston State University, USA

Increased student access to research practice is a pillar of Vision and Change for increased biological literacy. Yet, not all students can access classical mentored research experiences. A way to expand access to research opportunities is course based undergraduate research experiences (CUREs). For 3 years, we have implemented a novel CURE approach. In Fall '21, we began a collaboration between Sam Houston State University and University of Houston-Downtown students to share analyses, results, and ideas. Here, we not only utilize the high-impact CURE practice, but also try to capitalize on building a peer community to improve STEM identity and positively impact student retention. Specifically, we use Zoom and on-line file sharing technologies so that students at the two institutions collaborate on a common problem, presenting to each other in a "lab meeting" setting. Our aim is to create a space for students to see peers as "scientists", feel belonging within a peer scientific community, and thereby increase their own STEM identity, while experiencing authentic research and engaging in a realistic remote collaborative process. Scientifically, a prior mentee identified 92 conserved, but poorly characterized, candidate genes for *Drosophila* eye or head development, which our students test. With this approach, we now have a program that serves roughly 45 students each Fall; multiple students have presented their work at regional conferencesand we have identified 4 new eye development genes. In Fall '23, with SDB support, we expanded this experience by organizing a regional Developmental Biology Symposium where these students (and more) presented their research and interacted with faculty and graduate students. Here, we summarize the impact of these two interventions. Supported by SDB Education Grant 2023.

Program Abstract #72
Golgi protein Glg1 is required for ciliogenesis
Tynan Gardner, John Wallingford
UT Austin, USA
Cilia are microtubule-based organelles that are vital for proper vertebrate development, and mutations that disrupt their biogenesis or function lead to a variety of human diseases called ciliopathies. By serving as signaling hubs and driving extracellular fluid flow, cilia are necessary for developmental events including SHH signaling and left-right asymmetry. Two protein complexes, the Intraflagellar Transport (IFT) A and B complexes, build cilia by driving transport along the microtubule-based axoneme, delivering new protein cargoes to the growing cilium. While the dynamics of IFT proteins along the axoneme and at the basal body are well characterized, the full host of cytoplasmic factors required for their recruitment from the cytoplasm to the basal body remain unclear. Recent work in our labs investigating conserved eukaryotic protein-protein interactions revealed physical interactions between Glg1 protein and the IFT complexes. Glg1, also known as MG160, ESL-1, and CFR-1, is a transmembrane glycoprotein that localizes to the lumen of the medial golgi and the surface of the plasma membrane. Until now, this protein has not been implicated in ciliogenesis. Furthermore, its localization to the Golgi apparatus supports the hypothesis that IFT proteins travel to the basal bodies as vesicle coats. Through in vivo imaging of ciliated cells, we show that Glg1 is required for ciliogenesis. Knockdown of Glg1 reduces the number of cilia in multiciliated cells and results in a build-up of IFT-B proteins at the proximal region of axonemes. This work implicates a novel regulator of cytoplasmic recruitment of IFT imperative for ciliogenesis and development. NIH 5R01HD085901-05

Program Abstract #73
Frizzled 7-dependent regulation of planar polarity and cell migration during zebrafish gastrulation
Joy Creighton
Middle Tennessee State University, USA
Planar polarity is a conserved context-dependent process necessary for shaping the body axes, wherein cells communicate to coordinate orientation and organization within the plane of a tissue. During zebrafish (*Danio rerio*) gastrulation, a planar polarity-dependent phenotype of polarized cell morphological behaviors regulates collective cell migration toward the dorsal axis as part of convergence and extension. Yet how planar polarity coordinates and regulates migrating cells on the cellular and subcellular level is still unclear. Previous studies in fly epithelia identified a core group of proteins required for planar polarity. Here we explore the requirement for the core transmembrane protein Frizzled 7 (Fzd7) in gastrulation cell movements. A cellular phenotype was first identified where loss of *fzd7* inhibits cell orientation and migration causing a severe phenotype evident by a shortened, wider dorsal axis and misshapen body plan. Interestingly, the *fzd7* phenotype is more severe than the embryonic lethal mutant phenotype of another core transmembrane polarity protein (VANGL planar polarity protein 2), but *fzd7* mutants largely recover after gastrulation stages. While these data support Fzd7 as necessary for planar polarity, phenotypic recovery in *fzd7* mutants highlights the importance of investigating subcellular function. Current research shows altered membrane protrusion polarity in *fzd7* mutants, providing potential reasoning for loss of cell orientation. Additionally, *fzd7* mutants exhibit increased fibrillogenesis, warranting further investigation into cell adhesion molecules as an explanation for impeded cell migration.

Program Abstract #74
***Emx2* Lineage Tracing Reveals Antecedent Patterns of Planar Polarity in the Mouse Inner Ear**
Ellison Goodrich, Michael Deans
University of Utah, USA
The planar polarized organization of vestibular hair cells in the utricle and saccule is unique because these sensory organs contain two groups of hair cells with oppositely oriented stereociliary bundles that meet at a Line of Polarity Reversal (LPR). EMX2 is a transcription factor expressed by one hair cell group that reverses the orientation of their bundles, thereby forming the LPR. Using *Emx2*-Cre mediated genetic labeling in the mouse, we demonstrate that the onset of *Emx2* expression occurs at embryonic day 11.5 (E11.5) within the developing otic vesicle before vestibular hair cells are specified. At this stage *Emx2*-Cre

genetic labeling reveals a continuous field of *Emx2* expression within the inner ear prosensory domain that will subsequently segregate to give rise to the utricle and saccule. To determine whether this early pattern of *Emx2* expression corresponds to the boundary of EMX2 and hence the position of the LPR seen in the mature utricle and saccule, we generated *Emx2*-CreERt2 transgenic mice for complementary genetic lineage tracing assays. Precursors labeled by *Emx2*-CreERt2 at E11.5 give rise to hair cells located along one side of the LPR in the mature utricle or saccule indicating that this boundary is indeed established in the prosensory domain and prior to sensory organ segregation. Consistent with this, *Emx2*-CreERt2 lineage tracing in *Dreher* mutants where the utricle and saccule fail to segregate labels a continuous field of cells located along one side of a fused utricular-saccular-cochlear organ. These observations reveal that LPR positioning is pre-determined in the developing prosensory domain of the otic vesicle, and that EMX2 expression distinguishes two lineages of hair cells with oppositely oriented stereociliary bundles. This work was supported by NIH R01DC013066.

Program Abstract #75

Palmitoyltransferases and Golga7 in Fat/Dachsous mediated growth control in Drosophila

Alex Murphy[1], Xing Wang[1,2], Yifei Zhang[1,3], Seth Blair[1]

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Heterophilic protocadherins Fat and Dachsous (Ds) and their downstream effectors regulate growth and planar cell polarity by modulating the levels and localization of Dlish, a SH3-containing protein, and its binding partner, the atypical myosin Dachs. Loss of Fat or its intracellular domain (ICD) increases Dlish/Dachs levels at the subapical cortex where Dachs negatively regulates Warts, the final kinase of the Hippo pathway. Conversely, subapical accumulation of Dachs/Dlish requires the DHHC palmitoyltransferase (DHHC) Approximated (App). App palmitoylates Dlish and the Fat ICD, tethering Dachs/Dlish to the subapical cortex and weakening Fat's impact on Dachs/Dlish localization. Fat ICD palmitoylation persists in *app* null mutants, suggesting the involvement of additional DHHCs. Most of Drosophila's 20 DHHCs concentrate in the Golgi or ER, but dZDHHC8, like App, can localize to the plasma membrane. We assessed dZDHHC8's effects on spacing between the crossveins (CV) in adult wings, as reductions are common in mutants of Fat/Ds pathway components, including App knockdowns. We found that dZHHC8 knockdown weakly reduced CV spacing in WT and *app* null mutants, indicating its involvement in the Fat/Ds pathway, potentially acting redundantly to App. We also found that dZDHHC8 (and App) binds Dlish though little is known about their regulation. App and dZDHHC8 resemble yeast Erf2 and mammalian ZDHHC9 and 5, which bind GOLGA7/Erf4 cofactors thought to aid in target recognition and palmitoylation. We found that the sole Drosophila GOLGA7 (CG5447) is required for normal CV spacing and WT accumulation of Dachs/Dlish. Knockdown of dGolga7 also strongly reduced App and dZDHHC8 levels. This suggests dGolga7 regulates the Fat/Ds pathway through control of DHHC stability. We observed GOLGA7-DHHC binding *in vitro* and are testing conserved binding motifs to provide tools for further analyses. Supported by NIH R01GM151072 and the UW-Madison Genetics Training Program.

Program Abstract #76

Deconvolution of the G protein-coupled receptor signaling pathway that mediates lower jaw development

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During vertebrate craniofacial development, extracellular signaling cues specify neural crest cells (NCCs) into diverse skeletal progenitors of the head and face. The bone and cartilage of lower jaw structures are specified by a secreted ligand Endothelin 1 (Edn1), which induces expression of lower jaw patterning genes in NCCs through activation of Endothelin receptor type A (Ednra). However, the signaling effectors and mechanisms downstream of the Edn1/Ednra signaling pathway that regulate patterning gene expression remain poorly understood. Ednra is a G protein-coupled receptor (GPCR) that can activate all four families of Gα subunits ($G_{q/11}$, $G_{i/o}$, G_s, and $G_{12/13}$). One model has proposed that lower jaw patterning is mediated by a combination of $G_{q/11}$ and other Gα family members. Here, we tested this model with a comprehensive analysis of $G_{q/11}$ function in zebrafish lower jaw development. We generated mutant alleles for three genes encoding $G_{q/11}$ proteins, *gnaq*, *gna11a* and *gna11b*, finding

that loss of *gna11a* and *gna11b*, but not *gnaq*, recapitulated the craniofacial phenotype of *edn1*$^{-/-}$ animals. Further, by inducing expression of a constitutively active G_q protein in *edn1*$^{-/-}$ animals, we restored expression of lower jaw patterning genes and rescued the craniofacial phenotype. These results indicate that the $G_{q/11}$ signaling pathway is necessary and sufficient for lower jaw development. Establishing $G_{q/11}$ as the sole mediator of the Edn1/Ednra signaling pathway in lower jaw development now allows us to deconvolute the complex signaling network downstream of $G_{q/11}$ that regulates patterning gene expression. These studies will shed light on the molecular mechanism of cell fate determination by $G_{q/11}$ in craniofacial development and potentially reveal broader roles for $G_{q/11}$-coupled GPCRs in other developmental processes than is currently appreciated. Funded in part by the National Institute of Dental and Craniofacial Research (F32DE029406 and K99DE032428).

Program Abstract #77

SH2 domain protein E and ABL signaling regulate blood vessel size

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Blood vessels in different vascular beds vary in size, which is essential for their function and fluid flow along the vascular network. Abnormally enlarged vascular lumens are associated with different types of vascular disorders such as venous malformations (VM). Molecular mechanisms involved in the formation of a vascular lumen of appropriate size, or tubulogenesis, are still only partially understood. *Src homology 2 domain containing E (She)* protein was previously identified in a screen for proteins that interact with Abelson (Abl)-kinase. However, its biological role has remained unknown. Recent studies have demonstrated that ABL signaling is increased in VMs and inhibition of ABL signaling reduces lumen diameter in vivo. Here we demonstrate that She and Abl signaling regulate vessel size in zebrafish embryos and human endothelial cell culture. Zebrafish *she* mutants displayed increased endothelial cell number and enlarged lumen size of the dorsal aorta (DA) and defects in blood flow, eventually leading to the DA collapse. Vascular endothelial specific overexpression of *she* resulted in a reduced diameter of the DA, which correlated with the reduced arterial cell number and lower endothelial cell proliferation. Chemical inhibition of Abl signaling in zebrafish embryos caused a similar reduction in the DA diameter and alleviated the *she* mutant phenotype, suggesting that She acts as a negative regulator of Abl signaling. SHE knockdown in human endothelial umbilical vein cells resulted in a similar increase in the diameter of vascular tubes, and also increased phosphorylation of a known ABL downstream effector CRKL. These results argue that SHE functions as an evolutionarily conserved inhibitor of ABL signaling and regulates vessel and lumen size during vascular tubulogenesis. This study is supported by NIH R01 HL163161 award to Saulius Sumanas and the AHA Postdoctoral Fellowship award number 24POST1192240 to Surendra Kumar Anand.

Program Abstract #78

Developmental atlas of calcium signaling induced gene expression in early zebrafish development

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Calcium signaling, pivotal from prokaryotes to eukaryotes, orchestrates crucial cellular functions and developmental states. Despite significant advances, detailed molecular characterization of calcium activity through development has been challenging due to the dynamic and transient responses, and the inadvertent triggering of calcium signals during cell dissociation. In our research, we employed single-cell RNA sequencing (RNA-Seq) techniques to unravel the role of calcium signaling in zebrafish early vertebrate development. We added actinomycin (Act), a transcription inhibitor, to mitigate artificial calcium transcription, enabling the sequencing of 16,843 cells at a depth of 2,408 unique molecular identifiers (UMIs) per cell. We covered three distinct pharmacological perturbances to calcium influx alongside a DMSO control and corresponding replicates. Our analysis identified a gene expression program (GEP) indicating calcium responsiveness. This program not only confirmed the role of well-documented genes like *fosab* and *junba* in calcium signaling but also identified novel candidate genes for further exploration. We ranked cell types based on responsiveness and found that epithelial cells

exhibit the highest sensitivity to calcium influx. Interestingly, the hatching gland and some epidermal cell types differ in response to calcium influx-inducing drugs, indicating sensitivity to calcium influx sources. We are developing new computational tools to characterize the diverse transcriptional responses to calcium signaling perturbations and their effectors, paving the way to accurately annotate calcium activation states in emerging datasets. This research will deliver a comprehensive molecular atlas of calcium-positive cell molecular identity and their calcium-responsive gene programs. It will offer unprecedented insights during early vertebrate embryogenesis and potential therapeutic avenues for managing developmental disorders tied to calcium signaling dysregulation.

Program Abstract #79

Notch receptors are involved in secretory cells production during intestinal development of Zebrafish.

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During the first half of embryogenesis, the intestinal epithelium is a simple layer of cuboidal cells surrounded by lateral plate mesoderm. As embryogenesis proceeds, the epithelium differentiates to include a variety of enterocytes and secretory cells. Notch signaling is used multiple times during intestinal development to specify cell fate decision and commitment. Using a Notch driven CreERT2 combined with a nuclear mCherry reporter, we identify at least three different periods where Notch signaling is active. The first two periods involve the choice between secretory and enterocyte cells. Zebrafish have 4 types of receptors (Notch 1a/1b, Notch 2, and Notch 3) and 5 ligands which are expressed homogeneously within the intestine. Notch signal transmits between adjacent cells by lateral inhibition. Activation of Notch by secretory cells initiates differentiation of enterocytes, while loss of Notch signaling in zebrafish intestine results in secretory cells development. We hypothesized that two Notch receptors work together redundantly to guide epithelial cells differentiation and production towards secretory or enterocytes cell fate. We are investigating which of the four Notch receptors are involved in this process by creating double mutant embryos using previously identified null mutants in each of four zebrafish *notch* genes. Screening of intestine and determining the average number of secretory cells in each null mutant combination allows us to decide which gene combination causes increased secretory cell numbers within the intestinal epithelium. Funding sources: NIH. **Keywords:** Notch, Secretory cells, Lateral inhibition, intestinal development.

Program Abstract #80

CD44 facilitates adhesive interactions in airineme-mediated intercellular signaling

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Specialized cellular protrusions facilitate local intercellular communications in various species, including mammals. Among these, airinemes play a crucial role in pigment pattern formation in zebrafish by mediating long-distance Notch signaling between pigment cells. Remarkably, airinemes exhibit large vesicle-like structure at their tips, which are pulled by a macrophage subpopulation and are delivered to target cells. The interaction between macrophages and Delta-ligand carrying airineme vesicles is essential for initiating airineme-mediated signaling, yet the molecular details of this interaction remains elusive. Through high-resolution live imaging and genetic *in vivo* manipulations, we found that adhesive interactions via the extracellular domain of CD44, a class I transmembrane glycoprotein, between macrophages and airineme vesicles are critical for airineme signaling. Mutants lacking the extracellular domain of CD44 lose their adhesiveness, resulting in a significant reduction in airineme extension and pigment pattern defects. Our findings provide valuable insights into the role of adhesive interactions between signal-sending cells and macrophages in long-range intercellular signaling. We acknowledge funding from NIH R35GM142791 to D.S.E., support from the Drosophila Genomics Resource Center (NIH Grant 2P40OD010949) and GAANN fellowship awarded to R.B.

Program Abstract #81

BOC activation of SRC family kinases impacts Sonic Hedgehog signaling

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The Sonic Hedgehog (SHH) morphogen is essential for proper development during embryogenesis. SHH is released from a small subset of producing cells to establish a signaling gradient across developing tissues. Receiving cells respond to the SHH ligand in a graded manner, allowing them to adopt distinct cell fates to drive tissue patterning. One method to establish this gradient is through cytonemes, which are a type of specialized filopodia that facilitate long-distance cell-to-cell communication. The molecular mechanisms leading to the development of these actin-based structures are not fully understood. Our previous work shows that SHH expression induces cytoneme formation. Here, we show that the adhesion protein BOC, which can function as a SHH co-receptor, induces cytoneme occurrence independent of SHH expression. This induction is lost in BOC knockout cells, and it is rescued by BOC re-expression. Here, we demonstrate that SRC tyrosine kinase over-expression can induce cytoneme-like extensions independent of SHH. Functional studies revealed that BOC is a substrate of SRC, suggesting SRC may contribute to BOC-mediated cytoneme induction. We identified the tyrosine residues on BOC that are phosphorylated by SRC and are currently testing which phosphosites are necessary for induction of cytonemes. We are also investigating the functional implications of ablating SRC-mediated phosphorylation of BOC to understand how SRC contributes to SHH transport. This work was funded by NIH R35GM122546 (SKO) and ALSAC of SJCRH.

Program Abstract #82
Wnt signaling and its role in the development and progression of colon cancer via macropinocytosis
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The Wnt pathway plays a critical role in regulating cell growth, differentiation, and apoptosis, and its dysfunction is closely related to the development of colon cancer. Wnt signaling activation is also associated with cellular macropinocytosis, a crucial process for the internalization and degradation of extracellular materials vital for tumor progression. Here, we found that the Na,K-ATPase levels are elevated in advanced colon carcinoma, that this enzyme is elevated in cancer cells with constitutively activated Wnt pathway and is activated by GSK3 inhibitors that increase macropinocytosis. In Xenopus embryos, brief Ouabain treatment at the 32-cell stage critical for the earliest Wnt signal in development inhibited brain development and could be reversed by Lithium chloride, a Wnt mimic. These strategies could represent promising approaches for colon cancer treatment and potentially other types of cancer. Ongoing research in this field is crucial for translating these findings into improvements in diagnosing and treating this disease.

Program Abstract #84
Dishevelled C-terminus Signaling in Developing Vertebrate Embryos
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The conformational dynamics of Dishevelled (Dvl) play a crucial role in modulating its interaction with binding partners, thereby regulating downstream signaling pathways. While extensive research has focused on the conserved domains of Dvl (DIX, PDZ, and DEP), comparatively less attention has been given to its C-terminal region. Previous studies have demonstrated that components of the non-canonical Wnt signaling pathway bind to the C-terminal region of Dvl2, inducing a conformational change that activates non-canonical Wnt signaling while suppressing canonical Wnt signaling. In this study, we aim to identify and elucidate the role of novel Dvl2 C-terminal region binding partners in modulating canonical and non-canonical Wnt signaling pathways. Through mass spectrometry analysis, we identified Kizuna (Kiz), also known as PLK1s, as a potential binding partner of Dvl2's C-terminus. Mutations in the Kiz gene have been associated with deformities in the retinal pigment epithelium (RPE) and retinal dystrophy, leading to retinal degeneration and eventual blindness in affected human patients.
Interestingly, *Xenopus* Kiz expression was also observed in the RPE layer and the gastrocoel roof plate

(GRP). Further investigation revealed that Kiz localizes to basal bodies of cilia, which is vital in RPE maturation and signaling gradient establishment for proper left-right patterning. Loss of Kiz function led to defects in RPE layer differentiation and aberrant left-right patterning in Xenopus tadpoles. Additionally, our findings suggest a link between non-canonical Wnt signaling, mediated through Dvl and PKC-delta, and Kiz function for cilia formation and tight junction formation in the GRP. Future studies will investigate the mechanistic insights of the interaction between Dvl's C-terminal region and Kiz, particularly in the context of cilia formation. This study was supported by the Intramural Research Program of the NIH/NCI (1ZIABC010006-26).

Program Abstract #85

TGF-Beta and non-canonical Wnt signaling interactions coordinate anterior-posterior and dorsal-ventral axis formation in sea urchin embryos

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In deuterostomes, the specification and patterning of the anterior-posterior (AP) and dorsal-ventral (DV) axes spatiotemporally overlap. However, we lack a clear understanding of the explicit molecular mechanisms that control these axes in any developmental model organism. In sea urchin embryos an integrated network of canonical (Wnt/β-catenin) and non-canonical (Wnt/JNK and Wnt/PKC) signaling pathways specify and pattern the early germ layers along the AP axis. During this progressive AP patterning process, ventrally localized Nodal signaling initiates DV axis specification, establishing opposing Nodal and BMP2/4 signaling gradients activating DV GRNs in all three germ layers. Previously, we have shown that non-canonical Wnt16-Fzd1/2/7 signaling plays a critical role in the sea urchin AP Wnt signaling network. Here, we use functional knockdown experiments to show that Fzd1/2/7 signaling and a different Wnt ligand, Wnt6, are necessary for early Nodal signaling activity, as well as the transcription of *bmp2/4* and the dorsal ectodermal GRN. Unexpectedly, our data indicate that *nodal* transcription is normal in Wnt6 and Fzd1/2/7 knockdown embryos, but phosphorylation of its downstream target Smad2/3 is perturbed. Furthermore, we show that dorsal BMP2/4 signaling is necessary for the activation of a secreted Wnt ligand antagonist, Wnt inhibitory factor1 (Wif-1), in dorsal-anterior blastomeres. Wif-1 functional knockdown assays indicate that Wif-1 is essential for the positioning of the anterior neuroectodermal GRN around the anterior pole during AP patterning. Together, our results illustrate that in sea urchin embryos there are direct interactions among components of the early AP Wnt signaling network and the DV Nodal-BMP2/4 signaling pathways. This work was funded by NIH and Auburn University.

Program Abstract #86

Characterizing the role of Claudin.j in *Ciona* Bipolar Tail Neurons

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Cell-to-cell adhesion is necessary for the proper establishment of epithelial tissues and organs during development. In vertebrate organisms tight junctions comprised of Claudin, Occludin and Junctional Adhesion Molecule (JAM) transmembrane proteins anchor cells to one another and serve as physical barriers to the transport of molecules between epithelial cells, regulating the epithelial polarity and paracellular transport. In addition, Claudin proteins within tight junctions interact with the actin binding protein Zona Occludin (ZO) to create a linkage between the cytoskeletons in adjacent cells. In the model chordate, *Ciona robusta*, Bipolar Tail Neurons (BTNs) undergo a complex morphogenesis during which nascent pairs of BTNs delaminate from the dorsal ectoderm and migrate as pairs of cells anteriorly alongside the neural tube. RNAseq profiling of BTNs has revealed a putative tight junction, Claudin.j (Cldn.j), is expressed when BTN cells are specified. As invertebrate chordates, *Ciona* bridges invertebrate and vertebrate taxa. Previous microscopic analysis of *Ciona* tight junctions has indicated the presence of vertebrate-like tight junctions in *Ciona* (Lane et. al 1986). However, whether Cldn.j participates in these structures is unknown and what role this may play in the morphogenesis of BTNs remains unclear. To determine whether Cldn.j is necessary to form a tight junction that links pairs of BTN cells during their collective migration, we examined the distribution of fluorophore-tagged Cldn.j, JAM and ZO in transgenic BTN cells. To more directly assess whether Cldn.j might interact with ZO proteins to facilitate

migration of BTN cell pairs, we also generate transgenic embryos expressing truncated Cldn.j proteins which lack the PDZ-binding domain required for binding to ZO. Our results are necessary for understanding the specific role of cell junction-associated proteins, such as Cldn.j, in the collective migration of neuronal cells. (NIH Award #RO1HD110544-01)

Program Abstract #87

Rab7-dependent FGFR storage during asymmetric heart progenitor induction in *Ciona*

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Spatial and temporal control of signaling components plays a critical role in the regulation of cell signaling during development. In *Ciona*, the mitotic kinase, cyclin-dependent kinase-1 (CDK1), inhibits the degradation of Fibroblast Growth Factor Receptors (FGFRs) during asymmetric division of pre-cardiac founder cells. This mitotic storage of FGFRs is required for proper heart progenitor cell fate induction. However, the precise mechanism by which CDK1 promotes the mitotic storage of FGFRs remains unclear. Here, we show that CDK1 inhibits mitotic degradation of FGFRs via phosphorylation of the Rab7-GTPase, Rab7a, which is required for the fusion of late endosomes to lysosomes. Using site-directed mutagenesis, we mutated the putative CDK1 phosphorylation site in *Ciona* Rab7a to produce phospho-deficient and phospho-mimetic Rab7a expression plasmids. We generated transgenic *Ciona* embryos expressing these mutant Rab7 proteins and examined their effects on both FGFR degradation and cardiac cell fate induction. Degradation and induction assays showed that expression of the phospho-deficient Rab7a mutant proteins in transgenic *Ciona* embryos results in increased FGFR degradation and reduced heart progenitor fate induction. Conversely, expression of the phospho-mimetic Rab7a mutant protein resulted in decreased FGFR degradation and increased heart progenitor cell fate induction. Both CDK1 and Rab7a are highly conserved proteins whose roles in regulating mitotic entry and late endosome to lysosome transport, respectively, are preserved across species. As such, the mechanism of CDK1-dependent control of FGFR in the asymmetric division of the heart progenitor cell may translate to other cell types, providing a new model for how cells carry growth factor signaling components through cell divisions. (This work is funded by NSF Grant #2052493.)

Program Abstract #88

Cellular trafficking of Fibroblast Growth Factor receptors during and after cell division in cultured keratinocytes

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Cellular trafficking of receptors and associated membrane proteins is crucial for regulating cell signaling. It has long been thought that membrane trafficking shuts down during cell division, thus the regulation of membrane trafficking during mitosis remains poorly characterized. In the model chordate, *Ciona robusta*, active cyclin-dependent kinase 1 (CDK1) prevents the degradation of Fibroblast Growth Factor receptors (FGFRs), responsible for inducing cardiac cell fate during asymmetric pre-cardiac founder cell division (Cota *et al.*, 2021). However, it is unclear whether this mechanism is conserved in other systems or unique to *Ciona robusta*. Therefore, we aim to investigate whether cell cycle-dependent regulation of FGFR trafficking is conserved in mammalian organisms. To achieve this goal, we examined the trafficking of mammalian wild-type FGFR2 in cultured mouse keratinocytes both during and after cell division. Furthermore, our analysis of FGFR degradation in *Ciona* suggests that CDK1-dependent inhibition of mitotic FGFR degradation may result from the phosphorylation of the Rab7-GTPase, a protein necessary for delivering FGFR to lysosomes for degradation (Cota *et al.*, 2021). Using site-directed mutagenesis, we mutated the putative CDK1 phosphorylation sites in the mammalian Rab7 plasmids to generate phospho-deficient and phospho-mimetic Rab7a mammalian expression plasmids. We expressed these mutant Rab7 proteins in cultured keratinocytes to determine the impact of the Rab7 phospho-mutants on mitotic FGFR2. Our results provide critical insight into the dynamics of FGFR2 trafficking in keratinocytes during and after cell division. Moreover, these findings represent a necessary first step in determining whether the CDK1-dependent inhibition of mitotic FGFR degradation observed in *Ciona robusta* is conserved in mammalian systems. This research project is funded by the National Science Foundation under Award #2052493.

Program Abstract #89

CRYPTIC PRECOCIOUS plays a role in establishing herkogamy in distylous *Turnera subulata* (Passifloraceae)

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Herkogamy, physical separation of the stigma from the anther, is one of several approaches plants use to promote outcrossing by discouraging self-pollen from landing on self-stigma. In addition to self-incompatibility, distylous species exhibit reciprocal herkogamy, which includes an additional reverse herkogamous morphotype where the anther resides above the stigma. In *Turnera*, reverse herkogamy is controlled by two genes, *BAHD* (style length) and *SPH1* (filament length). While *BAHD* has been characterized, little is known of *SPH1* in *Turnera* and essentially nothing is known about the *SPH* family outside of the founding member in *Papaver*. Here, we use a multi-omic approach to explore the role of SPH1 in filament elongation. Utilizing phosphoproteomics, proteomics, and transcriptomics we identified pathways and genes of interest for future empirical work. Our analyses suggest that both cell elongation and proliferation are important for establishing filament dimorphisms in *Turnera*. We propose that overexpression of CRYPTIC PRECOCIOUS in the L-morph is responsible for establishing herkogamy; gain of *SPH1* in the proto-S-morph returned the proto-S-morph to a non-herkogamous state and subsequent gain of *BAHD* created the reciprocal herkogamy that's present in the modern S-morph. Altogether, we report the first exploration of the *SPH* family outside of *Papaver*, the first multi-omic analysis of a distylous species to date, and the first hypothesis unlinked modifier L-morph of any distylous genera. This work was funded by a National Science Foundation PRFB grant awarded to Paige M Henning (#2208975).

Program Abstract #90

Understanding male infertility in *bbs-5* mutant *C. elegans* using fluorescent tagging of sperm cells

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Bardet-Biedl syndrome (BBS) is a genetic disease classified as a ciliopathy, which is a group of diseases that arises due to abnormal function or structure of cilia. BBS is associated with a host of different phenotypes including early onset obesity, retinal degeneration, renal malformations, polydactyly, learning disabilities, and reduced fertility, specifically in sperm-producing individuals. In recent years, researchers have identified 18 genes that, when mutated, can each cause BBS. One of these genes, *BBS5*, is a minor contributor to BBS in humans and has therefore been historically understudied. *BBS5* is well-conserved in the model organism *Caenorhabditis elegans*, allowing us an opportunity to learn more about how mutations in this gene can cause such a wide array of developmental defects. Previous work in our lab has indicated that male *C. elegans* with a loss of function mutation in *bbs-5* are infertile, yet little is known about the mechanism driving this phenotype. Past research has drawn a strong link between male *C. elegans* fertility and mating behaviors and serotonin signaling. Therefore, we have designed a study to examine whether serotonin signaling is disrupted in *bbs-5* mutant males, leading to infertility and whether exposing them to exogenous serotonin can reverse this phenotype. To assess changes in male fertility after exposure to exogenous serotonin, the sperm of both wildtype and *bbs-5* mutant males was tagged with MitoTracker red fluorescent dye and sperm motility was tracked by imaging sperm movement in mated wildtype hermaphrodites before and after exposure. This work can help draw important connections between *BBS* gene mutations and infertility, with the potential to identify new treatment options for individuals with Bardet-Biedl syndrome. This work was supported by funds from Southern Oregon University.

Program Abstract #91

TCER-1 Coordinates Immunity and Reproduction through Modulation of Lipid Metabolism

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The detrimental relationship between fertility and immune function is evident across species. Yet, its underlying mechanisms remain elusive. Previously, our lab discovered that TCER-1, homolog to human Transcription Elongation & Splicing Factor 1 (TCERG1), links reproductive fitness to innate immunity in

Caenorhabditis elegans. *tcer-1* mutants exhibit impaired fertility but enhanced innate immunity against the human opportunistic pathogen, *Pseudomonas aeruginosa strain* PA14 (PA14). This study explores how TCER-1/TCERG1 manages these roles, finding it influences lipid metabolism for both immune suppression and fertility enhancement. Through multi-omic approach of RNAseq and lipidomics, we identified lipid-metabolic genes and lipid species whose levels are altered specifically in the TCER-1/TCERG1 in the maternal and embryonic tissues. Based on these genome-scale analyses, we investigated the in vivo expression and function of two TCER-1/TCERG1-regulated, lipid-metabolic genes during PA14 infection, *mxl-3* and *lbp-1*, which were predicted to be decreased and increased in expression, respectively. *mxl-3* encodes a conserved MAX-like transcriptional repressor of lipolytic gene expression, whereas, *lbp-1* encodes an extracellular lipid chaperone. *mxl-3* expression decreased in intestinal cells' nuclei, key immune sites, while *lbp-1*'s expression barely changed. However, mutants of both genes showed improved PA14 survival, suggesting a nuanced interaction. Additionally, TCER-1 loss lowered egg viability and essential fatty acid levels in embryos, impacting eggshell permeability. Experiments with BODIPY dye highlighted eggshell defects in TCER-1-deficient, PA14-exposed mothers. Our findings demonstrate TCER-1's role in balancing immunity and reproduction through lipid metabolism adjustments, pointing to new research avenues for organismal health over a lifetime. This work is funded by the NIH grants (RO1AG051659, 1R56AG066682, R214G083329) to Dr. Ghazi.

Program Abstract #92

Tissue-intrinsic signaling affects spermatogonial niche formation in *Drosophila*

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Stem cells play an important role in tissue maintenance and self-renewal. A cellular microenvironment, the niche, is required to maintain stem cells. Understanding how a niche forms and functions is key to stem cell biology. The *Drosophila* testis niche provides an optimal model to study niche formation. *Drosophila* are easily genetically modified, and this model enables complete *in vivo* visualization of niche formation with subcellular resolution. The testis niche forms at the anterior of the embryonic gonad. At this stage of development, the gonad is a spherical arrangement of germ cells encysted by somatic cells. Directly opposite the niche, at the posterior, is a group of somatic cells called male specific gonadal precursor cells (msSGPs) that eventually develop into the terminal epithelial (TE) cells of the larval gonad. We have ablated these msSGPs using two different genetic means and found that the niche has disrupted morphology or clusters away from the gonad anterior. Niches that fail to compact do not correctly polarize the F-actin cytoskeleton, which is required to establish proper niche morphology. These results, in combination with the specific positioning of msSGPs at the posterior, suggests a possible repulsive cue originating in these cells that serves to localize the compacted niche at the gonad anterior. We have turned to recent scRNA sequencing data (Mahadevaraju et al., 2022) to identify candidate signaling genes expressed in TE cells. Our data confirms that Nord, a secretory protein that modulates diffuse signals, is expressed in msSGPs and plays a role in niche compartmentalization at the anterior. Our goals include defining the mechanisms by which msSGPs, and msSGP expression of *nord*, enable proper assembly of the testis niche. As concepts in stem cell niche biology have repeatedly translated from the *Drosophila* testis to other systems, we aim to uncover novel concepts required to establish a stem cell microenvironment. NIH funding, ECU startup

Program Abstract #94

Inside-out integrin activation is essential for early mammalian development

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The ability of cells to attach to either other cells or to the extracellular matrix (ECM) is essential to ensure proper cell arrangements in three dimensional tissues. The integrin family of transmembrane adhesion receptors are the main mediators of the attachment of cells to the ECM. Integrins bind directly to ECM ligands outside the cell through their extracellular domain and link to the cytoskeleton inside the cell through their intracellular domain. Although integrin activity is thought to be under precise regulation during animal development, these regulatory mechanisms are poorly understood. The best characterized mechanism for integrin regulation is through conformational activation of integrins by extracellular signals ('outside-in activation') or by intracellular signals ('inside-out activation'). The large cytoplasmic protein

talin is an essential component of the integrin adhesion complex and is required for forming a direct link between the integrins and the cytoskeleton, as well as for inside-out activation. We used mutations in talin that disrupt integrin activation to demonstrate, for the first time, that modulation of integrin function through conformational change is essential for early mammalian development. We find that integrin activation mutants die by E8.5-9.5 and show severe defects in size and shape. Intriguingly, disrupting integrin regulation does not impinge on embryonic patterning, in particular patterning of the mesoderm and the endoderm. We explored the cellular basis of these phenotypes in embryonic stem cells isolated from integrin activation mutants. We observed only mild defects in the number of focal adhesions in ES cells derived from integrin activation mutants, but normal cell spreading and focal adhesion formation. Our working hypothesis is that disrupting integrin activation impacts ECM organization in early mouse embryos and we are currently analyzing laminin deposition in the integrin activation mutants.
Funding:CIHR.

Program Abstract #96
Muscle Filopodia Relay Motor Axon to Target Muscle Field for Functional Synapse Formation
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Precise positioning of axons is crucial for appropriate synaptic targeting for proper brain development. The glutamatergic neuromuscular synapse of Drosophila is a great model for studying the mechanisms of axon targeting and synapse formation. During axonal outgrowth into the muscle field, a motor neuron meets various muscle cells most of which are not the correct target cell. Yet, the two non-matching cells still have the ability to interact using their respective filopodia. Earlier reports have shown that filopodia at the synaptic side of the muscle contribute to the identification of the partnering presynaptic neuron. However, whether they play any role in axon guidance and targeting remain unclear. Our study investigates the filopodial activity and function across the musculature in the Drosophila body wall which consists of 30 muscles and ~36 motoneurons. To precisely map each muscle's filopodia, we genetically labeled each muscle with membrane bound GFP. We find that filopodia on the muscle is not restricted to only the presynaptic contact side, but there are also additional hot spots of filopodial activity along the edges of the muscle corresponding to the axonal trajectory during guidance. The presence of filopodia on either side raises the possibility that they have a dual response for redirecting axons into target muscle field. Indeed, we observe with live imaging that muscle filopodia are dynamic on either side and non-synaptic filopodia can interact with non-partner neurons. Additionally, through genetic manipulation of filopodia we find defects in axon guidance and targeting across motor axons in the body wall. Following up on these observations, we propose to test a model in which filopodia on the muscle act to relay the axon from one side to the other side by opposing behaviors such as *pulling* versus *pushing* to refine axon guidance and targeting for functional synapse formation. This work is supported by NIH R01 grant NS107558.

Program Abstract #97
Neurons in Disguise? Cell-cell junctions in the *Drosophila* follicular epithelium
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Epithelial tissues are commonly organized into monolayers, sheets of cells that are one cell thick. During tissue development, mitotic epithelial cells frequently move in the apical-basal axis, likely to make room for division. In the *Drosophila* follicular epithelium, reintegration of the daughter cells relies on a suite of evolutionary-conserved Immunoglobulin cell adhesion proteins called Neuroglian (Nrg), Fasciclin II (Fas2), and Fasciclin III (Fas3). These proteins are well-studied for their roles in axon fasciculation, and we have previously demonstrated mechanistic overlap between this process and reintegration. Nrg, Fas2 and Fas3 are also identified at neuromuscular junctions, synapses that appear subsequent to fasciculation, and in epithelia at septate (occluding) junctions, which do not develop in the follicular epithelium until after proliferation has ceased. These observations suggest both a mechanistic and an evolutionary relationship between neural and epithelial tissue development. We are currently exploring this possibility using structure-function analysis, genetic rescue experiments, and advanced imaging techniques. This work is funded by an NSF CAREER (PI: Bergstralh) and NIH NIGMS R01 (PI: Bergstralh).

Program Abstract #98

Identifying a Role for *miR-8* in Drosophila Border Cell Migration

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Collective cell migration is an essential process in which cells coordinate their movement, and it fulfills many physiological roles. Cell movement during immune responses, formation of tissues during development, and metastasis of cancerous cells are all examples of processes that require collective cell migration. We study the mechanisms of collective cell migration in *Drosophila melanogaster* due to the many available genetic tools and applicable imaging methods, in addition to their shared signaling pathways with humans. During oogenesis, within a developing fruit fly egg chamber, a cluster of border cells migrate through a substrate of germline cells before reaching the oocyte and contributing to egg development. MicroRNAs (miRNAs), short RNA fragments which suppress protein production through transcript destruction or by blocking translation, are potential regulators of border cell migration. Using a miRNA target seed prediction tool, TargetScanFly, we predicted which miRNAs may target genes implicated in border cell migration. We hypothesized that the knockdown or overexpression of these miRNAs may impact speed or other migration phenotypes. We identified *miR-8*, whose predicted target genes, including *slbo* and *apt*, are known to be required for border cell migration. We used the Gal4/UAS system to target *miR-8* and induce both upregulation and downregulation of the miRNA in several egg chamber cell types. Altering expression levels of *miR-8* within a subset of follicle cells, which includes the border cells, changed border cell migration speed and may impact cluster pathfinding. In these genotypes, we are currently assaying expression levels of several predicted *miR-8* targets to confirm which may be contributing to the migration phenotypes. This study will improve our understanding of how miRNAs, which are abundant in eukaryotic cells, interact with environmental cues to alter cell migration.
This work was funded by NSF-IOS Award 2303857.

Program Abstract #99

Innexins proteins regulate the breaking and making cell-cell interactions during collective cell migration

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In the study of cell movement, much of the attention has been focused on observing how cells traverse from one location to another. However, there remains a substantial gap in the understanding of how cell collectives break away from their initial neighbors in the process of delamination. Even less is known about how cells make new connections upon arrival at their ultimate destination. To be concise I name this process neolamination. I have established an *in vivo* model using the border cells in the *Drosophila* ovary to study both delamination and neolamination. Utilizing the powerful tools of *Drosophila* genetics, coupled with newly emerged optogenetics techniques and high-resolution live imaging, I have delved deep into the intricacies of collective border cell migration. In prior research, I investigated mechanisms by which border cells separate from the follicular epithelium in the process of delamination and how they make new connections upon reaching the oocyte, a process of neolamination. This research led to the identification of key stages and the regulatory genes involved in each process. A standout discovery was the crucial role of innexins proteins, responsible for forming gap junctions between cells. These proteins facilitate the diffusion of ions and small molecules between cells. Intriguingly, we observed that during the neolamination process, innexins function in a channel-independent manner, partially through the regulation of microtubule (MT) abundance and its post-translational modifications. In this work, I will exploit our newly-developed live imaging to study the border cell MT dynamics to uncover how innexins regulate MTs. Innexins are also essential for delamination and I will compare and contrast their contributions to these two processes.

Program Abstract #100

Intraepithelial migration drives cell dispersal through the developing zebrafish skin

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Specialized cells are often distributed evenly and broadly within a tissue to effectively carry out their functions. For example, mucus-secreting cells (MCs) and ion-transporting cells (ionocytes, ICs) must be broadly distributed in mucosal epithelia in the lung, gut, kidney, and many other organs to maintain tissue

health. In larval fish skin, MCs and ICs arise from basal progenitors and become broadly distributed in stereotyped patterns in the superficial epithelium, called the periderm. How these cells transit from one epithelial layer to another and achieve their broad distribution is unknown. We serendipitously discovered a cell population in zebrafish that arises from basal cells through epithelial-to-mesenchymal transition, migrate for several hours in the intraepithelial space between basal and periderm cells, and intercalate into the periderm. Live imaging and marker analysis confirms that these cells differentiate into MCs and ICs upon integration into the periderm cell layer. Time-lapse imaging reveals that migrating cells repel one another through a process reminiscent of "contact inhibition of locomotion". Surprisingly, migratory cell paths are also altered by contact with sensory axons that grow in the same intraepithelial territory. In the absence of sensory neurons, migratory cell properties are altered and MCs and ICs are aberrantly distributed. Thus, contact-mediated interactions between multiple cell types determines distribution patterns in the epidermis. To identify molecular regulators of these processes, we purified MC/IC migratory precursors and conducted single cell RNA-seq. Using these data, we aim to define the transitions between epithelial, migratory, and differentiated cell states, and to identify candidate genes that mediate the motility and cell-cell interactions of these migratory cells. These studies were supported by NIAMS and NIGMS.

Program Abstract #101

Reappraising the Role of E-cadherin in Neural Crest Epithelial-Mesenchymal Transition and Migration

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Epithelial-Mesenchymal Transition (EMT) is a highly dynamic process that occurs in embryogenesis, regeneration, and cancer metastasis. A well-known signature for cancer EMT is E-cadherin to N-cadherin switch and similar event is believed to mediate neural crest (NC) EMT. However, we and others have observed continued expression of E-cadherin in migrating Cranial NC cells of frog embryos, and loss of E-cadherin leads to defective migration of NC cells. In this study, we explored the roles of E-cadherin in Cranial NC migration. We noticed that E-cadherin is not expressed ubiquitously in NC cells, but rather expressed at high levels in a group of leading-edge cells. Cell tracking analysis demonstrated that this group of NC cells migrate more persistently, suggesting a role for E-cadherin in directed cell migration. High-resolution imaging analysis showed that parallel protrusions were formed between front row cells from this high E-cadherin expressing population. Mosaic labelling experiment confirmed that these E-cadherin expressing protrusions are double-membraned structures that extended from one cell into the neighboring cell. Our preliminary results indicate that these protrusions have actin filaments in the center, and actin filaments from neighboring cells aligned through these protrusions, suggesting a potential mechanical role mediating collective migration of NC. Live imaging and endocytosis assays also revealed that vesicles shed off from the tips of these E-cadherin expressing protrusions, suggesting a role for these protrusions in cell communication. Future experiments will elucidate the mechanical and signaling functions of these E-cadherin expressing protrusion during collective migration of NC cells. We expect this study to advance our understanding of the molecular mechanisms underlying cell migration and its implications for developmental biology and cancer research. The project is supported by NIH GM136892.

Program Abstract #102

Coordinated regulation of Cdc42ep1, actin, and septin filaments during neural crest cell migration

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The septin cytoskeleton has been demonstrated to interact with other cytoskeletal components to regulate various cellular processes, including cell migration. However, the mechanisms of how septin regulates cell migration are not fully understood. In this study, we use the highly migratory neural crest cells (NCCs) of frog embryos to examine the role of septin filaments in cell migration. We found that septin filaments are required for the proper migration of NCCs by controlling both the speed and the direction of cell migration. We determined that septin filaments regulate these features of cell migration by interacting with actin stress fibers. In NCCs, septin filaments coalign with actin stress fibers, and the loss of septin filaments leads to impaired stability and contractility of actin stress fibers. In addition, we showed that a partial loss of septin filaments leads to drastic changes in the orientations of newly formed actin

stress fibers, suggesting that septin filaments help maintain the persistent orientation of actin stress fibers during directed cell migration. Lastly, our study revealed that these activities of septin filaments depend on Cdc42ep1, which colocalizes with septin filaments in the center of NCCs. Cdc42ep1 interacts with septin filaments in a reciprocal manner, with septin filaments recruiting Cdc42ep1 to the cell center and Cdc42ep1 supporting the formation of septin filaments. To further investigate how septin filaments regulate actin stress fibers, we currently examine the interaction between septin filaments and non-muscle myosin II, the motor protein that regulates actin stress fiber formation and contractility. Our preliminary work suggests that septin filaments interact with myosin II in NCCs and this interaction influences the septin-actin association. This work is supported by the National Institutes of Health (R01GM136892) to SN and DT, the National Science Foundation (CMMI 1942561) to DT, and GAANN Fellowship to MK.

Program Abstract #103

Molecular mechanisms underlying axon target selection during regeneration in zebrafish vagus nerve

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Due to limited regenerative potential, peripheral nerve damage in humans can result in long-term sensory or motor deficits. To regenerate, axons must re-extend towards their targets, and little is known about target reinnervation. Zebrafish have a highly regenerative nervous system, making them an ideal model to study regeneration. We study axon target selection in the zebrafish vagus nerve which projects five major branches to the pharyngeal arches in an anterior-posterior pattern. While the mechanisms regulating target selection during development are well understood, how this intricate innervation pattern is reconstructed after injury is unclear[AI1]. We developed a novel single-axon injury model to examine regenerative target selection at the single-cell scale. Our previous work established that injured axons robustly regrow to correct branches, but the cues required for vagus axon guidance during development are not required during regeneration, suggesting a unique regenerative mechanism. To understand the intrinsic molecular differences allowing axonal projections to distinct targets during regeneration, we conducted RNAseq on larval neurons projecting to each vagus branch and examined differentially expressed genes (DEGs). Gene Ontology analysis identified 20 candidate DEGs with putative roles in axon guidance. RNA in situ hybridization confirmed differential expression of candidate genes. To examine the function of these candidates, we created CRISPR/Cas9 knockouts of each DEG. We will use our single-axon injury model to injure mutant axons at specific A-P positions and observe axon guidance defects. We hypothesize that knockouts of anteriorly-enriched genes will disrupt guidance to anterior branches, while knockouts of posteriorly-enriched genes will disrupt guidance to posterior branches. This work will allow us to identify the molecular mechanisms that guide axon target selection in the regenerating zebrafish vagus nerve. Funding: R00 NS121595 (AJI).

Program Abstract #104

Sonic hedgehog activates *fibrillin 2b* expression for fin ray branching

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Adult zebrafish fins rapidly and robustly regenerate, including restoring their characteristic branched bony ray skeletons. Ray branching proceeds by splitting of progenitor osteoblast (pOb) pools adjacent to *sonic hedgehog a* (*shha*)-expressing basal epidermal cells (bEps). Sonic hedgehog / Smoothened (Shh/Smo) signaling is active transiently in both cell types within a narrow, distal domain of the fin regenerate. Inhibiting Shh/Smo signaling specifically blocks ray branching. However, how Shh/Smo signaling promotes pOb pool splitting is poorly understood. We established an intubated adult live imaging method to visualize pOb and bEp behaviors for up to 24 hours during fin regeneration. *shha*-expressing bEps moved collectively over immediately adjacent pOb pools. pObs varied in morphology and directional cell movements. Distal pObs often formed cell protrusions that contacted passing-by bEps, followed by their co-movement. Split pOb pools gradually became more apparent over the imaging period, defining future ray branch points. Smo inhibition increased bEp movement rates and disrupted interactions between moving bEps and pObs. In some cases, Smo-inhibition caused partially split pOb pools to re-fuse. We conclude Shh/Smo signaling likely promotes transient, heterotypic interactions between pObs and moving bEps that ratchets pObs into split pools. We then used transcriptomics to identify candidate

Shh/Smo target genes. We found germline mutation of one candidate, *fibrillin 2b (fbn2b),* phenocopied Shh/Smo inhibition in disrupting ray branching during fin regeneration. Smo inhibition decreased *fbn2b* expression specifically in *shha*-expressing bEps. Additionally, *fbn2b* mutants have increased bEp movement rates, matching the effects of Smo inhibition. We propose Shh/Smo-upregulated Fbn2b cooperates with integrin adhesion complexes to bridge pObs and bEps and enable their co-movements underlying ray branching morphogenesis. R01GM149999 and F31HD113401 provided support.

Program Abstract #105
Investigating the role of GPR125 during zebrafish gastrulation
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The structure of adhesion GPCRs (aGPCRs), unlike other classes of GPCRs, features a highly conserved GPCR autoproteolysis–inducing (GAIN) domain and large extracellular adhesive subdomains which bind to a diverse set of ligands known to affect a variety of biological processes as diverse as growth, tissue development, and tumor metastasis. Here we investigated the role of orphan adhesion GPCR, Gpr125/Adgra3, an ortholog to human GPR125/ADGRA3, which we previously implicated using antisense morpholinos in the Planar Cell Polarity pathway and demonstrated that it interacts with Dishevelled via a PDZ binding motif (Li et. al., 2013). To provide genetic evidence and understand the functional domains of Gpr125/Adgra3 during zebrafish gastrulation, we generated a Gpr125/Adgra3 allelic series using TALENs/CRISPR/Cas9, including premature stop codons at the N-terminus (*stl720, p.A26Stop*), GPCR proteolytic site (*stl40, p. Q687fsX711Stop*), intracellular domain (*stl712, p. C1028Stop*), and at the C-terminus to remove the PDZ binding motif, ETTV, (*stl713, E1343_V1346del_Stop*). To assess the phenotype of these mutants during gastrulation, we analyzed convergence and extension (C&E) of embryonic tissues in live embryos and performed whole mount in situ hybridization using the *myoD* and *ntl/tbxta* probes to measure the length and width of somites and notochord respectively. Our experiment revealed that zygotic mutant Gpr125 embryos for the above alleles are viable and without an obvious phenotype, however, maternal zygotic (MZ) mutant Gpr125 embryos show shorter and wider body axes at late gastrulation and early segmentation stages. This evidence is consistent with C&E defects commonly observed in PCP mutants such as *trilobite/ vangl2* and supports our hypothesis that Gpr125 is required for C&E movements during zebrafish gastrulation. This work was supported in part by National Institutes of Health grant [R35GM118179 MIRA to L.S.K.].

Program Abstract #106
Protocadherin-mediated cell-cell adhesion maintains the integrity of a proto-placode in the tunicate *Ciona robusta*
Sriikhar Vedurupaka, Sydney Popsuj, Alberto Stolfi
Georgia Institute of Technology, USA
Tunicates are the closest living relative to vertebrates and their simple embryos and compact genomes make them prime model organisms to study molecular mechanisms that underlie chordate development. The oral siphon of the tunicate *Ciona robusta*, homologous to the vertebrate mouth, originates from the anterior neuropore, which gives rise to the Oral Siphon Placode (OSP). Like vertebrate placodes, the OSP is a thickening of the surface ectoderm, formed by a small rosette of tightly clustered cells that is distinct from the surrounding epidermis. Interestingly, the oral siphon of adult tunicates possess hair cell-like sensory cells. Thus the OSP might be similar to cranial placodes that give rise to various sensory cells and ganglia in vertebrates. Protocadherins are cell adhesion molecules that represent the largest group within the cadherin superfamily. We have recently shown that the OSP specifically expresses a protocadherin, *Protocadherin.e* (*Pcdh.e*) as the cells begin to coalesce and separate from other surface ectoderm cells. We hypothesized that *Pcdh.e* mediates homotypic adhesion between the cells in the OSP such that they form these self-segregating rosettes. Using tissue-specific CRISPR/*Cas*-9-mediated mutagenesis and overexpression of *Pcdh.e*, we show evidence that Protocadherin.e (*Pcdh.e*) maintains the structural integrity of the OSP and allows it to separate from related epidermal cell lineages in the tunicate *Ciona*. With this study, we work towards understanding the functions of protocadherins in tunicate development. Funding source: This is funded by NIH grant R01HD104825

Program Abstract #107

Uncovering synapse proteins in the planarian nervous system

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Regeneration of the nervous system requires both the creation of missing cell types and the reconnection of cells through creation of new synapses. Planarians, including the species *Schmidtea mediterranea*, have been used for studies of brain and nervous system regeneration due to their ability to regenerate their entire brain *de novo*. After injury, planarian stem cells produce diverse neuronal cell types and glia. New cells are present in predictable ratios and locations throughout the planarian nervous system, which includes a brain, pair of ventral nerve cords, and peripheral neuronal networks. Despite progress in understanding regenerative neurogenesis, less is known about how the planarian brain is rewired and how new neurons are connected faithfully with their partners. As a starting point for understanding these processes, an undergraduate team of researchers is characterizing planarian homologs of conserved genes known to be important in synapse formation and function in other organisms. We characterized Syntaxin family members, determining that *syntaxin-1* (*stx-1*) and *syntaxin* (*stxbp-1*) *binding protein-1* mRNAs are expressed throughout the planarian nervous system. We hypothesized that Syntaxin was important for neurotransmitter release at chemical synapses. Consistent with this hypothesis, knockdown of either *stx-1* or *stxbp-1* results in paralysis. We also identified homologs of the Neuroligin and Neurexin families, showing that *neuroligin-2* and *-4* are required for movement of the animal and for regeneration of brains of typical size. We hypothesize that Neuroligin-Neurexin pairs could promote synapse formation after injury and will test this hypothesis using antibodies targeting planarian Synapsin. Once we establish the key structural and functional components of the planarian synapse, we will be positioned to study how synapses are restored after injury. This work was funded by the McKnight Foundation and the Center for Undergraduate Research Opportunities.

Program Abstract #109

Towards a statistical analysis of expression patterns on cell lineage trees

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Cell lineage trees indicate where and when fate is determined in developing cellular systems. Phenotypic expression on these trees involve complex patterns of correlation between cells and phenotypes, reflecting the coordinated processes of fate determination. A general framework for the statistical analysis of such tree-structured patterns is, however, an open problem. Simple visualisations usually fail because the trees are too noisy, while dimensionality reduction techniques generally do not respect the topology of the tree. Here an approach is proposed to quantify tree-structured variation using a linear model with a covariance matrix that encodes the tree topology. Statistical contrasts are defined that quantify the importance of particular expression patterns. The method is benchmarked using data from C. elegans. This work was supported in part by the Australian Research Council and the National Health and Medical Research Council.

Program Abstract #110

Divide and Develop! Mitotic regulators and cell fate specification in early embryogenesis

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Early embryonic development is driven by mitosis and differentiation. Aberrations in early mitoses result in altered cell fate specification of the daughter embryonic cells, aneuploidy, and in severe cases, congenital disorders like microcephaly. We are investigating the role of mitotic machinery components like centrosomes, cytoskeletal regulators, and molecular motors during early mitoses. Experiments from our lab show that organelles such as the centrosome serve as key developmental signaling hubs and regulate cell fate specification during early zebrafish development. Here, we investigated the developmental and tissue-specific expression and functional role of the centrosomal protein *Rapgef1* using zebrafish embryos as a model. We show that *Rapgef1* is maternally expressed and the two paralogs and their alternately spliced isoforms show development and tissue-specific expression. CRISPR-Cas9 and

morpholino-based targeting of *Rapgef1b* resulted in developmental defects in the brain, eye, and somites of the developing embryo. The *Rapgef1b* morphants showed altered expression of lineage determinants of the neural crest, myogenic program, and mitotic defects such as supernumerary centrioles and chromosome mis-congression during early embryonic mitoses. Thus, our results show that *Rapgef1b* is required for early mitoses, lineage specification, and differentiation during early embryonic development. This research has been funded by the Council of Scientific and Industrial Research- Centre for Cellular and Molecular Biology (CSIR-CCMB), Govt. of India, and the Department of Science and Technology (DST), Govt. of India (DST/INSPIRE/04/2016/001436).

Program Abstract #111

Embryonic competence regulated by hydrostatic pressure in a Yap-dependent manner

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Embryonic induction is a key mechanism in development that corresponds to an interaction between a signaling and a responding tissue, causing a change in the direction of differentiation by the responding tissue. Considerable progress has been achieved in identifying inductive signals, yet how tissues control their responsiveness to these signals, known as competence, remains poorly understood. While the role of molecular signals in competence has been studied, how tissue mechanics influence competence remains unexplored. Here we investigate the role of hydrostatic pressure in controlling competence in neural crest cells, an embryonic cell population. We show that neural crest competence decreases concomitantly with an increase in the hydrostatic pressure of the blastocoel, an embryonic cavity in contact with the prospective neural crest. By manipulating hydrostatic pressure in vivo, we show that this increase leads to the inhibition of Yap signalling and impairs Wnt activation in the responding tissue, which would be required for neural crest induction. We further show that hydrostatic pressure controls neural crest induction in amphibian and mouse embryos and in human cells, suggesting a conserved mechanism across vertebrates. Our work sets out how tissue mechanics can interplay with signalling pathways to regulate embryonic competence.

Program Abstract #112

Analysis of *Sox2* Dynamics and Regulation in Preimplantation Mouse Embryos

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Understanding the establishment and regulation of pluripotency is a vital tool to furthering our knowledge on development, stem cell maintenance and cell lineage specification. During murine development, pluripotency is believed to be regulated by the Hippo pathway and is specified by the cell fate determining factor *Sox2*. Hippo regulates the expression of both *Sox2* and *Cdx2*, transcription factors which specify inside and outside cell fate respectively, in parallel. However, the mechanism by which Hippo regulates these two differing cell fates in parallel is unknown. My recent immunofluorescence data has shown embryos with individual cells expressing conflicting cell fate determining factors. This new data has thrown into question our understanding of how the first cell fate decision and its downstream targets are regulated. Here I identify the conflicting cell fate markers in the data and possible causes. In my future studies, I will use CUT&RUN as well as RNA-sequencing to determine the mechanism of regulation and address issues causing irregularities in cell fate specification. Discovering the mechanism that regulates pluripotency will generate more areas of research in developmental biology both *in vivo* and *in vitro*. Research reported in this publication was supported in part by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number T32HD087166 and R01 HD108722, MSU AgBio Research, and Michigan State University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Program Abstract #113
Identification of Critical Components Missing from Standard Culture Protocols for Optimal Mouse Preimplantation Development
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The HIPPO pathway is a key signaling pathway involved in determining cell fate decisions. Preliminary data from the Ralston lab suggests components of embryo culture have the potential to modulate activity of the HIPPO pathway and cell fate determining factors such as SOX2 and YAP1 in developing embryos. Dysregulation of the HIPPO pathway has been shown to contribute to developmental defects highlighting the importance of understanding how components of culture impact this pathway. The goal of my project is to identify approaches to ensure proper gene expression during preimplantation embryo culture. I am examining the influence of the timing of embryo culture and differences between the maternal and ex utero environments. My preliminary results suggest both maternal oviduct environment and the timing of culture can influence the regulation of cell fate determining factors. Understanding components necessary for proper preimplantation development is crucial for improving the success rate of ART procedures in addition to advancing preimplantation development research.

Program Abstract #114
Translational repressor pre-complex assembly drives timely cell-state transitions during differentiation
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Translational repressor complexes promote timely cell-state transitions during differentiation by rapidly degrading obsolete transcripts, but mechanisms controlling their assembly and activation are poorly understood. The RNA-binding protein Brain tumor (Brat) promotes deadenylation and degradation of obsolete transcripts during the maternal-to-zygotic transition in syncytial embryos and in uncommitted intermediate neural progenitors (immature INPs) in Drosophila. We identified Ubiquitin-specific protease 5 (Usp5) as a Brat interactor essential for the degradation of Brat target mRNAs in both cell types. Usp5 functions independently of its enzymatic activity to promote Brat pre-complex assembly in mitotic neural stem cells (neuroblasts) by bridging Brat and the scaffolding components of deadenylase complexes. The adaptor protein Miranda binds Brat limiting its RNA-binding activity in mitotic neuroblasts. Cortical displacement of Miranda activates Brat-mediated mRNA decay in immature INPs. We propose that the assembly of an enzymatically inactive and RNA-binding-deficient translational repressor pre-complex poises mRNA degradation machineries for rapid activation, thus driving timely developmental transitions.
National Institute of Neurological Disorders and Stroke grants: R01NS111647 and R01NS107496; Canadian Institute for Health Research grants PJT-15970 and PJT-19012

Program Abstract #115
An ancient cation channel affects membrane potential and exit from pluripotency during gastrulation.
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Congenital heart disease (CHD) is the most prevalent congenital defect and arises from genetic and environmental triggers that push cardiac development off its normal course. Genomic screens of patient cohorts have revealed many candidate CHD genes and follow up studies in model organisms like *Xenopus tropicalis* can validate the disease relevance of candidates and assess their developmental functions. *X. tropicalis* has been particularly useful in defining mechanisms of cardiac heterotaxy (HTX), a form of CHD characterized by abnormal asymmetry of the heart in relation to the Left-Right (LR) axis. Golgi anti-apoptotic protein (GAAP) has emerged as a candidate CHD/HTX gene from multiple human genetic screens and three CHD patients have been identified with loss-of-function variants in GAAP. GAAP is a 6-7 pass transmembrane cation channel that exists across evolutionarily distant groups including viruses, vertebrates, plants, and bacteria. Interestingly, GAAP is more conserved than even actin across these groups, positing GAAP as an exciting CHD candidate. We sought to identify the role of GAAP in cardiac development by testing whether GAAP depletion reconstitutes the phenotypes of identified patients. GAAP depletion reconstituted patient phenotypes and led to gastrulation defects and defects in LR patterning. Moreover, we recorded the membrane potential of cells in the developing

embryo and found that GAAP depletion led to electrical depolarization of the marginal zone just prior to the onset of gastrulation, while preventing depolarization rescued gastrulation defects. Importantly, membrane depolarization in the setting of GAAP depletion led to a failure of gastrula stage embryos to exit the pluripotent state. Finally, we demonstrate that exogenous human GAAP and viral GAAP rescue cardiac phenotypes of GAAP depletion in frogs, implying that the developmental functions of GAAP have been retained across evolution. This work was funded by GR109098 and T32GM13665.

Program Abstract #116

Histone Variant H2A.J is a Key Epigenetic Regulator in Human Trophoblast Development

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Nearly 60% of early pregnancies are lost during the stages surrounding implantation. The process of implantation is guided by the trophoblast lineage of the mammalian embryo, which directly attaches to the uterine lining. Despite its immense clinical relevance, the human trophoblast lineage is poorly understood at this stage. We discovered that the histone variant H2A.J is uniquely expressed in human trophoblast tissue, contrasting with its absence in mice. Through ex vivo culture of a limited number of human embryos over the stages of implantation coupled with immunofluorescence (IF) analysis and high-resolution confocal imaging, we found that the number of trophoblast cells expressing H2A.J gradually increased over the developmental period, mimicking implantation, and that this pattern was reflected in stem cell-based models of the human blastocyst (hBlastoids) representing the pre-implantation stage. We observed that knockdown of H2A.J caused hBlastoids to fail to generate correct lineage ratios. Single-cell RNA sequencing further confirmed the observed shifts in cell population dynamics of the H2A.J KD hBlastoids. Further implantation assays with KD hBlastoids demonstrate a continuous trend of incorrect lineage ratio development. In summation, the biased lineage specification and unique expression of H2A.J in human trophectoderm tissue suggest that H2AJ has a pivotal role in regulating the spatiotemporal dynamics of human trophoblast lineage development. This study provides valuable insight into a largely unknown histone variant protein H2A.J and paves the way to explore the causal genes in early human embryo development failure using 3D stem cell-derived embryo models.
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Program Abstract #118

Bacterial ribosome induced multipotency in mouse fibroblasts that platformed *in vitro* transdifferentiation to multilineage cells

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"Moonlighting roles" of ribosomes like cell proliferation and differentiation, is a blooming field of research beside their function for protein translation. In accordance our lab previously reported a unique role of ribosome. It states, incorporation of bacterial ribosome could induce multipotency in somatic cells and cancer cells which leads to conversion of cell lineages. Being inquisitive to know the universality of exogenous ribosome effect in other cells, we tested a less appraised primary cell-Mouse Adult Fibroblast (MAF) in this study. We have observed, bacterial ribosome incorporation into trypsinized MAF lead to ribosome induced cell clusters (RICs) formation. This clusters showed strongly positive Alkaline Phosphates staining. Under *in vitro* differentiation condition, RICs-MAF were differentiated into Adipocytes (stained by Oil red O, immunostained by Lipi-Green), Osteoblasts (stained by Alizarin Red, immunostained by *Runx*, and Chondrocytes (stained by Alcian Blue). Besides, generation of neural cells from RICs-MAF were also observed through expression of neural markers i.e., *Tuj1* (Neurons), *NG2* (Oligodendrocytes) and *GFAP* (Astrocytes) individually. Strikingly, noticeable expression of stemness markers like *Oct4*, *Nanog*, *Sox2* etc. was not observed here. Later RNA-sequencing data revealed, RICs-MAF expressed markers like *Sox5*, *DNMT3L* and *Leo1,* these genes are also known to played role in pluripotency. To sum up, this study reports a distinctive approach of exogeneous-ribosome mediated transdifferentiation of MAF through multipotency acquisition. For future cell-based regenerative applications, this study merits further investigation. Grant Support: The Support for Pioneering Research Initiated by the Next Generation

(SPRING) Program, Kyushu University and Sasakwa Scientific Research Grant by Japanese Society of Science.

Program Abstract #119
Maternal Foxi2 and Sox3 control early ectoderm gene programming and cell state specification
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During embryogenesis, maternal transcription factors (TFs) occupy cis-regulatory modules (CRMs), initiating the activation of zygotic genes through interactions with histone modifications and the chromatin architecture. Although significant insights into the maternal TFs governing mesendoderm specification have been reported, those pivotal for early ectoderm specification remain poorly understood. Since Foxi2 and Sox3 are animal pole enriched maternal TFs involved in ectoderm gene regulation in Xenopus, we hypothesized that these TFs function at the top of the TF hierarchy in regulating ectodermal cell lineage commitment. ChIP-seq analyses demonstrate the enrichment of H3K27ac and H3K4me1 activating histone markings in Foxi2-Sox3 colocalized regions of open chromatin. RNA-seq analyses on knockdowns reveal that Foxi2 and Sox3 directly co-activate critical genes for neural and non-neural ectoderm development, while repressing unique sets of mesendodermal lineage genes, suggesting their dual functional roles in gene regulation. Single-nucleus transcriptomics of both wild type and morphants revealed maternal control over the specification of numerous ectodermal cell states, which we detect emerging at earlier embryonic stages than previously reported. We propose a model where ectodermal CRMs are bound by localized maternal factors that orchestrate the initiation of neural and epidermal gene regulatory networks. Our work highlights the essential role of Foxi2 in the establishment of TF regulatory networks governing anterior-posterior neural and inner-outer layer epidermal specification. This work is supported by the following NIH grants: R21HD109696, R35GM139617.

Program Abstract #120
Deciphering the functional role of lipid droplets in the developing mammalian endoderm
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Cell fate and function is tightly controlled by different factors including genetics, epigenetics, cell signaling, and metabolism. Lipids, a class of metabolites, have been shown to correlate with and control cell identities by modulating the greater metabolic landscape, influencing internal signaling, and modifying chromatin. One of the unique features of lipids is that they are often packaged in an organelle called lipid droplets. Previously, lipid droplets were thought to be inert, but recent literature points to their critical role in modulating cell fate; they have been shown to be critical in preventing the naïve to primed transition in mouse embryonic stem cells, mESCs. In addition, this organelle has been shown to be essential for pre-implantation mouse embryonic development. Their function post implantation and in other stem cell populations, though, remains unclear. During mouse gastrulation, the visceral endoderm acts as a support system both absorbing nutrients from the maternal environment and coordinating the signals necessary for gastrulation onset. Single cell RNA sequencing results of gastrulating mouse embryos indicate high expression of genes associated with lipid droplet formation and maintenance in the visceral endoderm, but not in other lineages. Due to this, and the previous literature showing the importance of lipid droplets in other stem cell contexts, I am investigating the functional role of lipid droplets in mXEN cells, visceral endoderm derived cells. We hope to uncover how lipid droplets might function in these extraembryonic cells during gastrulation by manipulating gastrulation specific signals like WNT and BMP and by inhibiting lipid droplet formation in these cells. **Funding**: NIH Director's New Innovator Award DP2-HD112040 and Yale Training Program in Genetics 1T32GM148332-01

Program Abstract #122
The Role of Basigin in Stem Cell Differentiation of the Mucociliary Epithelium
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The mucociliary epithelium (MCE) is a layer of differentiated cells that line organs and respiratory tracts, serving as a necessary defense mechanism against pathogens and inhalants. The function of the

MCE is dependent on the correct differentiation of basal cells to ionocytes, multiciliated, secretory, and goblet cells. While we know many diseases, such as cystic fibrosis, arise from MCE defects, mechanisms that drive stem cell differentiation within the MCE remain unclear. Our lab has identified Basigin (BSG), also known as extracellular matrix metalloproteinase inducer (EMMPRIN), to play a role in differentiation of skin cells in the Xenopus mucociliary epidermis. Our data shows that BSG colocalizes with ionocytes and some basal cells. Morpholino-mediated knockdown of BSG results in a decrease in basal cells and an increase in ionocytes and multiciliated cells. BSG morphant tadpoles also display an increase in epidermal intracellular pH. From our current data, we hypothesize that BSG plays a role in regulating intracellular pH and maintaining β-catenin stability, which in turn regulates basal cell differentiation and subsequent differentiation of MCE cells. This study was supported by the Intramural Research Program of the NIH/NCI (1ZIABC010006-26).

Program Abstract #123

Uncovering essential genes for neurogenesis in *Schmidtea mediterranea*

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Regeneration is an essential function for many organisms to respond to injury. Mammals repair damaged skin and bone, deer can regrow antlers after shedding, and axolotls can regrow whole limbs. However, most organisms have limited regenerative capabilities. Improving regeneration continues to be a goal of medical science, especially for the central nervous system, where regeneration is extremely limited in humans. *Schmidtea mediterranea* is a species of planarian, an aquatic flatworm, with the unique ability to regenerate its entire body from a small fragment in just days. Robust regeneration is made possible by adult pluripotent stem cells found in the planarian, which give rise to all adult cell types after injury. Stem cells can become any one of dozens of neuronal cell types, including serotonergic, GABAergic, and dopaminergic neurons. The regeneration of diverse neuronal cell types in predictable ratios makes the planarian a great model with which to study neurogenesis and neuronal repair. To improve our understanding of neuronal regeneration, we are currently optimizing a tool to birthdate neurons and uncover the timeline of lineage specification from stem cell to neuron. Using RNA interference (RNAi), we are also knocking down genes with enriched expression in proposed neuronal progenitors and measuring the effect on regeneration and maintenance. To date, we have two hits from our screen, distal-less and six6, and we expect to find additional hits as we continue this screen. With RNAi, we discover the regulators of regenerative neurogenesis during brain regeneration in the planarian. Our data will increase our understanding of stem cell fate specification after injury. This research is supported by R01NS128096 funding from NIH/NINDS.

Program Abstract #124

Regulation of oligodendrocyte precursor cell specification by *gsx2*

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In vertebrate development, oligodendroglia, the myelinating cell type of the central nervous system, emerge from specific subsets of brain and spinal cord progenitors initially dedicated to neuron production. The mechanisms orchestrating the transition from neuron to oligodendroglia formation, and whether these mechanisms are conserved across different regions of the central nervous system, remain elusive. Previous studies have elucidated the role of the transcription factor *gsx2* in governing the timing of this neuron-oligodendroglia switch within the mouse forebrain. Our own investigation, employing single-cell RNA sequencing in zebrafish, unveiled the expression of *gsx2* in spinal cord pre-OPCs, a subset of progenitors that give rise to oligodendrocyte precursor cells (OPCs), hinting at a potential conservation of *gsx2*-mediated neuron-glia transition across species and neural tissue compartments. To test this hypothesis, we conducted loss-of-function experiments in zebrafish utilizing CRISPR/Cas9 mutagenesis to assess the necessity of *gsx2*in oligodendrogenesis. Similar to observations in the mouse forebrain, disruption of *gsx2* function in zebrafish led to formation of precocious and excess OPCs. We therefore hypothesize that *gsx2* regulates the timing of OPC specification, perhaps by regulating a switch between neuron and OPC specification by ventral spinal cord progenitors. Currently, we are investigating if *gsx2* also regulates oligodendroglia differentiation and performing gain-of-function experiments to further investigate how *gsx2* controls the timing of OPC formation.

Program Abstract #125

Regulation of granule neuron precursors in the developing cerebellum

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The cerebellum is essential for motor coordination and higher cognitive activities such as learning, attention and motion. The developing cerebellum consists of two progenitor zones, the ventricular zone (VZ) and the rhombic lip (RL), giving rise to GABAergic and glutamatergic neurons, respectively. In the RL, the proneural bHLH transcription factor ATOH1 directs the generation of granule neuron precursors (GNPs) that form the external granule layer (EGL). In the EGL, GNPs first proliferate and then differentiate into granule neurons (GNs), which migrate to the internal granule layer (IGL). Derailment of GNPs proliferation leads to 30% of medulloblastoma, which is the most common pediatric brain tumor. Therefore, the proper control of GNP proliferation is crucial during normal cerebellum development. Here, we study how the TEA domain (TEAD) family of TFs—the main DNA binding factors in the Hippo pathway—and insulinoma associated-1 (INSM1) regulate GNPs in the developing cerebellum. We found that TEAD1/2 and INSM1 are expressed in GNPs in the RL and EGL but not expressed in differentiated GNs or Purkinje cells. In addition, TEAD1 but not INSM1 is expressed in progenitors in the VZ and in glial cells. Deletion of *Tead1;2* and *Insm1*from GNPs by using *Atoh1-Cre* shows that both TFs are required for cerebellum development. Both mutants show reduced proliferation and differentiation. RNA-seq analysis revealed that these two mutants exhibited similar gene expression changes. Consistent with histological analysis, genes associated with neural development are downregulated in both mutants. Surprisingly, genes associated with muscle development are upregulated in both mutants. These results suggest that TEAD and INSM1 ensure the fidelity of the granule neuron lineage by suppressing muscle genes while promoting neural genes. **Funding:** St. Jude Children's Hospital, National Institute of Neurological Disorders and Stroke

Program Abstract #126

Converging identities: shared competence of neural crest and mesoderm in the little skate

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In vertebrate embryos, much of the craniofacial skeleton derives from the neural crest while the appendicular (fin/limb) skeleton derives from lateral plate mesoderm. In fishes, the gill arches are located at the head-trunk interface, and in the little skate (*Leucoraja erinacea*) the gill arch cartilages are of dual neural crest and mesodermal origin. This hints at an equivalence in developmental potential of neural crest and lateral mesoderm at the head-trunk interface. Using CM-DiI labeling of the neural tube in skate embryos, we mapped the fate of pre-migratory neural crest cells (NCCs) in the hindbrain. We found that pre-otic NCCs populate the mandibular and hyoid arches, while post-otic NCCs contribute to the gill arches, a pattern that is conserved with bony vertebrates. To test for shared competence of neural crest and lateral plate mesoderm, we transplanted DiI-labeled hindbrain dorsal neural tube, containing pre-migratory NCCs, into the fin bud of skate embryos. We found that labeled cells migrated away from the grafts and contributed to chondrocytes within the typically mesoderm-derived fin skeleton. These findings point to a common competence of neural crest- and mesoderm-derived mesenchyme at the head-trunk interface. Further, they suggest that local environment and tissue interactions, rather than germ layer origin, are the ultimate determinants of mesenchymal cell fate. This work is supported by the BSD Dean's International Student Fellowship at the University of Chicago.

Program Abstract #127

Expansion of a neural crest gene signature following ectopic MYCN expression in sympathoadrenal lineage cells in vivo

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Neural Crest Cells (NCC) are transitory multipotent stem cells that arise from the neural tube during vertebrate embryogenesis. NCCs give rise to a variety of cell types within the developing embryo, including neurons and glia of the sympathetic nervous system. It has been suggested that failure in NCC development leads to several diseases, including neuroblastoma (NB). During normal sympathetic

differentiation, MYCN is transiently expressed to promote NCC migration, and its downregulation precedes neuronal differentiation. Overexpression of MYCN has been linked to high-risk and aggressive NB progression. Interestingly, the effect MYCN overexpression has over sympathetic development remains largely unknown. In this study we found that overexpressing human MYCN within the SAP lineage in zebrafish led to the transient formation of an abnormal SAP population which presented expanded expression of NCC markers, while unexpectedly also co-expressing SAP and neuronal differentiation markers. In vivo time-lapse confocal imaging in zebrafish larvae during the early stages of SAP development, revealed transient expansion of *sox10* reporter in MYCN overexpressing SAPs. Additionally, we noted reduced activity in BMP signaling in these abnormal SAPs, suggesting that elevated MYCN expression disrupts BMP signaling and thereby affects the transition from NCC to SAP differentiation. Consistent with this finding, we found that pharmacologically dampening BMP signaling induced a similar abnormal NCC gene signature in SAP cells, mimicking the effects of MYCN overexpression. Our findings indicate that MYCN overexpression in SAPs interferes with their differentiation by inducing abnormal expression of NCC-related genes and attenuating the BMP signaling response, having potential developmental implications for NB priming in vivo. Funding for this project was provided by CPRIT grant RR170062 and NSF 1942019.

Program Abstract #128

Combinatorial mutation of select *hox* genes alters vagal neural crest cell differentiation during the enteric nervous system development

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Neural Crest Cells (NCCs) are a group of transient, multipotent stem cells that arise from the dorsal neural tube and extensively migrate during vertebrate embryogenesis. NCCs are subdivided into cranial, vagal, trunk and sacral subpopulations along the anterior-posterior neuraxis, and exhibit regionally specified migration and differentiation, eventually giving rise to many major cell types. Vagally-derived NCCs migrate into the gut, becoming enteric progenitor cells (ENPs), and give rise to the Enteric Nervous System (ENS). The ENS is a highly conserved, complex network of neurons and glia that locally regulates basic gut functions, such as water balance and peristalsis. It is known that ENS development is controlled extrinsically and intrinsically, including via transcription factors (TFs), however our understanding of how TFs control vagal NCC proliferation, migration, and differentiation is limited. Homeobox genes (*hox*) are a highly conserved family of genes, encoding for Hox TFs, that have been implicated in ENS development. Zebrafish single-cell RNA sequencing data has identified strong neural-expressed *hox* genes, including *hoxb5a* and *hoxb5b*, within NCCs and ENPs. Previous data has shown that *hoxb5b* overexpression in zebrafish was sufficient to expand NCC and ENP numbers, however it is not known what effect loss of *hoxb5a* or *hoxb5b* has on zebrafish ENS development. In this study, we leveraged CRISPR-mediated mutation of *hoxb5a* and *hoxb5b* to elucidate Hox TF roles in NCC migration and differentiation during ENS development. We discovered a failure of terminal neuronal differentiation within ENPs in *hox* mutants, while NCC and ENP migration was unaffected along the gut. These results suggest a potential decoupling of ENP colonization of the gut tube from neuronal differentiation upon mutation of select *hox* genes, providing new specific insights into ENS development and execution of neuronal differentiation. Funding provided by NSF-1942019 and NIH-R01DK124804.

Program Abstract #129

Defining the stage-specific role of SOX9 in avian neural crest cell development

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Neural crest (NC) cells are a population of stem-like, transient, multipotent cells originating from the dorsal side of the neural tube in developing embryos. After undergoing an epithelial to mesenchymal transition (EMT), where the cells transition from tightly bound to migratory invasive cells, they travel long distances

and differentiate into craniofacial structures and other derivatives. However, errors in NC development and function results in the formation of defects such as craniofacial cleft or the development of lethal congenital disorders such as Campomelic Dysplasia. Our project focuses on defining the role of SOX9, which is a transcription factor linked to NC EMT. Preliminary work from our lab identified that Sox9 transcripts are limited to premigratory NC cells, but that SOX9 protein expression is maintained throughout NC migration in two avian species. To determine the role of SOX9 in multiple phases of NC development, we performed SOX9 knockdown at two unique developmental stages in chick embryos, prior to NC specification and at the onset of NC EMT. We then used immunohistochemistry and histological analysis to observe the effects of SOX9 perturbation on various NC transcription factors and cell adhesion markers involved in NC EMT. We found that loss of SOX9 in later developmental stages resulted in shorter NC cell migration distances. We also saw that losses of SOX9 resulted in dysregulation of several cadherin molecules at the time of neural crest cell migration. This work will illuminate the molecular mechanisms that drive NC cell formation and increase our understanding of how cells transition from epithelial to migratory, invasive, and mesenchymal. Our future work will help to determine the conservation of SOX9 function across species and how congenital defects caused by abnormal SOX9 expression may occur. This work was funded by a Choose Development! Fellowship to HH and an NSF CAREER (2143217) to CDR.

Program Abstract #130

Single-cell profiling coupled with lineage analysis reveals vagal and sacral neural crest contributions to the avian enteric nervous system

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In amniotes, the enteric nervous system (ENS) is formed by two subpopulations of the neural crest. The vagal neural crest is the first to arise, emerges from the caudal hindbrain, and colonizes the entire gastrointestinal tract. The second population is the sacral neural crest, which develops in the caudal neural tube, enters the hindgut later, and contributes only to the post-umbilical ENS and peripheral ganglia. Although much of the ENS arises from the vagal neural crest, sacral neural crest contribution has not been fully identified. We coupled single-cell transcriptomics and axial-level-specific lineage labeling in avian embryos to compare the contributions of the embryonic vagal and sacral neural crest to the chick ENS and the associated peripheral ganglia (Nerve of Remak and pelvic plexus). In the embryonic day (E) 10 gut, the vagal and sacral neural crest derivatives form overlapping subsets of neuronal and glial cell types. However, the post-umbilical vagal and sacral neural crest profiles more closely resemble each other compared to the pre-umbilical vagal neural crest population, potentially indicating environmental rather than population differences. Additionally, there were some differences in cell types noted between the vagal and sacral derived cells. RNA trajectory analysis suggests that there is a vagal-derived neuronal/glial progenitor pool that is surprisingly absent in the sacral population. Instead, the sacral neural crest gives rise to numerous subtypes of enteric glia. The present findings identify sacral neural crest contributions to the hindgut ENS and associated peripheral ganglia and highlight the potential influence of environment in the development of the ENS. Funding sources: F31HD11128 to J.J.L, R01DK13348 to M.E.B.

Program Abstract #131

Understanding the role of SOX2 in shaping the regulatory landscape during cochlear development

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Hair cells, support cells and neurons are the basic components of an auditory epithelium, also known as cochlea, and function in tandem to enable hearing. These cell types trace their origin to a common progenitor pool however each follows a specific developmental trajectory. The transcription factor SOX2 remains a constant and crucial presence during this sensory progenitor differentiation. Our study aims to dissect SOX2 function during cochlear development and understand its role in directing these three cell fates. To dissect this function, we used timed deletions of SOX2 and characterized morphological changes alongside changes in cell type, number, and arrangement. We observed perturbation of cell cycle exit and hair cell differentiation. We also elucidate the molecular mechanism behind these observations by looking at SOX2 binding and studying the effect of its loss on the regulatory landscape

governing cell fate. This work has been carried out with funding from the Goverment of India allocated to National Center for Biological Sciences (NCBS).

Program Abstract #132

Conserved long-range Atoh1 enhancers are required for hair cell development and regeneration in the zebrafish inner ear

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The vertebrate inner ear consists of mechanosensory hair cells (HCs) and their surrounding support cells (SCs). During development, upregulation of *Atoh1* expression promotes HC differentiation, which in turn suppresses *Atoh1* expression in neighboring SCs through Notch-mediated inhibition. In contrast to non-regenerative mammals, SCs in zebrafish can initiate *atoh1a* expression and transdifferentiate into HCs after HC loss. To understand this difference, we performed cross-species single-cell profiling of gene expression and accessible chromatin in the inner ears of mouse and zebrafish. We identified a group of long-range *Atoh1*enhancers, including one conserved across vertebrates, that are accessible in SCs through adulthood in zebrafish, but only transiently open in mouse. Interestingly, these enhancers are not accessible in *Atoh1*-expressing HCs. Both mouse and zebrafish versions of these enhancers can drive SC-specific reporter activities in larval zebrafish and neonatal mouse inner ears. Moreover, deletion of these *atoh1a* enhancers in zebrafish resulted in a severe reduction in *atoh1a* expression, fewer HCs during inner ear development, and failure to regenerate inner ear but not lateral line HCs following ablation. These findings indicate a requirement for these long-range *atoh1a* enhancers in inner ear HC development and regeneration. Further, we identified similar putative enhancers near other HC genes that are selectively open in adult zebrafish but not mouse SCs, suggesting that the ability to maintain their accessibility may allow zebrafish SCs to upregulate HC-lineage genes and transdifferentiate into HCs following injury. Currently, we are investigating candidate transcription factors expressed in zebrafish but not mouse SCs that may maintain chromatin accessibility of these HC enhancers, and whether prolonging expression of these factors in SCs may enhance HC regeneration in postnatal mice. This work is supported by NIDCD F31DC020633.

Program Abstract #133

Unraveling the mechanisms of axonemal dynein transport.

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Cilia are complex microtubule-based projections with both sensory and motor functions. Axonemal dyneins drive the coordinated beating of motile cilia, which play critical roles in human airway biology and disease. Mutation of the human *LRRC56* gene has been linked to motile ciliopathies, and while Lrrc56 is essential for cilia beating in both humans and model organisms, the underlying mechanisms remain unclear. We have used the power of *Xenopus* multiciliated cells for live imaging and proteomics to investigate the cellular and molecular dynamics of Lrrc56. We found that Lrrc56 localizes within cytosolic foci known as DynAPs, basal bodies, and mature axonemes. We observed specific loss of outer dynein arms (ODAs) upon knockdown of *lrrc56,* suggesting that Lrrc56 is a crucial player on axonemal dynein transport. ODAs are pre-assembled in the cytoplasm and transported through the cytoplasm to the base of cilia, and our laboratory has shown that this is a dynamic and multiprotein process. Using affinity purification mass-spectrometry of *in vivo* interaction partners, we have also identified Ccdc63 and Ccdc151 as candidate interacting proteins of Lrrc56. Our findings will provide new depth and breadth to our understanding of axonemal dynein transport in vertebrates. This project was funded by the NIH (RO1HH117164) and the UT Austin Provost's Early Career Fellows program.

Program Abstract #134

Ybx2 as a novel regulator of motile ciliogenesis

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The cilium, a microtubule-based organelle, plays a pivotal role in embryonic development and maintaining physiological functions in the human body. Multiciliated cells (MCCs) possess bundles of motile cilia crucial for generating fluid flow across epithelia, essential for tissue homeostasis. The

differentiation of MCCs and the construction of ciliary structures necessitate diverse cellular mechanisms that occur in MCC cytoplasm, but their exact mechanisms remain poorly understood. Recent discoveries have unveiled intriguing interactions between the RNA-binding protein Ybx2 and the motile cilia-related protein Ribc2. While Ybx2 is known for its role in RNA storage during spermatogenesis, its function in motile cilia remains relatively unexplored. Knockdown experiments in *Xenopus laevis* embryos reveal disrupted multiciliary structures and impaired left-right asymmetry, with phenotypic rescue upon ectopic Ybx2 mRNA expression. Notably, Ybx2 localizes in the MCC cytoplasm, similar to the formation of DynAPs and stress granules crucial for MCC development. Ybx2 deficiency correlates with reduced granule numbers and disrupted ciliary phenotypes. Surprisingly, our investigations have unveiled the intricate interplay between Ybx2 and essential MCC genes, such as MCIDAS and Foxj1, shedding light on the dynamic landscape of RNA processing and translational control during MCC differentiation. These findings underscore the pivotal role of Ybx2 in orchestrating cellular mechanisms fundamental to ciliary dynamics. This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI). (grant number: HI19C1095)

Program Abstract #135

Histone methyltransferases, SET-2 and MES-4, contribute to sterility in *C. elegans* that inappropriately inherit histone methylation

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At fertilization, histone methylation must undergo maternal reprogramming to reset the epigenetic landscape in the new zygote. During maternal reprogramming of histone methylation in the nematode, *C. elegans*, H3K4me is removed by the H3K4 demethylase, SPR-5, and H3K9me is subsequently added by the histone methyltransferase, MET-2. Recently, it was demonstrated that SPR-5; MET-2 maternal reprogramming antagonizes H3K36 methyltransferase, MES-4, which maintains a transcriptional memory of a subset of germline genes between generations, and H3K4me3 methyltransferase, SET-2, a member of the COMPASS complex. Maternal loss of SPR-5 and MET-2 results in ectopic expression of MES-4 germline genes and overexpression of H3K4 methylation. Together, this leads to developmental delay and sterility. We recently demonstrated that knocking down *mes-4* and *set-2* rescues the somatic developmental delay. However, whether knocking down *mes-4* and *set-2* rescues sterility in *spr-5; met-2* double mutants has yet to be explored. To examine whether knocking down *mes-4* and *set-2* rescues sterility in *spr-5; met-2* mutants, we knocked down *mes-4* and *set-2* using RNAi then performed DAPI staining and DIC imaging of the germlines. Excitingly, we found that knocking down *mes-4* and *set-2* significantly increases the number of oocytes in *spr-5; met-2* mutants. These data demonstrate that ectopic H3K4me and H3K36me contribute to sterility in the absence of SPR-5; MET-2 maternal reprogramming. Our findings provide mechanistic insight into how evolutionary conserved histone methylation and maternal reprogramming ensure development of a germline. This project is supported by NIH R15 1R15GM148887-01A1 (PI: Carpenter) and NIH U-RISE 1T34GM140948-01A1 (PIs: Hudson and Griffin).

Program Abstract #136

The Effects of Inappropriate Inheritance of Histone Methylation on Muscle Structure and Function

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At fertilization, maternally deposited histone modifying enzymes establish germline versus somatic transcriptional states by adding and removing histone methylation. To do this, SPR-5, an H3K4 demethylase, removes an active H3K4 methylation, while MET-2, a methyltransferase, adds repressive H3K9 methylation to prevent germline genes from being expressed in somatic cells. Recent studies show that SPR-5; MET-2 maternal reprogramming is antagonized by the H3K36 methyltransferase, MES-4, which maintains H3K36 methylation at germline genes to ensure proper germline gene expression in germ cells. Maternal loss of SPR-5 and MET-2 allows MES-4 to ectopically maintain H3K36 methylation at germline genes leading to ectopic expression germline genes in somatic tissues and a range of developmental phenotypes including developmental delay and muscle defects. Here, we demonstrate that muscle motility is decreased in *spr-5, met-2* mutants and that this decrease is significantly rescued by knocking down *mes-4*. We further show that *met-2*, but not *spr-5*, single mutants display decreased motility levels. This exciting finding revealed the first tissue specific phenotype that doesn t overlap between *spr-5* and

met-2 mutants and suggests a possible muscle specific role for MET-2. By performing RNA sequencing in *spr-5;met-2* mutants, we found an overall dampening in the expression of genes required for proper muscle development. After knocking down *mes-4*, the average expression of these muscle affecting genes returned to wildtype levels. We are currently investigating whether the muscle phenotypes in *spr-5;met-2* mutants are due to perturbations in development or arise from an ongoing interference in muscle function. Together, our data provides mechanistic insight into how germline-to-soma conversions may cause developmental phenotypes in human neurodevelopmental disorders. This work is supported by NIH R15 1R15GM148887-01A1 (PI: Carpenter).

Program Abstract #137

Defects in maternal LSD1 reprogramming may contribute to phenotypes observed in human neurodevelopmental disorders

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The lysine demethylase 1A (LSD1) is an epigenetic reprogramming enzyme that removes H3K4me1/2 and is required maternally for proper development in C. elegans and mice. Complete knockout of maternal LSD1 in mice results in embryonic arrest at the 2-cell stage due to failure to shut off maternal genes during the maternal to zygotic transition. This led us to consider the possible roles of maternal LSD1 in inherited disease. To study this, we developed a hypomorphic LSD1 allele (M448V) in mice that decreases the ability of LSD1 to bind to CoREST, resulting in inefficient demethylation. When LSD1 reprogramming is partially compromised only maternally, progeny show a high rate of perinatal lethality, developmental delay, craniofacial abnormalities and abnormal behavior. Three of these phenotypes, developmental delay, craniofacial abnormalities and abnormal behavior, are also observed in LSD1 and related Kabuki Syndrome patients. This raises the possibility that maternal defects could contribute to these diseases. To take an initial look at what changes might be inherited when LSD1 is hypomorphic maternally, we also performed RNAseq in progeny at embryonic day 13.5. Despite the fact that these embryos showed no obvious phenotypes, the overall gene expression changes can be distinguished by maternal genotype. This suggests that failure to completely reprogram LSD1 maternally in the oocyte can result in heritable genes expression changes in the progeny. Intriguingly, using gene ontology analysis, we observe that mammalian phenotypes associated with the genes that are significantly upregulated in our RNAseq analysis include decreased body weight/postnatal growth retardation and preweaning lethality, both of which we observe later in development in the progeny of mothers with maternally compromised LSD1 reprogramming. This hints that genes that may be causing the perinatal lethality and developmental delay may already be starting to be misexpressed at e13.5 Fund: NSF and NIH

Program Abstract #138

Dynamics of Ezh2 binding and global H3K27me3 estabilshment during early zebrafish development

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Histone 3 Lysine 27 trimethylation (H3K27me3) is a histone tail modification associated with transcriptional repression. H3K27me3 is established by the Polycomb Repressive Complex 2 (PRC2), with enhancer of zest homolog 2 (Ezh2) acting as the primary catalytic subunit. While many studies have explored H3K27me3 regulation in cell culture systems, less is known about how this mark is regulated in the context of vertebrate development. Zebrafish offer many advantages for studying chromatin states, particularly during the earliest stages of embryogenesis. Here, we use the chromatin profiling technique CUT&RUN to define the landscape of H3K27me3 and Ezh2 binding across early zebrafish development with high sensitivity and temporal resolution. Consistent with previous reports, we find that H3K27me3 is first detected during activation of the zygotic genome during the mid-blastula stage of development. Our results further reveal establishment is rapid and synchronized with widespread H3K27me3 establishment across the genome within a brief 30-minute interval between the 256 and 1000 cell stages of development. As observed in more differentiated cells, we find that H3K27me3 predominates at gene clusters including Hox genes and gene promoters. We noted clear concordance of Ezh2 binding and H3K27me3 at many regions across the genome. Unexpectedly, we also identified sites with clear, persistent Ezh2 binding following zygotic genome activation, but no evidence of H3K27me3. This observation suggests that PRC2/Ezh2 recruitment may not be sufficient to promote H3K27 methylation

during early embryogenesis. This research is supported by the National Institute of General Medical Sciences of the National Institute of Health under award number 1T32GM142623 to AA and 1R35GM139556 to MG. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Program Abstract #139

Ectopic transcription due to inappropriately inherited histone methylation may interfere with the ongoing function of terminally differentiated cells

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de novo mutations in the H3K4 demethylase LSD1/SPR-5/KDM1A and other related chromatin proteins lead to neurodevelopmental disorders, characterized by developmental delay, craniofacial defects and behavioral abnormalities. Two major assumptions are made about these patients. First, that the defect occurred developmentally and can't be reversed. Second, that the defect is strictly zygotic. Our work challenges both assumptions. A *C. elegans* double mutant in SPR-5/LSD1 and the H3K9 methyltransferase MET-2/SETDB1 has a severe chemotaxis defect caused by ectopic expression of germline genes in somatic tissues. Taking advantage of the invariant *C. elegans* lineage, we find surprisingly that these mutants have no lineage defects. This suggests that the altered chemotaxis behavior may be due to an ongoing defect in terminally differentiated cells rather than a defect in development. To test this, we shut off the ectopic expression of germline genes in *spr-5; met-2* larvae. Strikingly, we see a rescue of normal chemotaxis behavior in the same adult worms that previously had a defect at the L2 stage. This suggests that ongoing ectopic transcription can block normal behavior in a fully intact nervous system. If this mechanism also underlies patients with neurodevelopmental syndromes, it is possible that these syndromes may be reversible. In mice, we showed that the maternal loss of LSD1 results in arrest at the 2-cell stage, suggesting that LSD1 maternal reprogramming is conserved. Therefore, to determine if a defect in LSD1 maternal reprogramming might contribute to human disease, we developed a hypomorphic allele of *Lsd1*. Our data show that progeny from mothers with maternally hypomorphic LSD1 exhibit increased developmental delay and craniofacial abnormalities. The overlap of these phenotypes with human LSD1 patients raises the possibility that a defect in maternal reprogramming may contribute to disease in these patients. Funding: NSF1931967, GM149422, and R56NS122964.

Program Abstract #140

A novel modus operandi for mRNA translation regulation in zebrafish early development

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In the first hours of zebrafish early development, maternal products omnipotently direct almost all biological processes. For the zygotic genome activation, three transcription factors have been found to be indispensable. When Soxb1, Nanog and Pou5f3 are concomitantly knocked out, around 75% percent of genes fail to activate (Lee, M., Bonneau, A. et al., Nature, 2013). Yet, how the transcription factors themselves are translated remains unknown. It has been established that motifs lying wherein mRNA 3'UTR regulate or have an impact on translational efficiency, localisation, and regulation, due to binding sites or motives found in the UTR (Kuersten, S., Goodwin, E., Nat Rev Gen, 2003). When a transcript is shortened, the loss of a few nucleotides can greatly impact its fate. However, the exact mechanism behind this crucial yet scarcely studied event, is not self-evident. In our research, we show that *pou5f3* shortens before the maternal to zygotic transition by polyA tail test assay, proving it to be a regulation process as opposed to a degradation step. RNA sequencing revealed this to not be a single occurrence happenstance but a more general phenomenon with 46% of genes studied exhibiting similar features from 0 to 4 hours post fertilization. The same experiment allowed us to discover new, non-annotated alternative 3' UTR regions, proving that zebrafish transcriptome remains largely inaccurately annotated or uncharted. Puromycin proximity ligation assay of GFP-long *pou5f3* and GFP-short *pou5f3* transgenic lines showed the translation sites of said reporter to be predominant in short lines. These results are not due to a difference in mRNA number. Henceforth, based on our studies, we propose a new rationale that 3'UTR processing of transcripts is a translation regulation mechanism (Takada, Y., Fierro, L. et al, Sci Adv, 2023). This work was supported by JST SPRING (to L.F.) and KAKENHI n°16K07242 and 21H02398 (to T.K.) from JSPS

Program Abstract #141

3' untranslated region (UTR) shortening activates the translation of maternal mRNA that is necessary for early development

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In zebrafish, Pou5f3 is one of the transcription factors that activates zygotic genes during zygotic genome activation (ZGA). Studies have revealed that *pou5f3* is stored in the oocytes as maternal mRNA and the translation is repressed until after fertilization (Sato *et al*., 2022; Takada *et al*., 2023). Previously, we discovered that *pou5f3* 3'UTR is shortened after fertilization, prior to translational activation. Additionally, mass sepectrometry analysis revealed that different RNA-binding proteins (RBPs) bind to *pou5f3* 3'UTR after its shortening. We hypothesized that when the 3'UTR is shortened, the changes in structure allows the binding of RBPs that facilitate translation, activating the expression of Pou5f3 protein. In this research, we tested this hypothesis by inhibiting *pou5f3* 3'UTR shortening and analyzing the effects. First, we injected morpholino oligonucleotide (MO) that binds to the 3' end sequence of *pou5f3* 3'UTR into the embryos. qPCR validified that the injection of the MO inhibited 3'UTR shortening. Western blot showed that MO-injected embryos had decreased Pou5f3 protein expression compared to the control. These embryos expressed a fatal phenotype that resembles Pou5f3 translation-inhibition embryos, suggesting that 3'UTR shortening is necessary for translation activation. We then selected Pabpc1l as a model for RBPs that may facilitate translation. Immunoprecipitation and RT-PCR revealed that after ZGA, Pabpc1l expressed in control embryos bound to *pou5f3* mRNA, while Pabpc1l expressed in MO injected embryos did not. Together, these results suggest that the translation of *pou5f3* is activated when the 3'UTR is shortened, newly allowing RBPs that facilitates translation to bind to the mRNA. We propose this as a novel mechanism that activates maternal mRNA translation during early development. This work was supported by JST SPRING.

Program Abstract #142

Increased Enhancer—Promoter Interactions during Developmental Enhancer Activation in Mammals

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Remote enhancers are thought to interact with their target promoters via physical proximity, yet the importance of this proximity for enhancer function remains unclear. Here, we investigate the 3D conformation of enhancers during mammalian development by generating high-resolution tissue-resolved contact maps for nearly a thousand enhancers with characterized *in vivo* activities in ten murine embryonic tissues. 61% of developmental enhancers bypass their neighboring genes, which are often marked by promoter CpG methylation. The majority of enhancers display tissue-specific 3D conformations, and both enhancer–promoter and enhancer–enhancer interactions are moderately but consistently increased upon enhancer activation *in vivo*. Less than 14% of enhancer–promoter interactions form stably across tissues; however, these invariant interactions form in the absence of the enhancer and are likely mediated by adjacent CTCF binding. Our results highlight the general significance of enhancer–promoter physical proximity for developmental gene activation in mammals. National Institutes of Health grants R00HG009682 and DP2GM149555 (to E.Z.K.), R01HG003988 (to L.A.P.) and F31HD112201 (to G.B.). Z.C. was supported by NSF grant DMS1763272 (to Qing Nie) and Simons Foundation grant 594598 (to Qing Nie). J.L-R. is funded by the Spanish Ministerio de Ciencia e Innovación (grant PID2020-113497GB-I00 and institutional María de Maeztu grant CEX2020-001088-M).

Program Abstract #143

Zelda pioneer activity directs the establishment of Polycomb mediated domains

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Initially the early embryonic genome lacks cues for transcription. The large scale de novo binding of RNA Polymerase 2 (Pol II) initiates the bulk zygotic transcription known as Zygotic Genome Activation (ZGA) that in the Drosophila embryo occurs over nuclear cycles (NC) 12-14. During ZGA, the genome concurrently gains chromatin accessibility and is decorated with histone post translational modifications that influence later transcriptional activity. We aim to describe how H3K27me3, a hallmark of gene silencing, changes over the course of ZGA and whether it depends on the pioneer factor and ZGA regulator, Zelda (Zld). Timecourse ChIP-seq indicated that H3K27me3 appeared at early NC14 and was further enriched at regions throughout NC14. Interestingly we found by live-imaging that E(z), the H3K27 methyltransferase and subunit of Polycomb Repressive Complex 2 (PRC2), was nuclear beginning at NC10 suggesting a delay in the acquisition of H3K27 methylation. PRC2 is known to be targeted to DNA through Pho, a DNA binding protein, to Polycomb response elements (PREs) which are motif rich regions found through functional assays. These motifs include those for Zld. We hypothesized that Zld regulates the onset of H3K27 methylation through modifying PRC2 access to chromatin or PRC2 binding to chromatin. To determine the impact of the loss of Zld, we generated Zld germline clones (zld). A subset of PREs, as inferred by E(z) ChIP peaks, lose chromatin accessibility in zld. Of the ~300 broad H3K27 domains we detect, a small but significant fraction of these depend on Zld for their establishment. In contrast to canonical transcription logic, there is no gain of opposing H3K27ac and Pol II binding is reduced at transcribed genes in these H3K27 domains. This investigation links pioneer factor dependent accessibility to H3K27 methylation, an indicator of transcriptional repression. This research is funded by NIH/NICHD, Pew Charitable Trusts, and Northwestern University.

Program Abstract #145
Exploring the relationship between heterochromatin and *nanog* expression during zebrafish embryogenesis
Dilani Rajapakse, Audrey Calvird, Mary Goll
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DNA wraps around proteins known as histones, forming the structural unit known as a nucleosome. Nucleosomes further aggregate to create chromatin. The addition of modifications to histone tails can help promote condensed, transcriptionally repressive regions of chromatin, called heterochromatin. One such modification is trimethylation of histone 3 lysine 9 (H3K9me3), which serves as a canonical marker of heterochromatin at repetitive DNA elements and its establishment is crucial for early metazoan embryogenesis. However, the mechanisms governing the establishment of heterochromatin in the embryo and the developmental consequences of altering the timing of its establishment remain limited. Embryonic stem cells exhibit reduced hallmarks of heterochromatin alongside elevated expression of pluripotency genes compared to differentiated cells. Previous work has demonstrated that forced overexpression of the pluripotency factor Nanog can disrupt heterochromatin in differentiated cells. However, the relationship between Nanog and heterochromatin establishment has not been explored in the context of embryogenesis. We find that *nanog* downregulation correlates with the onset of bulk heterochromatin establishment during zebrafish embryogenesis, suggesting it may be important for heterochromatin regulation in vivo. Here, we present our progress in testing the impact of sustained Nanog expression on heterochromatin formation during zebrafish embryogenesis. This research is supported by funding from the National Institute of Health (Grant No. R35GM139556) to Mary Goll.

Program Abstract #146
Investigating transport of miRNA and its targets to the mitotic spindle
Malcolm Arnott, Jia Song
University of Delaware, USA
Mitosis is a critical and fundamental process in development, which requires tight spatial and temporal regulation. We have previously shown that miR-31 and three of its validated targets, *Fascin*, *Rab35*, and *Gelsolin*, localize to the mitotic spindle of mammalian cells and sea urchin embryos. We hypothesize that these transcripts are transported to the mitotic spindle along the cytoskeleton, mediated by motor proteins and RNA-binding proteins (RBPs). To test this hypothesis, we treated sea urchin cleavage stage embryos with pharmacological inhibitors and discovered that subcellular transport of miR-31 and its target transcripts to the spindle depends on the cytoskeleton and motor proteins. Previously, we bioinformatically identified a cytoplasmic polyadenylation element (CPE) in the 3'UTR of the *Aurora B*,

which is essential for RNA localization to the mitotic spindle in the sea urchin embryo. This motif is also present in *Fascin*, Rab35, and *Gelsolin* 3'UTRs. We hypothesize that subcellular localization of these transcript are in part mediated by CPE-binding protein (CPEB). We will assess the impact of blocking the CPE motif, and the effect of knocking down CPEB on transcript localization, cell division, and early development. Additionally, we have bioinformatically identified candidate RBPs, human orthologs known to function in RNA transport, to have predicted binding sites in *Fascin*, *Rab35*, and *Gelsolin* transcripts. We will test the role of these RBPs in RNA transport and early development with knockdown experiments. Results from this experiment will reveal evolutionarily conserved molecular mechanisms of RNA trafficking in a rapidly dividing embryo. Since proper cell division is paramount for all cells, organismal development, and human health, a basic understanding of regulation of RNA transport during cell division is critical. This work is funded by NSF MCB (2103453) to JLS.

Program Abstract #147

Localized RNAs at the mitotic spindle are critical for proper early development

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University of Delaware, USA

All living organisms, from bacteria to humans, localize RNAs in regions of the cell to control local gene expression. We found select microRNAs and their targets localize to the mitotic spindle to regulate local translation during early cleavage stage of development. One of the RNAs that we examined is the *Fascin* RNA, which encodes a protein that is involved in actin bundling and microtubule polymerization. Interestingly, we found the localization of *Fascin* RNA and its regulatory miR-1 and miR-31 to be enriched on the mitotic spindle in dividing blastomeres and perinuclear in non-dividing blastomeres. Localization of these transcripts to the mitotic spindle is evolutionarily conserved, as we also observe this spindle localization in mammalian cells. We found miR-31 regulates local translation of these transcripts to ensure proper cell division. Forced ectopic translation of Fascin at the cell periphery in dividing embryonic cells resulted in chromosomal segregation defects, developmental delay, and even embryonic death, demonstrating the importance of local translation at the spindle. Perturbation of Fascin resulted in profound changes in the cytoskeleton. However, the molecular mechanism of how Fascin mediates cytoskeletal changes is still unknown. These exciting results prompted us to investigate how miRNAs and their target transcripts get transported to the mitotic spindle. Subcellular transport of these RNAs are dependent on microtubules and motor proteins. Additionally, we have bioinformatically identified candidate RBPs to have predicted binding sites within *Fascin*, *Rab35*, and *Gelsolin* RNAs. We will assess the impact of blocking the RBP binding sites and the perturbation of RBPs on transcript localization, cell division, and early development. Overall, this work contributes to fundamental understanding of RNA localization and cell division which are critical biological process used by all organisms. This work is funded by NSF MCB (2103453) to JLS.

Program Abstract #148

Uncovering the formation of enhancer-promoter interactions during ZGA and early *Xenopus* embryogenesis

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After fertilization, the newly formed zygote is transcriptionally silent, and its genome is largely unstructured. During zygotic genome activation (ZGA), the embryonic genome begins to organize into three-dimensional structures consisting of topologically associated domains, chromatin loops, and enhancer-promoter interactions. Enhancer-promoter interactions are an important part of 3D genome organization and regulate gene expression by bringing cis-regulatory elements near their target genes. Although large-scale DNA organization has been characterized during development, how proper enhancer-promoter interactions specifically form during early embryogenesis is not well-known. Thus, this project aims to characterize the formation of long-range enhancer-promoter interactions and their roles in regulating gene expression during ZGA. We used H3K27ac HiChIP-seq to identify DNA loop formation during early gastrula. Of the 2229 long-range interactions (greater than 10kb) found, 641 were determined to be enhancer-promoter (E-P) interactions. Some E-P interactions are observed over distances exceeding 1Mb, but 50% occur within less than 50kb. Importantly, the majority of E-P

interactions are associated with transcriptionally active genes, including a significant number associated with germ-layer enriched gene expression, such as *sox2, sox3, msx1, and fgf4*. We found that the anchors of these E-P interactions are bound by Ep300 and maternal transcription factors (TFs) such as Sox3, Vegt, and Foxh1, suggesting a potential role for these TFs in establishing E-P interactions. This work is supported by NIH grants, R21HD10969, R35GM139617, and T32 GM136624-02.

Program Abstract #149

Bicoid-nucleosome competition sets a concentration threshold for transcription

Eleanor Degen

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Concentration gradients of maternal transcription factors establish patterns of gene expression during a time in early embryonic development characterized by frequent mitotic divisions, rounds of genome replication, and chromatin reorganization. In the Drosophila embryo, an exponential gradient of the transcription factor Bicoid (Bcd) activates the earliest expressed patterning genes across the anterior-posterior axis. Bcd successfully navigates the chromatin of the replicating genome to bind its sites and facilitate transcription. However, we do not fully understand how genomic context leads to differential transcriptional outputs across Bcd concentrations. We modeled how enhancer sequence, chromatin, and DNA replication together determine the Bcd concentration-sensitivity of the transcriptional process. By live-imaging an MS2-MCP transcriptional reporter for the Hunchback P2 (HbP2) enhancer, we found that the length of the delay between mitosis and the initiation of transcription uniquely reflects Bcd concentration-sensitive regulation. We defined a stochastic model of transcriptional regulation that accurately predicts transcriptional onset times using mathematical descriptions of Bcd-nucleosome competition for occupancy and the probability of DNA replication at HbP2. Our work suggests that Bcd's ability to outcompete nucleosomes dictates a Bcd concentration threshold for expression, while DNA replication limits transcriptional activation at high concentrations where Bcd readily outcompetes nucleosomes. Disrupting nucleosome stability by promoting pioneer factor binding to HbP2 both expands the MS2 expression domain and preserves onset time variance at high concentrations, supporting these hypotheses. Understanding the critical parameters of chromatin and replication dynamics should allow for genome-wide predictions of the concentration-sensitivity of Bcd binding events. Funding: the NIH/NICHD, Pew Charitable Trusts, NSF GRFP, NU CMBD T32, Startup Funds.

Program Abstract #150

Comprehensive analysis of the role of prostaglandins in endometrial receptivity and pregnancy success

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Defective prostaglandin (PG) synthesis is linked with implantation failure, particularly in patients with recurrent pregnancy loss. The peri-conceptional use of non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit PG synthase (PTGS) enzymes, PTGS1 and PTGS2, poses a possible pregnancy risk. *Critical Knowledge gaps* include how PG impacts the 3D organization and function of the uterine compartments and which endometrial cells produce PG. Using innovative imaging methods combined with mouse genetic models and pharmacological inhibitors, we found that preimplantation indomethacin (PTGS1 and PTGS2 inhibitor) treatment disrupts uterine gland organization, implantation chamber formation, and vascular permeability at implantation sites and causes embryo resorption. Furthermore, indomethacin declines Leukemia inhibitory factor (*Lif*) expression, *a* critical factor for embryo attachment, prior to implantation and serum progesterone (P4) levels post-implantation. Our data indicates that ovarian PTGS1 and PTGS2 have a role in implantation. Also, we show that object-stimulated *Ptgs2*$^{-/-}$ pseudo-pregnant mice fail to show gland organization and vascular permeability, suggesting the vital role of PTGS2 in implantation. However, epithelium and blood vessels' specific deletion of PTGS2 does not risk pregnancy. Ovarian, oviductal, and uterine-specific deletion of PTGS2 in the epithelium, stroma, and smooth muscle using *Pgr*$^{cre/+}$*; Ptgs2*$^{f/f}$ delay implantation and restrict embryonic development to the blastocyst stage without affecting P4 levels. Our future work will assess the effect of pre-implantation PGs on ovarian and oviductal function and the post-implantation role of stromal PTGS2 on decidualization and decidual angiogenesis. Our study may resolve discrepancies surrounding NSAIDs, leading to informed decision-making on pain medication use during pregnancy. This study is supported by the March of Dimes grant #5-FY20-209, NIH R01HD109152 to RA, and T32HD087166 to NM.

Program Abstract #151

Analysis tools for intravital confocal imaging: segmenting tissue architecture and measuring protein distribution in time and space.

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Understanding how tissue organization and cell division intersect is critical for unraveling developmental mechanisms that shape tissues. In this pursuit, intravital imaging through confocal microscopy is an efficient tool for observing cells dynamics in three dimensions through time during development. Yet, the analysis of these rich multidimensional datasets poses significant challenges, necessitating computational methods to extract meaningful insights. Here, we use the germline stem cells in the developing *C. elegans* gonad to investigate the interplay between cell division and gonadal development. Our work introduces an open-source suite of image analysis tools that combine available and custom scripts to segment images and derive quantitative descriptors of cellular and tissue-level structures. These tools enable tracking of mitotic spindle orientation in relation to various tissue and cellular surfaces and quantification of fluorescence intensity and spatial distribution of relevant molecular regulators over time. Through the application of our tools, we achieved precise measurement, in three dimensions, of mitotic spindle orientation in relation to different cellular and tissue axes within dividing germ cells. Additionally, we measured the cortical distribution of force generators relative to these axes. Our methodology facilitates a deeper understanding of mitosis during tissue development in situ and may be expanded for use in other organisms amenable to intravital confocal imaging. Funded by a grant from the Canadian Institutes of Health Research (PJT-153283) to JCL and ARG.

Program Abstract #152

Using expansion microscopy to analyze mitochondrial network morphology during *Drosophila* gastrulation.

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The workhorse of developmental biology is the confocal microscope, which allows researchers to determine the three-dimensional localization of tagged molecules within complex biological samples. While confocal microscopy allows one to resolve two adjacent fluorescent point sources located a few hundred nanometers apart, observing the finer details of subcellular biology requires the ability to resolve signals on the order of tens of nanometers. Numerous hardware-based methods for "super-resolution" microscopy have been developed to allow researchers to sidestep such resolution limits, although these methods require specialized microscopes that are not available to all researchers. Alternatively, one can use expansion microscopy (ExM) to effectively increase the resolving power of conventional microscopes by swelling the sample itself to istropically increase the distance between constituent molecules, while preserving their relative spatial organization. Here we used ExM in whole-mount *Drosophila* embryos to examine the localization patterns of proteins within subcellular structures that have fine details below the typical resolution limit of confocal microscopy: GFP-tagged non-muscle myosin II, part of the actin cytoskeleton, and ATP synthase, a core component of mitochondria. Notably, we were able to resolve parallel lines of myosin in adjacent cell cortices and visualize mitochondria as ring-like structures. We then used this technique to characterize the morphology of mitochondrial networks during cellularization and gastrulation in wild-type embryos, as well as embryos in which mitochondrial fission was disrupted, to better understand the role of mitochondrial dynamics during normal embryonic development. (This work was supported by funding to A. Paré through NIH R15 GM143729.)

Program Abstract #153

Molds for imaging cardiac morphogenesis in zebrafish embryos.

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Zebrafish embryos, due to their external fertilization and optical transparency, are an ideal model organism for using *in vivo* imaging to study complex morphogenetic processes. This imaging can require mounting embryos in specific orientations. This is particularly true for heart development where cardiac morphogenesis occurs in different planes. At the beginning of cardiac development cardiomyocytes collectively migrate along the lateral-medial axis, however this is followed by lumen formation which

occurs along the dorsal-ventral axis, which is then followed by cardiac looping and chamber formation on the ventral side of the embryo along the anterior-posterior axis. To facilitate this imaging, we have developed a set of specialized molds for mounting zebrafish embryos. These molds are used to create agarose casts that position embryos for imaging different stages of heart development including cardiac fusion, heart tube formation, cardiac looping and chamber formation. Moreover, each mold - which is reusable - creates multiple agarose casts for high-throughput imaging of multiple embryos simultaneously. We have also developed molds for general dorsal, ventral or lateral imaging of embryos at successive stages of zebrafish development. While most molds have been developed for inverted microscopes, several molds have also been developed for upright microscopes. STL files for these molds are freely available to allow for further customization. We hope these molds will be useful to the Developmental Biology community, please email josh@olemiss.edu if you are interested using them for your imaging. This work is supported by the NIH (P20GM130460).

Program Abstract #154
Effect of eNOS on coronary smooth muscle maturation
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Coronary arteries supply oxygen-rich blood to the heart. Defects in these arteries, known as coronary artery anomalies, can lead to atherosclerosis, sudden cardiac death, and myocardial ischemia. The aim of this study is to understand how the coronary arteries remodel as they connect to the aorta and are exposed to shear stress. A marker of high shear stress and the recruitment of vascular smooth muscle (vSM) cells were analyzed in chick embryos using immunohistochemistry during coronary strand recruitment (HH 31-32) and coronary artery remodeling (HH 34-35). Coronary artery diameters were measured to determine whether diameter correlated with the presence of the shear stress marker endothelial nitric oxide synthase (eNOS) or vSM recruitment and maturation. Data were analyzed by two-way ANOVA and Tukey post hoc analysis. As immature coronary were recruited to the aorta, larger coronary artery diameters were significantly correlated with strong eNOS expression in the coronary arteries and aorta (pas the coronary arteries remodeled. During coronary strand recruitment, the vSM layer was fragmented and disorganized. As the coronary arteries remodeled, the coronary arteries had a thicker, more organized layer of vSM. Arteries with this thicker, organized vSM also expressed high levels of eNOS. Because eNOS is associated with areas of high shear stress, these results suggest that the coronary arteries see increasing levels of shear stress as they connect to the aorta and are remodeled. Future manipulation of blood flow through in ovo vascular surgeries will confirm whether shear stress determines which immature coronary arteries become the mature coronary stems. Understanding how the coronary arteries respond to the physical force of blood may help prevent or reverse coronary artery anomalies. This project is funded by NIH grant HL167279-01.

Program Abstract #155
Patterned distribution of extracellular matrix in the embryonic heart is regulated by Smarcc1a
Ivy Fernandes, Abby Lee, Deborah Yelon
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Essential patterning processes transform the linear heart tube into a compartmentalized organ with distinct chambers and an atrioventricular canal (AVC) at their junction. The AVC is morphologically, physiologically, and molecularly distinct from the adjacent chambers, and these distinctions are necessary for proper cardiac function. For example, the heightened deposition of particular extracellular matrix (ECM) components in the AVC contributes to the morphogenesis of the endocardial cushions and facilitates signal transduction for pathways that promote AVC differentiation, such as the Wnt pathway. However, the mechanisms that pattern ECM localization in the developing heart are not fully understood. Our prior studies have found that the zebrafish gene *smarcc1a*, encoding a BAF chromatin remodeling complex subunit homologous to mammalian BAF155, plays a key role in refining the gene expression patterns that distinguish the AVC from the cardiac chambers. Here, we show that *smarcc1a* function is also required to regulate the distribution of the ECM component hyaluronic acid (HA). In *smarcc1a* mutants, HA organization is aberrant: instead of displaying heightened deposition of HA within the AVC, *smarcc1a* mutant hearts instead exhibit excessive and broad distribution of HA. This is likely due to the ectopic expression of *has2*, a key player in HA synthesis, throughout the *smarcc1a* mutant endocardium.

In addition to their broad expression of *has2*, *smarcc1a* mutants also display abnormally broad expression of several other endocardial genes, including *klf2a*, *wnt9b*, and *raldh2*. The expanded presence of HA in the *smarcc1a* mutant heart is also accompanied by ectopic Wnt signaling within the cardiac chambers, extending beyond its normally restricted localization in the AVC. Together, our data support a model in which Smarcc1a-containing chromatin remodeling complexes play an important role in patterning the cardiac ECM by restricting the expression of key endocardial genes.

Program Abstract #156
Investigating the dynamics of the cardiomyocyte glycocalyx in developmental cell-state transitions
Marjan Farsi, Joshua Bloomekatz
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Cell-state transitions (changes in cell-identity, physiology and morphology) are essential for organogenesis and disease pathogenesis. Changes in the glycocalyx, the pericellular layer of glycans that surrounds a cell, has been shown to be important for cell-state transitions. For example, changes in both glycocalyx composition and height have been shown to facilitate epithelial-to-mesenchymal transitions (EMT), while changes in specific sialylated glycans have been shown to occur during the fibrosis stage of zebrafish cardiac regeneration and during cardiomyocyte differentiation (Spelat et al. 2002, Ashwood et al. 2020). Using the zebrafish model-system, we are investigating how the cardiomyocyte glycocalyx changes during cell-state transitions and the role of those changes during cardiac development. To identify the composition of the cardiomyocyte glycocalyx during development, we are utilizing proximity ligation to pull down cardiomyocyte membrane proteins and then extracting and analyzing the attached glycans via mass-spectrometry. Furthermore, we are using unnatural alkyne/azide-containing monosaccharides in combination with bio-orthogonal click chemistry along with lectins to identify spatial and temporal changes in monosaccharide and glycan enrichment during heart development via high-resolution microscopy. Our analysis is focused on terminal sialic and fucosylated glycans, along with mucins as we've hypothesized that they play an important role in the function of the cardiomyocyte glycocalyx. This work is supported by funding from the NIH (P20GM130460).

Program Abstract #157
Collective movement and robustness during heart tube formation
Rabina Shrestha, Tess McCann, Harini Saravanan, Prashanna Koirala, Joshua Bloomekatz
University of Mississippi, USA
A series of coordinated and stereotypical changes involving cell movement, cell shape and cell identity lead to the final architecture of a functioning organ. One of the first steps in the formation of the heart is the collective movement of bilateral myocardial precursors to the midline, a process known as cardiac fusion. Extrinsic influences such as the adjacent anterior endoderm are known to be required for cardiac fusion. Here, we show that an intrinsic mechanism involving platelet-derived growth factor receptor alpha and phosphoinositide 3-kinase (Pdgfra-PI3K) signaling is also important for directing the movement of the myocardium towards the midline. Inhibition of the PI3K pathway throughout the zebrafish embryo or tissue-specifically in the myocardium results in a failure of myocardial cells to properly move towards the midline. *In vivo* imaging further reveals midline-oriented dynamic myocardial membrane protrusions that become unpolarized in PI3K-inhibited embryos where myocardial movements are misdirected and slower. Moreover, we find that PI3K activity is dependent on and interacts with Pdgfra to regulate myocardial movement. Intriguingly, our studies further reveal that the heart can recover despite cardiac fusion defects caused by Pdgfra-PI3K inhibition, however defects in ventricular and atrial chamber morphology occur later in development. Together our findings reveal an intrinsic myocardial steering mechanism important for cardiac fusion and the existence of a robustness mechanism that facilitates heart development under abnormal conditions. Our ongoing studies are directed at elucidating the basis of this recovery and understanding how defects in cardiac fusion can lead to chamber formation defects. This research is supported by the American Heart Association and the National Institutes of Health.

Program Abstract #158

Role of NuRD complex during cardiac patterning and formation in *Drosophila melanogaster*

Mia Jones, Armeta Hadjimirzaei, Sara Khadraoui, Ariana Craft, Sarah Anglin, Scott Nowak
Kennesaw State University, USA

Congenital heart defects are among the most prevalent birth defect present in the human population, with an occurrence as high as 10 in 1000 live births. A number of these cases have been ruled as sporadic or resulting from different interactions of many independent genetic loci and alleles. Gene combinations and chromosomal changes are known to play a crucial role in the development of congenital heart defects, but the precise genetic and environmental factors involved in this process still remain poorly understood. Among the metazoans from fruit flies to humans the heart is one of the earliest and most fundamental organ structures to form during embryogenesis. In *Drosophila*, the process of heart specification and formation is entirely controlled by Tinman/Nkx2.5 and Mef2, working together with the nuclear co-factor Akirin to mediate cardiac gene expression. Preliminary work in the Nowak Laboratory found that Akirin likely regulates gene expression during cardiac development by working together with the Nucleosome Remodeling and Deacetylase (NuRD) complex to remodel cardiac gene loci. We used forward genetic screening and imaging techniques to quantify, characterize, and categorize the various types of heart formation defects that are observed in NuRD complex mutants. Embryos bearing mutations in different NuRD subunits produce hearts, but they are often severely misshapen, poorly patterned, and have reduced numbers of cardiomyoblasts in the finished organ. We have pioneered a novel live, confocal-based imaging technique to enable us to assess whether these mutant hearts that display cardiac defects are capable of coordinated contraction and/or hemolymph flow. Excitingly, our results suggest that Akirin serves as a link between chromatin remodeling complex activity and these developmentally critical transcription factors to regulate organ patterning and development. This work was supported by NHLBI Award 1R15HL161738 and NIGMS Award 1R16GM145448.

Program Abstract #159

Arginine Kinase 1 is required for *Drosophila melanogaster* eye growth and patterning

Amber Reynolds, Courtney Farrington, Josceline Tenido, Kaycee Torres, Dr. Mardelle Atkins
Sam Houston State University, USA

In *Drosophila*, Arginine Kinase is a highly conserved ortholog of Creatine Kinase. Both enzymes work cohesively with corresponding phosphagens (arginine or creatine) to create an indispensable cell energy reserve. These two phosphagens are found in a wide distribution of organisms; Arginine Kinase is primarily found in invertebrates, while Creatine Kinase is found primarily in vertebrate species. These enzymes have primarily been studied in skeletal muscles, the brain, and the heart, yet their function in epithelial tissues is poorly defined. In a candidate screen for novel eye genes, we have recently found that depletion of the Arginine kinase ortholog ArgK1 in the developing *Drosophila* eye causes a range of phenotypes including defects in growth and patterning as well as a 10-15% rate of trans determination. These results suggest that Argk1 function is essential for normal eye development and cell fate restriction. We will present the initial characterization of the expression pattern through eye development and describe the phenotypic response and when Arginine Kinase is knocked down. Future work will include generation of a null allele to validate the knockdown results. The data that we will get from this will shed new light on the developmental role of Argk1, and potentially phosphagen systems in epithelial tissues. I would like to thank to Rowland Black Endowed assistant professorship under Dr. Mardelle Atkins and the Department of Biological Sciences at SHSU for funding this project.

Program Abstract #160

Characterizing the expression of metabolic genes in pituitary gland organogenesis

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The pituitary gland is frequently called the master gland because of its central role in regulating physiology. Congenital hypopituitarism is the loss of one or more pituitary hormones at birth, which can cause significant morbidity for affected people. Sixty genes are associated with congenital hypopituitarism, but the genetic cause for 80% of cases is unknown. Using publicly available data for embryonic lethal homozygous null mice generated by the International Mouse Phenotyping Consortium, we identified fifty genes that cause embryonic pituitary gland malformations. These genes were not

previously associated with pituitary gland organogenesis and represent a candidate gene list for congenital hypopituitarism. Among the fifty genes are *Psat1*, *Psph*, and *Gldc*, which are all enzymes that function in amino acid metabolism. Homozygous null mouse embryos for each of these genes have a similar pituitary phenotype where the pituitary progenitors in Rathke's pouch, the precursor of the pituitary anterior lobe, are expanded. This phenotype is similar to other mouse models where a signaling center in the infundibular region of the ventral diencephalon that is necessary for induction of pituitary progenitors is expanded. We are beginning a characterization for how of these metabolic enzymes impact pituitary morphology by characterizing their expression at key embryonic time points. We hypothesize that *Psat1*, *Psph*, and *Gldc* are expressed in the infundibular region of the ventral diencephalon. We will present updated RNAscope in situ hybridization data that characterizes the temporal and spatial expression of *Psat1*, *Psph*, and *Gldc* in mouse embryonic pituitary glands. This work is supported by NIH 1R01HD108156-01A1.

Program Abstract #161

Region-specific roles of EVC2 in regulating hedgehog signaling and the development of taste organs

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Fungiform taste papillae in mammals are repeated structures in the anterior oral tongue that have a unique stereotypic pattern and each hosts a single taste bud in rodents. Understanding of molecular mechanisms governing the taste papilla formation and patterning is beneficial to understand the development of taste sensitivity that is associated with the density of taste buds. Hedgehog (Hh) signaling plays essential roles in taste organ development and maintenance throughout lifetime. Ellis–van Creveld (EvC) syndrome, a genetic disorder caused by mutations of *Evc* or *Evc2*,of which patients exhibit impaired Hh signaling activities and defects of several organs including taste papillae. In this study, we took advantage of the lacZ-knockin alllele of *Evc2* to depict a dynamic expression patten during embryonic development of the taste organs in mice. At E12.5, $Evc2^{lacZ/+}$ mice showed weak X-Gal signals in the anterior tongue epithelium and at E14.5 signals became robust in fungiform taste papillae and surrounding tissues. The phenotypic analyses showed that *Evc2* knockout ($Evc2^{-/-}$) leads to region-specific alterations of Hh signaling activity and taste papillae. In E12.5 $Evc2^{-/-}$ compared to the wild type littermates, Gli1 RNA expression was lower in the anterior tongue epithelium, but not in other regions; and fungiform papillae were higher in number and size at E12.5 and E15.5. The $Evc2^{-/-}$-caused phenotypic changes of taste papillae in the anterior region only contrast Hh disruption-caused taste papilla increases in both anterior and intermolar eminence of the tongue. RNA-sequencing data showed higher expressions of many genes encoding extracellular matrix proteins in the null mutants. Our results indicate that EVC2 regulates the development of taste papillae in a region-specific manner by fine-tuning Hh signaling activity and extracellular matrix protein expression. (Supported by NIH R01DC012308 to HXL and R01DE020843 to YM)

Program Abstract #162

Coordination of cell cycle and morphogenesis during organ formation

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Organ formation requires precise regulation of cell cycle and morphogenetic events. Using the *Drosophila* embryonic salivary gland (SG) as a model, we uncover the role of the SP1/KLF transcription factor Huckebein (Hkb) in coordinating cell cycle regulation and morphogenesis. The *hkb* mutant SG exhibits defects in invagination positioning and organ size due to the abnormal death of SG cells. Normal SG development involves distal-to-proximal progression of endoreplication (endocycle), whereas *hkb* mutant SG cells undergo abnormal cell division, leading to cell death. Hkb represses the expression of key cell cycle and pro-apoptotic genes in the SG. Knockdown of *cyclin E* or *cyclin-dependent kinase 1,* or overexpression of *fizzy-related* rescues most of the morphogenetic defects observed in the *hkb* mutant SG. These results indicate that Hkb plays a critical role in controlling endoreplication by regulating the transcription of key cell cycle effectors to ensure proper organ

formation. **Louisiana State University, Louisiana Board of Regents (LEQSF(2019-22)-RD-A-04), National Science Foundation (MCB 2141387).**

Program Abstract #163

Effects of low oxygen conditions during embryogenesis on growth in zebrafish

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A decline in oceanic O_2 concentrations associated with global warming is thought to have negative effects on fish growth, thus reducing fisheries yields and disrupting the marine environments. While the idea is based on the effect of O_2 concentrations after hatching, how the history of O_2 concentrations before hatching affects early development and subsequent growth remains largely unaddressed. To elucidate the effects of hypoxic exposure during fish embryogenesis, we studied zebrafish *Danio rerio* whose embryogenesis completes in 72 hours in normal O_2 conditions that are well-equilibrated under standard atmospheric pressure and incubation temperature of 28.5 °C. Freshly laid zebrafish eggs were divided into control and hypoxia groups (3.0% O_2) and maintained at 28.5 °C for 77 hours post-fertilization (hpf). We found that at 77 hpf, hatching rate, heart rate, and embryo length were significantly reduced in the hypoxia group. Hypoxia also had deleterious effects on organogenesis, leading to delayed formation of spinal columns and pharyngeal arches. Using *o*-dianisidine staining, we also found a reduction of hemoglobin synthesis in the hypoxia group. Furthermore, from acridine orange staining, we detected cell death throughout the body in the hypoxia group by 4 dpf. These aberrances recovered with growth, and there were no differences observed between the two groups in the anatomical structures of the heart, brain, and eyes, as well as in optokinetic and optomotor responses in adult fish. We conclude that once the embryo survives the embryonic stage, pre-hatching O_2 concentrations have little consequence on the adult fish's anatomy and behavior. Our observations suggest a cell competition mechanism that compensates for a temporary delay in the growth of the juvenile and organogenesis. This research was supported by HFSP Research Grant RGP0051/2021.

Program Abstract #164

Liver explant cultures to model biliary proliferation and maturation *in vitro*

Brianna Goodloe, Hannah Hrncir, Adam Gracz
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Hepatoblasts differentiate into hepatocytes or biliary epithelial cells (BECs) during liver development. While hepatoblast differentiation is known to initiate intrahepatic bile duct (IHBD) development, the contribution of BEC proliferation on IHBD expansion is not well defined. Additionally, recent large-volume light sheet imaging in our lab identified distinct developmental IHBD morphology that has not reached mature adult morphology even by postnatal day 14 (P14). To better understand how BECs contribute to IHBD branching morphogenesis, we quantified BEC proliferation from embryonic day 15.5 (E15.5) to P14 using immunofluorescence (IF) for KI67. We found that BEC proliferation is highest at E17.5 (41.0 ± 3.4%), decreases postnatally (20.3 ± 1.4% at P14), and reaches a low rate of homeostatic turnover in 10wk old adults (3.3 ± 1.1%). To evaluate BEC maturation through development, we conducted IF for NCAM1, a marker of immature BECs. There was a gradual increase in co-localization of NCAM1 with BEC marker EpCAM from E15.5-P1, which then decreased from P5-6wks. Mice with liver-specific loss of *Sox9*, which is known to cause IHBD defects, have no change in BEC proliferation rates during development. However, they exhibit increased NCAM1 expression in adult IHBDs, demonstrating that BEC proliferation and maturation can be uncoupled. We developed an embryonic liver explant model to define the regulation of IHBD morphogenesis further. Optimized culture conditions preserve BEC survival and growth in 500 µm slices of E15.5-17.5 livers for 24 hrs. Our data suggest that BECs are key contributors to IHBD morphogenesis and that proliferative expansion of IHBDs can be distinct from BEC maturation. Further development of explant cultures will facilitate live-imaging studies of IHBD development and better distinguish the dynamics of BEC maturation and proliferation. This work is funded by NIH R01 DK132653 (ADG), F31 DK134199 (HRH), T34 GM149511 (BG).

Program Abstract #165

***Sox9* inhibits Activin A to promote biliary maturation and branching morphogenesis**

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Branching morphogenesis couples cellular differentiation with the development of tissue architecture. During liver development hepatoblasts give rise to hepatocytes and biliary epithelial cells (BECs). BECs line the intrahepatic bile ducts (IHBDs) which are essential for liver function. The IHBD network is hierarchical in structure with smaller, peripheral "ductules" draining into larger "ducts" that eventually exit the liver. Despite proposed models from histological and genetic studies, mechanisms differentiating local "duct" or "ductule" hierarchies and driving the global IHBD architecture are poorly understood. The transcription factor SOX9 is an early and ubiquitous marker of BECs. Previous studies on liver specific *Sox9* knockout (Sox9cKO) mice showed that *Sox9* is important for timing of BEC specification during development and prevents cystogenesis in aged mice. It is possible that SOX9 regulates IHBD architecture, as it promotes branching morphogenesis in other tissues. Using whole-lobe 3D imaging and iDISCO+, we find that adult and postnatal Sox9cKO mice demonstrate ductal paucity with bias towards ductule, rather than duct, loss. We find in wild-type mice that IHBDs emerge as a webbed structure by E15.5 and undergo morphological maturation through 2 weeks of age. Developmental knockout of *Sox9* leads to decreased postnatal branching morphogenesis, manifesting as loss of ductules in adult livers. In the absence of *Sox9*, BECs fail to mature and exhibit elevated TGF-β signaling and Activin A. Activin A induces developmental gene expression and morphological defects in BEC organoids and represses ductule formation in postnatal livers. Our data demonstrate that adult IHBD morphology and BEC maturation are regulated by the *Sox9*-dependent formation of precursors to ductules during development, mediated in part by downregulation of Activin A. Funded by NIH R01 DK132653 & F31 DK134199.

Program Abstract #166

Characterizing Apoptosis in lung-specific conditional transgenic mice that up-regulate RAGE during lung organogenesis

Derek Clarke, Katrina Curtis, Ashley Chang, Juan Arroyo

Brigham Young University, USA

Receptors for advanced glycation end-products (RAGE) are multi-ligand cell surface receptors of the immunoglobulin superfamily primarily expressed by lung epithelium. Significant lung simplification has been observed when RAGE is upregulated throughout embryonic and fetal periods of development, potentially via increased apoptosis and altered mitochondrial activity. Here we investigate RAGE stimulated apoptosis in developing lung tissue. H&E staining confirmed aberrant development of murine lungs upon RAGE upregulation from conception (embryonic day, E0) to sacrifice date on E18.5 (long-term) and when RAGE was specifically upregulated from E15.5 to E18.5 (short-term). Multiple array analyses revealed common pathways of apoptosis when comparing short-term and long-term groups. In both groups, we discovered increased TNFR1, TNFRII, CD40 ligand, p21, and TRAIL R2—factors that modulate apoptosis. TNRFII inhibits anti-apoptotic signaling leading to an increase in TNFRI. TNFRI induces an increase in CD40 ligand expression, which causes elevated p21 activation and increased Trail R2. P21 activity is implicated in alveolar type 2 cell turnover and apoptosis and Trail R2 augments apoptosis via caspase activation. We also discovered interesting differences in apoptotic pathways in both groups. In the short-term mice, we found increased expression of the pro-apoptotic factors Smac and HTRA2 and decreased expression of the anti-apoptotic BCL-W. In long-term mice, we found decreased IGF-2 which leads to tissue malformation and diminished expression of an anti-apoptotic factor, HSP27. Understanding the role of RAGE in mediating apoptosis during lung formation further characterized an important role for RAGE in normal and abnormal lung formation. This work was supported by funding from the National Institutes of Health (NIH 1R15-HL152257, PRR).

Program Abstract #167

Building Pancreatic Tubes: The Hippo modulator Merlin is required for pancreatic development

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The central goal of regenerative medicine (RM) is to be able to build organs in a petri dish, to then be used for therapeutic purposes. There is a particular need for RM strategies for the pancreas due to the prevalence of diabetes. Our goal is to uncover mechanisms of pancreatic organogenesis using murine in vivo and ex vivo approaches. Pancreatic development relies upon de novo lumenogenesis, where in cells establish an apical membrane through vesicular trafficking and undergo apical constriction, giving rise to a microlumen. These microlumens fuse to give rise to an epithelial plexus, which harbors islet progenitor cells. How do cells coordinate the formation and fusion of lumens? We hypothesized that mechanical stimuli arising from apical constriction during lumen formation act as cues to guide plexus formation. To test this hypothesis, we focused on Merlin, akeyregulator of the Hippo pathway, which senses mechanical cues. We conditionally deleted Merlin from the pancreas and found that loss of Merlin leads to severe defects, including an expansion of ductal and endocrine cell populations, and a concomitant failure of plexus formation. To investigate the role of Merlin in microlumen formation, we utilized a 3D-sphere forming assay that enables live imaging of lumenogenesis. We found that spheres deficient in Merlin collapse by96hours of culture. To evaluate the role of Merlin in establishing the apical membrane, we utilized a novel live imaging system that enables us to track individual vesicles in explant culture. Intriguingly, we found that Merlin is required to restrain vesicular trafficking. Our current work focuses on understanding how Merlin is regulated through actomyosin tensionandidentifying the downstream targets for Merlin. This work provides insight into how pancreatic tubes are built in vivo and highlights the need to consider mechanics in RM approaches. Funding Sources: JDRF 3-PDF-2023-1327-A-N and NIDDK R01DK121408.

Program Abstract #168

Mosaic analysis with double markers reveals *Grb10* dosage cell-autonomously regulates pancreatic expansion

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Many of the small subset of mammalian genes regulated by the epigenetic phenomenon of imprinting strongly influence growth control networks. Dosage changes of these monoallelically expressed genes can profoundly alter body size, but the tissue specific and cell autonomous functions of dosage control by imprinting are not well known. The influence of these genes is particularly critical to normal growth of organs like the pancreas, which has limited regenerative capacity. To answer these questions, we have generated uniparental disomy (UPD) of mouse chromosome 11 in Pdx1+ multipotent pancreatic progenitors using Mosaic Analysis with Double Markers (MADM). Mouse chromosome 11 harbors the well characterized *Grb10* imprinting control region. Maternal ablation of *Grb10* has previously been shown to lead to newborn mice that are 30% larger, but not all organs increase proportionally with overall mass. We found that pancreas cells with paternal UPD of chromosome 11, expressing no *Grb10*, are significantly more abundant than maternal UPD cells expressing twice the normal dosage of *Grb10*. By tracking individual MADM11-labeled cells by 4D confocal microscopy, we determined cells with low dosage of Grb10 divide significantly faster than cells with a doubled dosage. We also determined the differentiation outcomes of patUPD and matUPD multipotent progenitors. We propose a model where the dynamic regulation of Grb10 titrates multipotent pancreatic progenitor expansion. These findings are significant for the effort to efficiently manufacture islet tissue from human pluripotent stem cells as therapy for type 1 diabetes. We are currently testing the effect of altered *GRB10* dosage in an established human pancreatic differentiation protocol. This work was supported by the Marie Sklowdowska-Curie Actions and the Novo Nordisk Foundation.

Program Abstract #169

Zebrafish with different rates of postembryonic development have different intestinal microbiota

Pijush Sutradhar

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During embryonic zebrafish development, fish develop at a constant rate when raised at the same temperature and time can be used to stage fish. However, as zebrafish transition into the postembryonic stage, they develop at different rates even when kept in similar conditions. Consequently, time

postfertilization is no longer a good predictor of the developmental stage, instead, individuals with the same developmental traits have been grouped and compared during experimental analysis. We suggest that **differences in intestinal activity**, whether it be due to genetic or environmental factors, may play a role in the rate at which post-embryonic fish proceed through developmental stages. To investigate this theory, we determine whether fish growing at different rates have intestinal differences in motility, microbiome composition, and inflammation. We compare two groups of post-embryonic fish who reach the same developmental stage but diverge by a week in their journey. The first group which we term on time grows at a constant rate through the first two weeks of post-embryonic development, reaching the previously characterized 5.7 mm stage at the end of the second week of post-embryonic development (19 dpf). The second group reaches the same developmental stage at the end of the third week (26 dpf) and is characterized as slow developing. We are investigating the gut microbiome composition using 16S rRNA Minion Nanopore sequencing. We are also analyzing gut motility and changes in inflammation. We find that the slow-developing group has a higher rate of intestinal motility, a greater microbiome diversity, and increased intestinal inflammation.

Program Abstract #170
Cellular and Molecular Characterization of Enteric Muscle Regeneration in *Holothuria glaberrima*
Alejandra Beltran Rivera, Joshua G. Medina-Feliciano, Yamil Miranda-Negron, Paula Sepulveda-Arsuaga, Aurora Torres-Perez, Griselle Valentin-Tirado
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Formation of the enteric muscle is a key event in the organogenesis of the digestive tract. During intestinal regeneration in the sea cucumber *Holothuria glaberrima*, the muscle layer is thought to be formed by dedifferentiated cells that originate from the adjacent mesentery. How this process takes place and whether the dedifferentiated cells retain their muscle characteristics remains unknown. Here we make use of new cell markers, tissue level, and single cell (scRNAseq) RNA sequencing to provide some insights into the enteric muscle regeneration process. Newly generated monoclonal antibodies identified muscle-specific markers that pose as potential candidates to follow the dedifferentiation process. Transcriptomic data from regenerating organisms have shown the expression profile of genes associated with the regenerative signaling pathways. Complementarily, data obtained from Single-Cell Sequencing analysis has enabled us to effectively identify distinct cell clusters that exhibit the expression of muscle-specific components very early in the regenerative process. We have integrated the available information into a working model for intestinal muscle regeneration and its interactions with other cells and tissues that lead to successful regenerative organogenesis. **Funding:** PR- LSAMP--> Bridge to the Doctorate Program (BDP) --> Award Number: HRD-2008186--> BDP Cohort XIV: EES-2306079

Program Abstract #171
Characterization of intestinal development during post-embryogenesis
Anisa Iftikhar
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In zebrafish embryogenesis, a consistent developmental rate is maintained when reared at a constant temperature. However, during the post-embryonic period, individual growth rates differ significantly, which presents challenges in comparing development due to the emergence of various developmental stages within cohorts of the same chronological age. We propose that once post-embryonic fish reach the developmental stage of SL5.5 they will once again grow at a constant rate. We have compared growth rates of post-embryonic fish that reach SL5.5 at different times and compared whether they will reach developmental milestones at comparable times. Comparable growth rates during later post-embryonic periods could then allow for comparison of age matched fish exposed to experimental treatments.

Program Abstract #172
Temporal single cell analysis reveals the requirement for Odd-skipped related 1 in regulating mouse embryonic bladder cell fate decisions
Vasikar Murugapoopathy[1], Rulang Jiang[2], Indra R. Gupta[1]
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The bladder is a hollow, muscular sac that stores urine and expands up to 6-fold while withstanding greater tensile force than the Achilles tendon. Bladder capacity is controlled by interactions between it's three layers: the epithelia which signals when full, the extracellular matrix (ECM)-rich lamina propria that propagates signals and bears mechanical load, and the muscle which contracts to expel urine. Bladder dysfunction is defined by aberrant ECM deposition, muscle contraction, and epithelial morphology, but mechanisms underlying these changes are largely unknown. Understanding how bladder cell types arise during development, and how they communicate to organize architecture is informative for understanding dysfunction and regeneration. Our lab has identified that the transcription factor Odd-skipped related 1 (Osr1) is expressed in the bladder at the onset of development, is required for development of all three layers, and that loss of *Osr1* decreases bladder capacity. Using histological and immunofluorescent analysis, we see that *Osr1*-KO embryonic bladders are smaller, fail to form a stratified epithelium, have decreased collagen, and a thin and underdeveloped muscle layer. Using single-cell RNA seq across three embryonic time points of *Osr1* KO and WT bladders we confirm a decrease in smooth muscle differentiation and increased cells arrested in a progenitor like state, suggesting Osr1 is required for terminal differentiation of mesenchymal progenitors. We also observed a loss of basal and intermediate epithelia cells. After performing pseudobulk analysis of mRNA expression across all cell types we observed aberrant upregulation of genes in Hedgehog, Wnt, and FGF signaling pathways suggesting Osr1 is important for regulating epithelial to mesenchymal crosstalk. Our research will provide a useful framework for understanding the cell types and genes important for embryonic bladder development. Funding sources: FRQS and Pierre Lavoie Foundation.

Program Abstract #173

Sexual Differentiation in 3D: An Interactive Atlas of Mouse and Human Genitourinary Tract Development Using Nanoscale Computed Tomography (nanoCT)

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Biological differences between males and females, known as sexual dimorphisms, have long been recognized in reproductive organs and secondary sex characters. The genitourinary (GU) system shows extensive morphological and physiological sexual dimorphisms, and numerous diseases of reproductive and urologic organs show sex differences in their prevalence, progression, responses to treatment, and outcomes. Early development of the mammalian GU tract is similar in males and females, which have bipotential gonads, a genital tubercle, and both Müllerian and Wolffian ducts. Sexual differentiation begins when the gonads differentiate into testes in males and ovaries in females, and, in turn, gonadal sex hormones influence morphogenesis of the internal and external genitalia and urologic organs. Identification of developmental anomalies and adult disease states requires a detailed understanding of normal organogenesis and structure in healthy males and females. As part of the GenitoUrinary Development Molecular Anatomy Project (GUDMAP.org), we used nanoscale computed tomography (nanoCT) to produce high-resolution, interactive, 3D models of normal genitourinary organogenesis in mice and humans. This developmental reference series includes males and females and reveals granular details of sexual differentiation. The data are freely available to the community via the GUDMAP site, where users can download the raw data for further analysis or use the interactive user interface to explore the 3D data using a standard web browser. This resource is intended to promote research into genitourinary development by providing reference specimens for phenotypic characterization of mouse models, interpretation of human structural birth defects, and comparative studies of mice and humans. Supported by NIDDK.

Program Abstract #174

T-box transcription factor Org-1 mediates signaling and identity to establish a spermatogonial niche

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Stem cells reside in microenvironments that regulate their location, identity, and division. This microenvironment, the stem cell niche, forms in human tissues like bone marrow and intestinal lining. We leverage the *Drosophila melanogaster* embryonic gonad to investigate the development of this paradigm. The spherical male gonad consists of germ cells mixed with somatic cells and somatically derived niche cells at the anterior. The position and morphology of this niche is important for proper

maintenance and regulation of spermatogonial stem cells to set up a compartmentalized region that separates differentiating germ cells along the adult testis. Our work has shown that Slit and FGF signals from visceral muscle are necessary for anterior assembly of niche cells (Anllo & DiNardo, 2022). Without FGF and Slit, we see reduced expression of the conserved transcription factor *islet*, and failed assembly. Since FGF and Slit act through receptors on somatic gonadal cells, and islet expression changes in response to these signals, there must be an intermediary required for niche assembly. *islet* expression during muscle development requires the Tbx1 ortholog Org-1 (Boukhatmi et. al., 2014), and we are testing a parallel mechanism in the gonad. We show that *org-1* mutants have multiple defects. Niche adhesion markers show abolished Fas3, dispersed E-cad, and fewer N-cad expressing niche cells. Fewer niche cells suggest a specification and/or identity problem. Indeed, our data show that *org-1* mutant niches have diminished hedgehog expression, a marker of niche cell identity, and *org-1* overexpression results in increased numbers of niche cells. Together, these data suggest that Org-1 is necessary and sufficient for niche specification. Our current work focuses on testing function of the few niche cells specified in the absence of *org-1*. This work identifies a novel regulator of niche identity that can be tested for parallel roles in other systems. NIH funding, ECU startup.

Program Abstract #175

Notch* and *Delta* Patterns of Expression in *Hymenolepis diminuta

Chandler Lowe

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Parasitic flatworms cause neglected tropical disease and are responsible for millions of dollars in healthcare and livestock damages annually, according to the World Health Organization. A hallmark feature of these worms is their ability to constantly grow and readily regenerate the strobilated region of their body, composed of repeated proglottids. We use the rat tapeworm, *Hymenolepis diminuta*, as a model system to understand the regeneration and developmental biology of these parasites. Ablation of cycling cells in the neck using irradiation is sufficient to cease growth and regeneration. However, what pathways guide the various stem cells to become proglottids remains elusive. Recent studies in a sister species, the mouse bile duct tapeworm *Hymenolepis microstoma*, point to a putative signaling center responsible for the morphogenesis of proglottids and includes *Notch*. Canonical Notch pathway involves membrane bound Delta and Notch signaling in a juxtacrine manner, activating target genes. Notably, Notch signaling plays a key role in somitogenesis by maintaining the segmental clock and establishing somite polarity. Does Notch signaling also play a role in orchestrating proglottid formation in *H. diminuta*? Here, we use antisense RNA *in situ* hybridization to characterize where both a *Notch* and *Delta* homolog are expressed in the neck and developing proglottids. We find that these components appear to be expressed in a position control gene-like pattern by both muscle and tegument progenitor cells. Both genes also express in accessory reproductive organs, indicating a possible role in maturation of these tissues. Future goals include characterizing other Notch components, as many *Notch*+ cells do not juxtapose *Delta*+ cells. This study provides the first steps towards characterizing Notch/Delta pathway and finding a critical signaling center in *H. diminuta*, an emerging model for regeneration and developmental biology. Funded by NIH/NIAID DP2: AI 154416-01.

Program Abstract #176

Trophoblast Differentiation in Mesoderm-Specific Tmed2 Mutant Placentas

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TMED2 is a member of the transmembrane emp24 domain protein family that is essential for cargo transport between the endoplasmic reticulum (ER) and Golgi and for placental labyrinth layer development. The placental labyrinth layer is where nutrient and gas exchanges happen between the mother and the embryo and is essential for embryonic survival. Placental labyrinth layer formation depends on attachment, fusion, and branching of the chorion and allantois, two extraembryonic tissues that express TMED2. Using an ex vivo chorioallantoic cultures system we showed that TMED2 was required in both the allantois and chorion for placental labyrinth layer development. The allantois and a portion of the chorion, the chorionic mesothelium, are both derived from extraembryonic mesoderm. We

hypothesize that TMED2 is required in extraembryonic mesoderm for placental labyrinth layer development. We generated conditional mutant mice with LoxP sequences flanking exons 2 and 3 of the gene. Using Mesp1-cre (Mesp1cre) we deleted Tmed2 from the extraembryonic mesoderm which results in embryonic death by embryonic day (E)12.5. Our preliminary data indicate that chorioallantoic attachment and fusion occur in Tmed2LoxP/LoxP; Mesp1Cre/+ mutant embryos. To examine placental labyrinth development, we performed histological analysis of E9.5 control and homozygous mutant placentas; to examine trophoblast differentiation, RNA probes for Tpbpa, Gcm1, and Pl1, were used for in situ hybridization. Our results reveal that expression of Gcm1 and Pl1 was similar in wild-type and mutant placenta at stages E9.5 and E11.5. However, at these stages, Tpbpa expression was reduced in mutant placentas. Our future work will allow us to identify the requirements for TMED2 in extraembryonic mesoderm during placental labyrinth layer development and to determine if abnormal placental development is responsible for the death of Tmed2LoxP/LoxP; Mesp1Cre/+ mutant embryos. This project is funded by NSERC.

Program Abstract #177

The requirement of TMED2 in placenta formation

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Exchange between the maternal and fetal compartments occurs via the placental labyrinth layer, which is formed by attachment and fusion of two extraembryonic mesoderm derived tissues, the allantois and the chorion. TMED2 is expressed in the allantois and chorion and is required for placental labyrinth layer formation. To examine the role of TMED2 in extraembryonic mesoderm we used *Mesp1-cre* and mutant mice with LoxP sequences flanking exons 2 and 3 of *Tmed2*. We postulate that TMED2 is required in extraembryonic mesoderm for labyrinth layer formation. To test our hypothesis, we collected *Tmed2*$^{LoxP/LoxP}$; *Mesp1*$^{Cre/+}$ embryos from Embryonic day (E) 9.5 – E12.5, for histological and morphological analysis. We used immunohistochemistry and *in situ* hybridization to examine expression of proteins and genes essential for placenta formation and function. *Tmed2*$^{LoxP/LoxP}$; *Mesp1*$^{Cre/+}$ embryos have placental labyrinth layer formation, but arrest at E12.5. At E9.5, expression of genes important for placental development are comparable in mutant and controls, while the spongiotrophoblast marker, *Tpbpa,* was decreased in mutants. The area of the placental labyrinth layer was reduced in a subset of E9.5 and E10.5 embryos, and this difference was significant at E11.5. The fetal and maternal compartments of the placental labyrinth layer had ectopic cells and were disorganized in E11.5 mutants. In addition, expression of fibronectin, a TMED2 cargo, was increased, more fibrillar and appears to be retained. Our data indicates an essential role for TMED2 in the extraembryonic mesoderm for proper formation and development of the placenta. This project is funded by NSERC.

Program Abstract #178

Placentation defects are common in murine organogenesis lethality: an examination of 22 murine homozygous lethal E9.5-12.5 novel knockout mouse project (KOMP) alleles.

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Despite complete sequencing of the mammalian genome for over 20 years, numerous gaps lie in understanding how these sequences function in the organism. The goal of the Knock-Out Mouse Project (KOMP) is to functionally annotate the mammalian coding sequence by gene deletion and null phenotype assessment. We characterized the phenotype of KOMP-generated null alleles of 23 understudied genes that are homozygous lethal between embryonic day (E)9.5-12.5. Most genes mutated in the alleles obtained are predicted to have broad cellular or metabolic roles and their null phenotypes include both embryonic and extraembryonic abnormalities. Almost all genes examined are specifically expressed in the fetal component of the murine placenta and their embryonic phenotypes fall into 3 broad phenotypic classes, suggesting that these genes play essential roles the placenta. One gene examined, *Glutamic-oxaloacetate transaminase 2*(*Got2*), the mitochondrial malate-aspartate shuttle is widely expressed throughout the conceptus and highly expressed in the nascent liver and

placenta. To assess the role of Got2 in the embryo proper, Sox2-Cre was used to delete a floxed Got2 locus in the embryo proper, retaining its expression in the placenta. Unlike the null allele that induces severe embryo defects by E9.5, the Got2 conditional knock-out is viable through E12.5, demonstrating that the first essential role for Got2 is in the placenta. Metabolic analysis of delayed but otherwise normal E10.5 conditional mutant embryos, reveals roles for Got2 not only in malate and aspartate levels but also the NAD/NADH ratio, purine and pyrimidine metabolites as well as glycolysis. By E10.5 a hypoplastic liver is also observed. We find that homozygous loss of function alleles that induce lethality between E9.5-12.5 mainly occurs in genes that play a role in placentation and hypothesize that many also play critical later roles in embryonic liver or vascular development. Funded by HD096073 to KDT.

Program Abstract #179

The function of the *gpr143* in the *A. sagrei* eye.

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Albinism is a genetic disorder most often characterized by a loss or reduction of pigmentation in the skin, hair, and eyes. In addition to pigmentation loss, albinism patients may experience reduced visual acuity due to fovea hypoplasia, where the fovea, a pit-like structure in the neural retina, fails to develop properly. However, the relationship between pigmentation loss and fovea hypoplasia in albinism remains poorly understood. This gap in knowledge is due to a lack of suitable animal model; the most commonly used animal models in biomedical research lack a fovea. We are developing the brown anole, *Anolis sagrei*, as a model for fovea development. This anole is a bi-foveated lizard from the Caribbean islands. *A. sagrei* has a large central fovea and a smaller, shallower temporal fovea making the anole an ideal model for studying fovea development. The albinism gene, *GPR143*, is responsible for melanosome biogenesis. In humans, loss of function mutations in this gene results in fovea hypoplasia. *GPR143* encodes for a G-protein coupled receptor, making it an attractive target to understand the cellular signaling that may be contributing to fovea formation. To this aim, we generated anoles with loss of function mutations in *gpr143* using CRISPR-Cas9 gene editing. Mutants were found to have both the temporal and central foveae present when iDisco was performed. However, further histological analyses indicate that the pigmented tissues of the retinal pigmentated epithelium (RPE), ciliary body, and iris were abnormally thick in *gpr143* mutant eyes when compared to wildtype. Neural retinal lamination and development was also perturbed with the RPE growing into the neural retina itself. We hypothesize that in *A. sagrei*, *gpr143* is required for the proper development of pigmented tissue and neural retina. This project is funded by Fight For Sight, Children's Glaucoma Foundation, Vision for Tomorrow, and NSF #1827647.

Program Abstract #180

Stem cell lineages in the *Drosophila melanogaster* ovary require glucose instead of trehalose as a primary sugar source for glycolysis

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Stem cell lineages are regulated by whole-body physiology, but less is known about their specific metabolic requirements. Unpublished data from our lab showed that glycolysis, but not fatty acid oxidation, is required for specific processes within the germline and follicle stem cell lineages in the *Drosophila* ovary. Trehalose (composed of two glucose subunits) is the predominant circulating sugar is *Drosophila*, while glucose is present at lower levels that fluctuate with diet. However, it remains unclear which of those sugar sources fuels glycolysis in the ovarian stem cell lineages. In this study, we examined the requirements for Glut1, which encodes a major glucose transporter, and Trehalase (treh), which encodes an enzyme that hydrolyses trehalose into two units of glucose, in these stem cell lineages using FLP/FRT-mediated genetic mosaic analysis. Surprisingly, treh is not required in the follicle stem cell lineage either on a normal or high sugar diet. Treh is mildly required for germline stem cell maintenance, but loss of treh has no other phenotypes in the germline. By contrast, loss of Glut1 results in various phenotypes on

both diets. Specifically, Glut1 is required in the germline for germline stem cell maintenance, survival of newly formed 16-cell germline cysts, and egg chamber growth, and in the follicle stem cell lineage for normal number of follicle cells. Altogether, our results suggest that glucose is the major sugar source for glycolysis within the germline and follicle stem cell lineages, and that trehalose has a minor role. These results are in contrast to the cell autonomous requirement for treh during larval organ growth. Future studies will expand our analysis of Glut1 in both stem cell lineages, as well as explore the role of additional sugar transporters in the *Drosophila* ovary. Funding sources: Morgridge Institute for Research and National Institute of General Medical Sciences.

Program Abstract #181
Unlocking the developmental potential of bovine fetal mammary cells
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With each new pregnancy, the mammary gland undergoes significant tissue growth and differentiation followed by programmed cell death and remodeling. These transformations are achievable because lineage-restricted progenitor cells lacking stem cell potential exhibit sufficient cellular plasticity to regenerate new tissue and restore lactation capacity. Adult stem cells were thought to be drivers of this process, however, their identities remain controversial. Fetal mammary cells are the only mammary cells known to exhibit high developmental potential, so we hypothesize that better understanding bovine fetal mammary development will facilitate new strategies advancing animal/human mammary health and function. Our objectives are to 1) identify gene networks governing fetal bovine mammary developmental potential, and 2) develop *in vitro* fetal mammary cell models for gene perturbation studies. To ascertain when the multipotent niche exists, fetal tissues were micro-dissected and partitioned into experimental groups by stage and crown-rump length (CRL, n= 5 fetal tissues per stage: hillock/bud, bud/teat, primary sprout, and secondary sprout). TRP63 and cytokeratin (5/8/14) immunolabeling indicate that luminal/ basal lineage segregation occurs prior to sprouting when P63+ cells segregate to the outer layer and inside luminal cells exhibit reduced TRP63 and CK14 expression. Multiplex fluorescent RNA *in situ* hybridizations reveal that mammary bud cells co-express specification (LEF1, SOX11), luminal (SOX9, ELF5), and basal (TRP63, LMO4) factors, indicating that they remain uncommitted yet transcriptionally primed for differentiation. Nine cell lines derived from fetal mammary epithelia were generated via conditional reprogramming culture. Together, this work will allow us to better understand how gestational influences including heat stress and malnutrition affect fetal mammary programming. (Funding: Maryland Agricultural Experiment Station.)

Program Abstract #182
Identifying the Origin of Innate Lymphoid Cells in Zebrafish Development
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The hematopoietic and immune systems are comprised of circulating and tissue resident cells critical for oxygenation, clotting, wound repair, and infection fighting. These lineages are established during embryonic development by multipotent Hematopoietic Stem Cells (HSCs) and more restricted Hematopoietic Progenitor Cells (HPCs). The relative contribution of HSCs versus HPCs to the embryonic immune and hematopoietic systems and the full repertoire of cells they generate is not delineated *in vivo*. To fill this critical knowledge gap, we used an inducible Cre-Lox lineage tracing system to label the emerging hematopoietic system during larval development followed by single cell RNA sequencing of HSC and HPC progeny. We detected the expected myeloid-erythroid lineage cells like macrophages, neutrophils, erythrocytes, and thrombocytes at 6- and 10- days post-fertilization. Surprisingly, in addition to rag1-positive T lymphocytes, we detected signatures of a diverse set of previously undescribed lymphoid cells expressing genes like *il13, il4, rorc, il23r, nkl.1* and *nitr* which are characteristic of Innate Lymphoid Cells (ILC)-like cells. We assessed the dependency of these ILC-like cells on the transcription factor Runx1 to determine if they were derived from the definitive wave of hematopoiesis. We performed a similar hematopoietic lineage tracing and single cell sequencing experiment in 6 dpf *runx1*^{W84X}mutants and sibling controls and showed that all ILC-like clusters are Runx1-dependent. Although ILCs were detected in adult zebrafish, our dataset provides some of the first evidence of ILC-like cells at earlier larval stages of zebrafish development than previously demonstrated. The work will provide fundamental knowledge on

the early establishment of immune hierarchies and open the zebrafish model to the exploration of ILC origination and function. Funding Source: F31HL167600, R01DK131445

Program Abstract #183

Dysregulated epithelial-mesenchymal crosstalk in the stem cell niche as mechanisms of intestinal polyp formation

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Epithelial-mesenchymal crosstalk is a crucial mechanism regulating morphogenesis, stem cell maintenance and tissue homeostasis. In the mammalian small intestine, distinct fibroblast populations are spatially organized to segregate the signals required around the stem cell niche (crypt) for epithelial proliferation and in the villus for epithelial differentiation. However, we have a limited understanding of how fibroblasts are organized and regulated to maintain tissue homeostasis. Studying fibroblast regulation is important as the loss of fibroblast Bmp signaling transforms the epithelium into hyperproliferative polyps via unknown mechanisms. Our inducible Bmp loss-of-function (LOF) mouse model reveals novel polyp-initiating events that involve ectopic tissue fold formation before epithelial hyperproliferation. This initial tissue architectural change separates the epithelium from its surrounding stroma, disrupting normal stem cell-niche interactions. Distinct villar and crypt fibroblasts mislocalize and cluster at ectopic tissue fold with excessive fibronectin deposition, indicating altered fibroblast compartmentalization. Furthermore, Bmp-LOF fibroblasts acquire a hypercontractile state and lead to epithelial hyperproliferation with increased Wnt signaling activity in vitro. The altered fibroblast cell mechanics, localization, and niche signaling could potentially transform tissue folds into hyperproliferative epithelial polyps. Taken together, our data suggest that fibroblasts require Bmp signaling to maintain proper cell mechanics and compartmentalization to critically regulate epithelial stem cell dynamics and tissue homeostasis. This work is funded by ACS Research Scholar Grant, institutional ACS Pilot Grant, NIH F31DK132866, George Robert Pfeiffer Fellowship and NCI T32CA193200.

Program Abstract #184

Investigating planarian parenchymal cells as a potential stem cell niche

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Schmidtea mediterranea is a species of flatworm with a capacity for whole-body regeneration, due to a pool of pluripotent stem cells. In many organisms, stem cells are regulated by neighboring differentiated cell types. However, the cells, if any, that serve this purpose in planarians are still unknown. In previous work, planarian stem cells were observed to reside adjacent to differentiated cell types called "fixed parenchymal" cells, a name based on the parenchymal tissue where they were found. However, the molecular identity of "fixed parenchymal" cell is not known, and their functions are not understood. We hypothesize that differentiated cells provide a niche important for maintaining stem cell identity and triggering differentiation during planarian regeneration. To test this, we first identified and characterized cell types found within the parenchymal space. These include the stem cells themselves, parenchymal, muscle, neuronal, intestinal, and phagocytic *cathepsin*$^+$ cells. We compiled a list of 29 genes that mark distinct cell types and found the cell type that most often neighbors stem cells are a subset of *cathepsin*$^+$ cells marked by *HSPGR3*, heparin sulfate proteoglycan related 3, expression. This neighbor status makes *HPSGR3*$^+$cells a good candidate cell type for a contributor to the niche. Second, we are completing a screen to identify genes that perturb *HPSGR3*$^+$ cells. Our goal is to investigate whether stem cells and regeneration are impacted when putative niche cells are perturbed. In our pilot screen, we determined that RNAi targeting *HSPGR3* reduces expression of *smedwi-1*, a broad stem cell marker. We hope that by revealing the microenvironment favorable to stem cell survival and function, we will improve pluripotent stem cell culture conditions for planarian cells and potentially stem cell culture conditions for other species. This work is funded by an NSF CAREER award and an NIH T32 Genetics Training Grant to Skylar Settles.

Program Abstract #185

Planar cell polarity signaling regulates stem cell division symmetry to control tissue growth self-termination

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How does tissue morphogenesis self-terminate at the appropriate time and space? Highly regenerative models, like planarians, present an exciting opportunity to uncover the molecular regulators of endogenous growth repression. Our previous data show loss of planar cell polarity (PCP) signaling in regenerating planarians promotes stem cell (SC) hyperproliferation and body-wide hyperplastic growth. Single-cell atlases and *in situ* analysis show PCP gene expression in all classified SC subtypes and diverse tissue lineages. Thus, we hypothesized PCP could serve as a growth termination signal to SCs. We used RNA interference (RNAi) to investigate effects of PCP loss on SC and progenitor populations and conducted differential expression/Gene Ontology (GO) analyses of bulk RNA-seq data from control and PCP-inhibited animals at 2- and 8-weeks to elucidate potential growth termination mechanisms. PCP inhibition caused increased early (*prog-1*$^+$) and late (*agat-1*$^+$) SC progeny numbers, consistent with increased epidermal cell density and reduced cell sizes observed at 8-weeks. Interestingly, PCP RNAi caused depletion of pluripotent *tspan-1*$^+$ SCs, which can replace all adult cell types after lethal irradiation. Our RNA-seq analyses point to PCP-mediated control of stem cell fate (by cell division symmetry) as a potential growth termination mechanism: GO analyses showed enrichment of Rho/Ras GTPases and GEFs, and regulators of cell division symmetry like lissencephaly-1 were differentially expressed following PCP loss. We quantified symmetric versus asymmetric cell division rates in controls over time and found that symmetric divisions expand the SC pool, whereas PCP loss increases the number of asymmetrically dividing cells, resulting in SC depletion and accumulation of differentiated tissue. Our data suggest that regulation of SC maintenance and fate decisions require PCP signaling during growth self-termination. Funding: NSF 1652312, NIH R15GM150073-01.

Program Abstract #186

A novel Robo signaling pathway regulates stem cell fate in planarians

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Both homeostasis and regeneration depend on the proper regulation of stem cell differentiation. Extrinsic signals ensure that stem cell differentiation occurs in a correct spatiotemporal context, but the identity of these molecules remains largely unknown. Planarians have adult pluripotent stem cells that can differentiate into all of the animals' organs, making them a unique organism to investigate regulators of stem cell differentiation. To explore extrinsic regulators of stem cell biology, our lab focuses on a single, anatomically distinct organ, the pharynx. The pharynx has unique cell types and robust regeneration readouts, allowing us to parse pharynx-specific differentiation from that of other organs. Our previous work established the Forkhead transcription factor *FoxA* as a critical determinant of stem cell differentiation toward pharynx lineages. To further determine what extracellular signals regulate *FoxA*-mediated differentiation, we analyzed a phenotype in which knockdown of a roundabout receptor, *RoboA*, causes animals to grow ectopic pharynges. Using lineage-specific markers, we find that *RoboA*(RNAi) animals abnormally generate extra pharynx neurons in the brain, where they otherwise never appear. Through RNAi or radiation-induced perturbations of stem cells, we show that the emergence of these additional pharynx neurons depends on stem cells and *FoxA*. Moreover, knockdown of *Slit-1*, the canonical ligand for Robo receptors, does not phenocopy *RoboA*, suggesting that a novel ligand may activate RoboA on stem cells, preventing them from adopting an abnormal pharyngeal fate. In summary, our findings suggest that *RoboA* restricts stem cell differentiation toward the pharynx lineage by repressing *FoxA*-mediated differentiation. This work is funded by NIHR01GM139933.

Program Abstract #187

Prospective isolation and molecular characterization of stem cells in the rat tapeworm, *Hymenolepis diminuta*

Corey Rennolds, Tania Rozario
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Tapeworms are successful parasites due in part to their ability to grow quickly, shed many reproductive body segments (proglottids), and regenerate segments rapidly. The cellular and molecular basis of such continuous, large scale tissue turnover remains poorly understood. The rat tapeworm *Hymenolepis diminuta* contains a sole population of proliferative cells with body-wide distribution that are required for growth and regeneration, like planarian neoblasts, indicative of stem cells (SCs). However, unlike planarians, *H. diminuta* regeneration is not body-wide, consisting only of proglottid regeneration from the neck. Understanding this regenerative ability requires characterizing the SCs, including determining their potency, functional diversity, and developmental relationships. We are conducting parallel approaches to isolate SCs for investigation. First, we are using basic stains and fluorescence activated cell sorting to enrich for SCs. We have found three populations of cells differing in nuclear DNA content, likely corresponding to cell cycle phases (G0/G1, S, G2/M), and we plan to use single-cell RNAseq (scRNAseq) of the putative 4N cells to distinguish SC subpopulations, including any pluripotent cells and lineage-restricted progenitors that may exist. Second, we are using existing scRNAseq datasets to discover SC markers, including cell surface receptors. We have already identified one candidate, a protocadherin-a ortholog, and are raising monoclonal antibodies against it. Single-nuclei RNAseq of anterior body fragments is also underway to improve our ability to detect subtle differences in gene expression between SC subpopulations in the neck. We anticipate that these approaches will yield novel insights regarding the molecular heterogeneity of tapeworm SCs and facilitate further work to understand SC function, potency, and plasticity. This work is supported by a NIH T32 Postdoctoral Fellowship to C. Rennolds and a NIH/NIAID DP2 to T. Rozario.

Program Abstract #188

Neuropeptide Processing Enzymes Contributions in Planarian Regeneration and Stem Cell Differentiation

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University of Georgia, USA

Neuropeptides play numerous roles in metazoans, including aiding in neurodevelopment and regulation of neurotransmitter release. Roles for neuropeptides in regulation of stem cells and regeneration are emerging. However, many questions remain. Planarian flatworms are among the only organisms that can fully regenerate all body organs *de novo*, making them an ideal model with which to explore neuropeptide function in regeneration and stem cell activity. Because neuropeptides have such diverse functions and the ability to act as long-range signaling molecules, they could promote regeneration across the entire body (Mendel et al., 2020). Prohormone convertase 2 (*PC2*) is an essential enzyme in neuropeptide synthesis which has been shown to impact behavior and regeneration when knocked down by RNA interference (RNAi) in planarians (Reddien, Bermange, et al., 2005; Collins, et al., 2010). We determined that *PC2(RNAi)* leads to a reduction in stem cell markers and a dramatic decrease in progenitor markers. Next, we investigated genes that encode other enzymes that cleave neuropeptides to analyze whether their perturbation causes similar phenotypes. Our data indicate that knockdown of genes encoding neuropeptide processing enzymes (*carboxypeptidase*, *peptidylglycine- a -amidation-like, furin*, *peptidylglycine- a -amidation-1*, and *furin-like*) leads to smaller brain size, diminished stem cell differentiation, and perturbed stem cell maintenance. We thus hypothesize planarian neuropeptide processors—potentially through neuropeptides— influence regeneration by impacting stem cell function. Discovering relevant neuropeptides and downstream signals will expand our knowledge of pluripotent stem cell regulation in vivo. This work is funded by an NSF CAREER award (#1942822).

Program Abstract #189

Checkpoints and strategies for improving human buffy coat-based iPSC reprogramming success

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Human pluripotent stem cells (hPSCs) are a valuable tool for disease modeling. Techniques utilizing induced pluripotent stem cells (iPSCs), which are reprogrammed from adult somatic cells, allow for studies of specific patient populations and disease-specific cell types, paving the way for ventures into personalized medicine, 'clinical trials in a dish,' and human disease modeling. Many current protocols describe how to reprogram iPSCs from patient cells, including skin cells (fibroblasts) and peripheral blood mononuclear cells (PBMCs). However, there are many issues that may arise in the reprogramming process that can limit the success of such protocols. In many cases with iPSC reprogramming, patient samples are

rare or difficult to obtain making it imperative to have thorough protocols enhancing the chance of success with limited patient samples. In our case, when attempting to reprogram human PBMCs from frozen buffy coats for a collaborator project, we found previously published protocols were not successful. While there are protocols detailing PBMC reprogramming, there is only one protocol that reprograms from frozen buffy coats. Buffy coats are a less purified blood sample, typically containing PBMCs as well as white blood cells and some red blood cells. Through rigorous trouble shooting, we identified key predictive markers of reprogramming success. Here, we identify checkpoints and troubleshooting strategies to enhance reprogramming success from frozen buffy coat samples. Recognition of these checkpoints and implementation of the troubleshooting strategies will help save researcher's time and money and protect against unnecessary waste of patient samples. These findings will allow researchers to identify predictors of favorable reprogramming outcomes and strategize alternative approaches to improve the chances of reprogramming success. This work was funded by NIH T32 training grant 5T32GM142623.

Program Abstract #190
Stem Cell Therapeutics and Modeling in Amyotrophic Lateral Sclerosis
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Amyotrophic lateral sclerosis (ALS) is a disease rooted in the brain caused by the deterioration of certain structural cells and glia needed to support the established neural network efficiently. Being the most fortified and protected organ in the human body, there are minimal means of reaching it to learn about or treat this disease further, even if it is beneficial. By utilizing stem cells in treatments for ALS, the progression of the disease may be slowed or halted. To reach human usage, trials must be conducted, and stem cells provide a method of stimulation. Organoid creation with induced pluripotent stem cells may provide a path to overcoming chronic cases of ALS because they recapitulate the patient's genetic background, which is invaluable for comprehending the highly heterogeneous nature of ALS. iPSC-based systems allow researchers the opportunity to scrutinize disease progression within a controlled and well-defined environment, providing the means to monitor cellular behavior over time making it the main methodology used in this study. In terms of results, these models enable the efficient screening of candidate drugs, expediting the process of identifying promising therapeutic agents. Preclinical evidence supports the applicability and potential of this strategy as seen while transplanting human oligodendrocyte progenitor cells derived from iPSCs which improved motor function and extended survival in the SOD1 mouse model of ALS. The potential provided by induced pluripotent stem cells is immeasurable. When looking into ALS, they bring up a vast number of theories and opportunities to expand horizons in the medical and research industries. The interaction between glia and the neurodegenerative aspect of ALS can be overcome with iPSCs in possible solutions like drug screening, oligodendrocyte regeneration supported by additional myelination, and TNFa prevention which further existing possible clinical treatments. This work was supported by CCIR.

Program Abstract #191
Context-dependent Interactions Between Immune Cells and Sensory Hair Cell Regeneration Programs
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Tissue regeneration following injury requires the concerted response of multiple cell types, including immune cells and stem cells. The cellular and molecular components of regeneration programs can be affected not only by the severity of the injury, tissue identity, but also the type of cell death. Determining the individual contribution of these parameters has remained challenging, largely due to the variable nature of mechanical injury paradigms. Here, we established a comparative approach of regeneration programs to induce either necrosis or apoptosis in zebrafish lateral line hair cells (HCs), respectively. Keeping the identity and quantity of the ablated cells consistent, this approach allows us to specifically interrogate the influence of the cell death modality on regeneration. High resolution live imaging led us to visualize the rapid recruitment of tissue-resident macrophages to the site of cell death, uncovering intricate differences in their phagocytic behavior depending on the cell death modality. Single-cell RNA sequencing revealed that these cellular differences were accompanied by distinct transcriptional signatures in both phagocytosing macrophages and lateral line support cells. While HC necrosis triggered

a robust injury response in support cells, it was greatly diminished following apoptosis. Despite these differences in the early response to injury, both paradigms eventually converge on similar genes involved in hair cell regeneration. Lastly, blocking recruitment of immune cells using a dominant-negative approach not only increased injury response gene expression but also injury-induced proliferation of support cells in response to apoptosis. In sum, our data provide evidence for distinct molecular and cellular responses to different cell death modalities in a regenerating organ. Taken together, this study provides invaluable insights into the context-dependent nature of regeneration programs. Funding: Stowers Institute for Medical Research

Program Abstract #192
Plasticity of Mantle Cells in the Regeneration of the Zebrafish Lateral Line Following Targeted Ablation
Josie Ward, Jason Meyers
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Mechanosensory cells of the mammalian auditory and vestibular systems do not regenerate after damage, leading to permanent sensory loss. Unlike mammals, sensory hair cells of the zebrafish readily regenerate following injury. Support cells act as the primary progenitor cells during hair cell regeneration, however mantle cells of the lateral line can regenerate sensory hair cells under certain conditions. Although the signaling pathways involved in regeneration from support cells has been extensively studied, less is known about the signals that mediate regeneration from mantle cells. We therefore performed a series of targeted laser ablation experiments combined with pharmacological manipulations to determine how mantle cells regenerate hair cells in the absence of a robust support cell population. We find that mantle cells have the ability to function as progenitor cells and regenerate both support cells and hair cells during regeneration in 3d larvae via a Wnt-dependent process. We next utilized pharmacological manipulations of the Wnt and FGF signaling pathways, both of which have been shown to be involved in hair cell regeneration from supporting cells. In 3d larvae, manipulating the Wnt and FGF signals during regeneration changes the behavior of mantle cells, such that they resemble migratory progenitor cells and crawl away from the original neuromast location. On the other hand, ablation of mantle cells along with support cells prevents neuromast regeneration in 3d larvae. Pharmacological manipulation of interneuromast cells does not initiate regeneration of hair cells, demonstrating the importance of mantle cells in neuromast regeneration. These data suggest that mantle cells have unique regenerative properties and that pharmacological manipulation allows mantle cells to be reprogrammed into a migratory progenitor state, pointing to signaling mechanisms that may be able to alter the plasticity of progenitor cells in hair cell epithelia. Funding: Colgate Univ.

Program Abstract #193
Dissecting the role of the primary cilium in the neural-glial interaction
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Neurons and glia interact with each other via neurotransmitters and extracellular signaling molecules to coordinate function in mature brain. Such interaction is critical to maintain the homeostasis of the central nervous system. However, it is not fully understood how the neurons and glia communicate with each other to coordinate development from the same pool of neural precursor cells. The retina is an architecturally simple part of the central nervous system specialized for vision. The light-sensing rod photoreceptors (retinal neuron) and Müller glia (glia) are the two last-born cell types from the same pool of retinal progenitor cells. Our previous research was focused on a congenital blinding disease caused by compromised biogenesis of the primary cilium, a ubiquitous organelle that harbors receptors for various signaling pathways. We established a retinal organoid model system from induced pluripotent stem cells of patients carrying mutations of a critical ciliary gene *CEP290* to study the disease in a human context. Although the compromised development of photoreceptor primary cilia was the most dramatic phenotype in patient-derived organoids, we surprisingly identified dysregulation of genes responsible for Müller glia cell fate commitment. We therefore hypothesize that the primary cilium mediates the interaction between retinal neurons and glia during retinal development. We will take the first step by determining if the Müller glia pathology is caused by the compromised primary cilia and/or photoreceptor dysfunction. As the photoreceptors and Müller glia closely interact with each other, we have established conditional knockdown human retina models with compromised primary cilia in Müller

glia, photoreceptors, or both cell type to dissect the role of the primary cilium. We are characterizing the development of photoreceptors and Müller glia in these models. This work is supported by Foundation Fighting Blindness.

Program Abstract #194
Neurogenesis and maintenance in the planarian peripheral nervous system
Taylor Medlock-Lanier, Kendall B. Clay, Rachel H. Roberts-Galbraith
University of Georgia, USA
How does successful nervous system regeneration occur? In our group, we look to planarians—freshwater flatworms capable of robust, whole-body regeneration—to study how a nervous system can be created de novo. Elucidating how the entire nervous system regrows can be a daunting task, so we are first focusing on understanding regeneration and homeostasis of neurons that produce dopamine. Our marker for dopaminergic neurons in planarians, tyrosine hydroxylase (th), is expressed in countable cells in both the central nervous system (CNS) and peripheral nervous system (PNS). With the use of available single cell transcriptomic data (Fincher et al 2018), we identified 73 candidate genes with enriched expression in th+ cells. We hypothesized that genes expressed in dopaminergic neurons could be important for their function and/or identity. We observed the expression pattern for each candidate gene through in situ hybridization and compared them to the expression of th. At least 85% of candidate genes are expressed in the nervous system. Through double fluorescent in situ hybridization, we are also confirming co-expression with th. Additionally, we used a reverse genetics screen to identify genes required for regeneration or homeostatic maintenance of dopaminergic neurons. We identified two genes required for regeneration of dopaminergic neurons in the brain and another five that are required for homeostasis and regeneration of dopaminergic neurons in the PNS. We are now characterizing the five transcription factor-encoding genes required for peripheral dopaminergic neurons: soxB1-2, irx-1, irx-2, fli-1, fli-1-like. We are also interested in whether the pathways we discover are conserved, as dopaminergic neurons are clinically relevant with loss of dopaminergic neurons being associated with Parkinson's Disease in humans. This project is funded by the NIH, the NSF, Alfred P. Sloan Foundation, and the McKnight Foundation.

Program Abstract #195
Uterine injury during diestrus leads to placental and embryonic defects in future pregnancies in mice
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The uterus is central to mammalian reproduction and gynecological health, and yet its capacities to regenerate itself and to interact with the embryo remain poorly understood. While the uterus is capable of scarless regeneration each menstrual or estrous cycle, C-sections and other uterine injuries contribute to scarring, resulting in either infertility or disorders like placenta previa and placenta accreta in subsequent pregnancies. With rates of C-section at approximately 30% of deliveries in the US and projected to continue to climb, a deeper understanding of the mechanisms by which these pregnancy disorders arise and opportunities for intervention are needed. Here we describe a rodent model of uterine injury on subsequent *in utero* outcomes. Using this mouse model, we recapitulated several features of human disorders, including implantation failure, previa-like embryo misspacing, and accreta-like overinvasive placentas. Strikingly, only uteri injured during the diestrus phase of the estrous cycle displayed subsequent embryo misspacing and thus embryonic underdevelopment. Using RNA-seq, we identified perturbations in the expression of components of the COX/prostaglandin pathway after recovery from injury, a pathway that has previously been demonstrated to play an important role in embryo spacing. Therefore, we demonstrate that uterine injury engenders a long-term molecular "memory" of damage that ultimately leads to numerous placental and embryonic developmental defects. This work was funded by an NIH grant (NICHD R01 HD094513) to J.C.B., an A.P. Giannini Foundation postdoctoral fellowship to E.T.Z., and an National Science Foundation Graduate Research Fellowship Program (DGE-1656518) to K.L.W.

Program Abstract #197

Investigating Inflammation in Newt Lens Regeneration: A Path to Understanding Chronic Fibrosis

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For decades, scientists have tried to better understand the scar-free injury response known as regeneration. This response is well exemplified by newt lens regeneration. Upon lens removal, dorsal iris pigment epithelial cells (iPECs) reprogram into lens cells. Transcriptomic analysis of irises during the early stages of lens regeneration depicted an inflammatory response program as well as the presence of macrophage markers and extracellular matrix remodeling genes. Previous data showed that early macrophage depletion not only resulted in a failure of lens regeneration, but also significant decrease in iPEC proliferation, induced an unresolved cellular accumulation, prolonged inflammation, and caused a fibrotic-like response, as well as abnormalities in extracellular matrix remodeling. The effect that macrophage depletion has on the inflammatory response led us to hypothesize that macrophages modulate inflammation during lens regeneration. Previous studies have shown that the initial inflammatory response is necessary for regeneration to occur. We used anti-inflammatory drugs FK506 and Celastrol after lentectomy to evaluate the function/importance of inflammation during lens regeneration. Additionally, we aimed to characterize the window of time after injury that inflammation is necessary for regeneration by treating lentectomized newts at various time points post-lentectomy. The excessive fibrosis present in the eye chamber after macrophage depletion in lentectomized eyes highlights this as an excellent model to dissect mechanisms involved in scar-free healing and will provide additional insight into advancing treatments for conditions such as chronic fibrosis and scarring. Funding: NEI R21 EY033916 and EY031865 (KDRT), the John W. Steube Professorship Endowment (KDRT), the Fight for Sight grant (AS), NINDS F99 NS129167 (JT) and Miami University DUOS (SR&JT).

Program Abstract #198

Novel Insights into Cutaneous Wound Healing: Remarkable Capabilities in Dolphins and Whales

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Cutaneous wound healing is a complex process including a series of specific phases: hemostasis, inflammation, proliferation, remodeling, and scar formation. In different species, the skin wound healing process exhibits species-specific variation. Fraser's dolphins (FD, *Lagenodelphis hosei*), in particular, demonstrate an extraordinary full-thickness wound healing capability. The infiltration of inflammatory cells during their inflammation phase is found to be limited. Moreover, the collagen structure in the remodeling phase resembles that in human hypertrophic scar. Instead of forming a mature scar, a skin restoration phase in FD has been identified, which restores the skin architecture nearly identical to that of normal skin, including adipose tissue. Pygmy sperm whales (*Kogia breviceps*) and dwarf sperm whales (*Kogia sima*) also exhibit similar but superior capability of complete skin recovery after full-thickness wounding. The skin architecture restoration in *Kogia* is observed to initiate during the early proliferation phase with adipocyte appearance while adipose tissue restoration would occur after the remodeling phase in FD. The difference could potentially be attributed to the substantial habitat disparity between them since *Kogia* may feed below 400-1000 m in depth and FD feeds below 200-500 m in depth. Collectively, these findings suggest that the skin wound-healing process in dolphins and whales may represent a novel scenario as other terrestrial mammals capable of skin restoration do not develop any scars. This research may shed light on new strategies for veterinary and human regenerative medicine. This work is funded by NTU 112L7805

Program Abstract #199

A multi-species strategy for deciphering the regulation and function of the short stature homeobox (*SHOX*) gene during tetrapod limb development

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Deficiencies of the human *SHOX* homeobox gene cause deformities of the middle segment of the limb, the zeugopod, that are characteristic of Léri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). Deletions of noncoding sequences within the gene desert downstream of the *SHOX* gene are a common cause of the limb defects of LWD and ISS. Since rodents lack the *SHOX* gene, identification of the relevant enhancer sequences in the deleted regions has been hampered by the lack of an animal model. Therefore, we adapted the domestic cat as a model to identify *SHOX* limb enhancers and their human orthologs. The conserved synteny of coding and noncoding elements in the human and cat pseudoautosomal region 1 where *SHOX* resides, and a similar pattern of *SHOX* expression in cat and human embryos support the use of the cat model for studying *SHOX* regulation. Toward this end, we used circular chromosome conformation capture (4C-seq) to identify enhancers regulating *SHOX* expression in cat embryonic limbs. The corresponding human orthologs were identified through sequence conservation and tested for enhancer activity using transgenic mice. Using this strategy, we have identified two previously uncharacterized human enhancers with specific activity in the proximal embryonic limb. Importantly, these enhancers are within an interval approximately 350 kb downstream of the *SHOX* gene that is deleted in a subset of LWD and ISS patients, suggesting that these sequences are critical for proper development of the human limb. The genomic distribution and spatial activity of the identified enhancers are remarkably similar to regulatory elements we have previously identified in the gene desert downstream of the mouse *Shox2* gene. To adapt these studies to a system where gene editing is feasible, we are pursuing the brown anole (*Anolis sagrei*) as a model for studying *SHOX* gene function and regulation. This project was funded by NIH grant R03HD097548 and NSERC Grant RGPIN-2019-04812.

Program Abstract #200

Development of Reproductive Organs in the Brown Anole

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Reproduction modes across vertebrates are diverse. Lizards and snakes have particularly diverse reproductive strategies yet the reproductive organs of squamates have a similar overall architecture. Here, we describe the development of the reproductive organs in one species of lizard, the brown anole (*Anolis sagrei*). Though the embryonic structures that develop into adult reproductive tract organs have been described in reptiles, a comprehensive understanding of morphological changes in these tissues across developmental time is lacking. The brown anole is popular as a research model and CRISPR genome editing techniques that rely on manipulation of the reproductive tract have been developed. Using histology and 3D imaging, we describe morphological changes to the embryonic precursors of the adult reproductive anatomy, the paired Müllerian and Wolffian ducts. In mammals, the reproductive organs develop from these ducts which are present in both sexes; however, Anti-Müllerian Hormone secreted by the male testis causes regression of the Müllerian Ducts, while testosterone facilitates differentiation of the Wolffian ducts into male reproductive tract organs. In females, the Wolffian duct degrades and the Müllerian ducts differentiate into female reproductive tract organs. We also describe preliminary results of the role of Anti-Müllerian Hormone on female reproductive tract development. Our data highlight morphological changes in the brown anole embryo that are unique to this species compared to mammals. Supported by NIH T32 HD098068 and NSF PRFB 2209150.

Program Abstract #201

A Comprehensive Staging Series of the *Trachemys scripta* Turtle

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Background: Turtles hold a unique place in vertebrate evolutionary history, making them valuable research assets in embryology. Despite this, turtle embryos remain understudied, in part due to the scarcity of experimental tools adapted to their use and the paucity of ways to obtain their eggs. Since *Trachemys scripta* is the most common invasive turtle species worldwide, they are more widely accessible than other species. Here, we have created a complete normal table of development to support careful and easy staging of *T. scripta* embryos for experimental manipulations. Results: The development of *T. scripta* embryos from 0 days post-oviposition (DPO) to hatching (~60 DPO) was described from approximately 300 viable eggs collected at California State University, Northridge during the 2021-2024 nesting seasons. Twenty-six stages between oviposition and hatching were identified, and anatomical structures were cataloged using the Standard Event System (SES) chart. Morphological characteristics were imaged using brightfield and DAPI. Conclusion: To facilitate research with Chelonian embryos, this staging series features a photographic, annotated glossary, details previously undescribed embryonic events, includes criteria from the SES, and still generally follows the accepted staging categories used with turtle embryos. Our funding sources include the Loyola Marymount University Rains Research Assistant Program and the Loyola Marymount University Kadner-Pitts grant.

Program Abstract #202

Wing development in the luna moth, *Actias luna*

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Size and shape are the most characteristic attributes of species. Yet, the developmental mechanisms that regulate the final sizes and shapes of appendages are for the most part unknown. Although the genetic and molecular mechanisms that control growth are becoming well-understood, we still lack much fundamental knowledge about how these play out at the organ and organismal level to control the final size and shape of tissues. The wings of Lepidoptera offer a particularly attractive and tractable system in which to investigate the morphogenesis and evolution of an epithelial structure. Species of Lepidoptera differ greatly in the size and shape of their wings, but all have the same venation pattern, consisting of a standard set of homologous veins that make it possible to map homologous locations on the wing throughout development. This makes the wing a particularly suitable system in which to study the interplay between development and evolution in morphogenesis because it is possible to ascribe changes in shape during development to quantitative changes in the growth of specific regions across the entire wing surface. Using a novel model system, the luna moth, *Actias luna*, we trace the development of forewing and hindwing shape during the last larval instar. We will discuss aspects of differential growth and ongoing research directions regarding the developmental genetics of lepidopteran wing shape. We thank High Point University for supporting this work through start up funds.

Program Abstract #203

Development of molecular genetic model of snail development in *Biomphalaria glabrata*

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Mollusks represent a large fraction of invertebrate species, but the molecular genetic regulation of their development remains largely unexplored, given their number and diversity. Brilliant work by many investigators has described unique features of the embryology and biology of snails. Our goal is to develop *Biomphalaria glabrata* as a molecular genetic model to allow us to examine the genetic basis of these unique features. *B glabrata* has many appealing features of a prospective model organism. They are small, very easy to keep and breed in the laboratory, the embryos are accessible and easy to observe without removing them from their protective capsules, and the genome has been sequenced and annotated. They are also an intermediate host for human schistosomiasis and are kept in laboratories around the world. We have refined the husbandry and methodology of this model. We have developed a recirculating culture system allowing intensive feeding and improved spawning rates. We have developed a description of developmental stages. We have developed methods for in vitro culture that support methods for microinjection and lineage tracing. We have optimized in situ hybridization in embryos to allow us to describe the temporal and spatial patterns of expression of mRNAs. Together, these methods will provide a robust tool kit that can be easily adapted to many laboratories for investigating mollusk embryogenesis.

Program Abstract #204

A new genome visualization resource for exploring and visually analyzing RNA-Seq gene expression data for tardigrades (*Hypsibius exemplaris* and *Ramazzottius varieornatus*)

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The tardigrade species *Hypsibius exemplaris* is an emerging model system for understanding the evolution of animal development. Its transparent embryos, rapid generation time, ease of cultivation in laboratory settings, and small genome size make this microscopic invertebrate amenable to genome-scale assays such as RNA-Seq gene expression analysis. Other tardigrade species, including *Ramazzottius varieornatus*, offer models for studying resilience in the face of extreme environmental stresses, such as ionizing radiation. However, advanced genomics visualization resources for tardigrade species are lacking in comparison to other invertebrates important in developmental biology and stress resilience studies. To address this, we developed a new genome browser resource for tardigrades using the Integrated Genome Browser, an open-source genome browser well-suited for visual analysis of functional genomics data sets, especially data from scaffold (incomplete) genome assemblies like those currently available for tardigrades. This new resource contains published RNA-Seq datasets downloaded from public archives as original sequence data and then re-aligned to tardigrade genome assemblies downloaded from Genbank. The re-processed data includes sequence alignments, useful for comparing genetic differences across species, and scaled coverage graphs, useful for observing differentially expressed genes between experimental samples. In addition, researchers can use the browser to visualize their own data alongside the public, re-processed data, making it easier to interpret their work and understand its significance and impact. The Integrated Genome Browser is freely-available from bioviz.org. An award from NIH NIGMS (R35GM139609) provided funding.

Program Abstract #206

Neural Regeneration in a Jellyfish Model System

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Regeneration is a widespread phenomenon across the animal tree of life, ranging from the regeneration of specific structures, to the regeneration of the entire body axes. Regardless of the type of regeneration, a common hurdle that is faced is the need to properly reintegrate newly built structures with the existing body. The majority of animals accomplish the integration of body parts through neural control systems, making neural regeneration fundamental to understanding how animals regenerate. To gain insights into neural regeneration, I am leveraging the simple neural architecture of the hydrozoan jellyfish *Clytia hemisphaerica*. *Clytia* are transparent animals that can regenerate its largely 2-dimensional nervous system using stem cells that reside in specific niches. *Clytia* comes equipped with tools such as transgenesis, RNAi, and whole-animal live imaging. Furthermore, neural networks within *Clytia* such as the RFamide peptide expressing (RFa+) subnetwork has been implicated in food passing behavior, providing a behavioral readout for proper neural reintegration. These attributes make *Clytia* an elegant system where neural regeneration can be dissected start to finish–from the very first divisions of their stem cells, to large-scale patterns of neural activity, to tractable behaviors. I am focusing on the regeneration of the RFa+ subnetwork using a transgenic line expressing mCherry and nitroreductase under the control of RFamide promoter. This allows for the selective ablation of the RFa+ network through the addition of metronidazole. Preliminary live imaging for the RFa+ neurons revealed global patterns of neural addition, with neurogenesis appearing to be elevated upon ablation. Future work includes the measurements of neural activity during regeneraiton, and the identification of molecular regulators for network patterning. Funding: Klingenstein-Simons Foundation and The Picower Institute for Learning and Memory.

Program Abstract #207

Novel Roles of Tubulin Autoregulation in Neurogenesis.

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Tubulin autoregulation is a well-establish phenomenon first discovered in the early 1980s. The mechanism responsible for this phenomenon was only recently elucidated with the finding that the TETRATRICOPEPTIDE REPEAT DOMAIN CONTAINING PROTEIN 5 (TTC5) protein is crucial for mediating this

process. Concurrent with this discovery, three patient cohorts were published identifying variants in *TTC5* that cause severe developmental brain malformations. We hypothesize that these cortical malformations are caused by neurogenesis (neuron development) defects as a result of dysfunctional tubulin autoregulation. To test this, we generated a novel *Ttc5* null mouse model to establish the first *in vivo* examination of tubulin autoregulation. Our model faithfully recapitulates human brain malformation phenotypes including hypoplasia of the corpus callosum and basal ganglia, ventriculomegaly, and hippocampal malformations. Early neurogenesis requires high levels of proliferation to create a large progenitor pool. Subsequent cortical development involves extensive neuronal differentiation and migration to form layers of neurons and the structure of the brain. Mechanistic studies performed on *Ttc5* null mice at both early (E12.5) and late (E14.5) embryonic (E) stages important in neurogenesis have shown decreased proliferation and increased intermediate progenitor cells. These findings support significant defects in early neurogenesis. Surprisingly, migration of neurons is unchanged. Cell fate, however, is altered with an increase in upper cortical neurons supporting defects in neurogenesis. Neurogenesis is reliant on tubulin structures such as the mitotic spindle and cilia. Defects in each lead to cortical defects and *in vitro* studies of *Ttc5* loss show errors in mitosis. Thus, we will examine these structures. This work is funded by a Nationwide Children's Hospital's Office of Trainee Affairs Research Development Grant and Wexner Research Institute recruitment funds.

Program Abstract #208

Altered nerve fiber growth in *Pax6*$^{+/Sey\text{-}Neu}$ mice cornea: cause or consequence of Aniridia Associated Keratopathy?

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Paired box 6 (PAX6) is a key gene for eye development. In mammals, complete loss of Pax6 protein leads to anophthalmia and heterozygous loss-of-function mutations leads to hypoplasia of iris as well as changes in cornea, lens, and retina. In humans, this latter condition is called Aniridia and in rodents it's called small eye. Over 90% of aniridia patients exhibit Aniridia Associated Keratopathy (AAK) leading to corneal scarring, opacity, and neovascularization. Despite several proposed pathophysiological mechanisms, the cellular and molecular changes driving AAK remain poorly understood. This study aims to investigate the hypothesis that corneal nerves play a role in the progression of AAK. As a first test of our hypothesis, we evaluated changes in nerve fiber growth between *Pax6*$^{+/+}$ and *Pax6*$^{+/Sey\text{-}Neu}$ mice corneas. Nerve fibers were visualized by immunofluorescent labeling and confocal microscopy. At birth, *Pax6*$^{+/+}$ and *Pax6*$^{+/Sey\text{-}Neu}$ mice both exhibit thick nerve fiber bundles in cornea. In wild type, by 2 weeks of age, these thick fibers are largely absent from the central cornea and numerous smaller fibers are observed. A stable pattern of innervation is observed by 8 weeks of age. In contrast, in *Pax6*$^{+/Sey\text{-}Neu}$ mice thick nerve fiber bundles are observed past 2 weeks. In these animals, different patterns of nerve fibers correlated with transparent and opaque corneal regions suggesting reorganization of nerve fibers correlates with disease progression. In addition to cornea, we find Pax6 expression in trigeminal ganglion. This leads to the question if Pax6 positive trigeminal ganglion is responsible for AAK phenotypes or if the changes observed in the nerve fiber pattern is a consequence of changes in corneal microenvironment or both. This project is funded by Children's Glaucoma foundation, Vision for Tomorrow Foundation, Fight for Sight.

Program Abstract #209

Comprehensive characterization of a 2D model of human whole eye development

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The human eye develops from the stepwise generation of specialized tissues derived from different primordial cell lineages, and disruption at any stage can result in a spectrum of ocular disorders.

Currently, human ocular cell cultures commonly used for disease modeling recapitulate later stages of specialized tissue differentiation, requiring the development of new models to test genetic variants affecting earlier embryonic time points. To overcome this, we are culturing human induced pluripotent stem cells to form multiple ocular cell types organized into four identifiable, concentric zones, known as self-formed ectodermal autonomous multi-zone of ocular cells (SEAMs; Hayashi et al., 2016). Each zone consists of specific cell types found in the eye and zone formation occurs gradually, mimicking the progression of human eye development. Despite standard conditions, individual SEAMs show variability that warrants careful characterization to determine reproducibility and establish a comprehensive timeline of SEAM formation. Here we provide a thorough evaluation of SEAM formation at three time points (Day 0; 14; 28) of a 28-day differentiation process, including measurements of morphological changes (cell density, zone thickness, overall size) and changes in protein expression visualized via immunocytochemistry. scRNA-seq profiling of >8500 cells from the same cultures identified >50 cell subtypes and the factors driving their developmental progression as visualized in pseudotime. Moreover, bulk RNA-seq analysis at the same time points delineated factors driving the overall progression, with 209 differentially expressed transcription factors at Day 14 and 279 at Day 28, including MEIS1/2, TP63, HES5, FOXG1, PITX2, FOXC1 and SIX3. Robust characterization of SEAM generation provides a system for determining how potentially disease-causing variants might disrupt early stages of eye development leading to more complex ocular phenotypes. NIH R21EY035121; R01EY015518.

Program Abstract #211

Developmental roles of *Nr2f1* and *Nr2f2* in the vertebrate cranial neural crest

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NR2F1 and *NR2F2* are members of a highly conserved nuclear receptor family. Heterozygous variants in both genes cause human disease. While the primary phenotypes do not overlap, mild facial dysmorphism is present in both patient groups. This, along with published epigenetic characterization of in vitro-derived human neural crest and our previously published zebrafish studies, suggests a redundant requirement for the *NR2Fs* in cranial neural crest cell (cNCC) development. We hypothesized a requirement for early cNCC survival in mice. Conditional *Nr2f1/2* mutant (cKO) perinatal mice, ablated with the cNC driver *Wnt1-Cre*, lose most of the facial skeleton. Their faces are cleft, with greatly reduced frontal, upper jaw, and palatal bones. Oddly, the mandible is largely unaffected. The earliest gross sign of the phenotype was an absence of the cNCCs migrating into the maxillary prominence at 16 somites (E9.0). Apoptosis analysis showed clear increases in cNCC and neural tube cell death starting at 8 somites (E8.5). No abnormal apoptosis was noted at 5 somites (E8.25). Investigating the preservation of the mandible, we hypothesized that *Wnt1-Cre* activated too late to affect the early-migrating mandibular crest. To test, we created cKOs with the early neural plate border gene *Pax3-Cre*. Surprisingly, the mandible was still preserved in *Pax3-Cre* cKOs despite a greater general loss of facial skeleton. Partial cKO analysis suggests *Nr2f2* is more crucial to the cNC, but while a reexamination of expression confirmed presence of NR2F1 at specification stages, NR2F2 was not noticeable until after the first mass migration at 5 somites. Thus, the earliest crest may not require the *Nr2fs*, explaining mandible preservation. We are currently examining proliferation and developmental markers of ablated crest, using bulk RNAseq and WMISH to discover downstream genes, and comparing timing of Cre activation and NR2F degradation in *Wnt1-* and *Pax3-Cre*. NIDCR 5R00DE026239 (LB) F31DE032261 (DP)

Program Abstract #212

Regulation of human neural crest cell formation by the Mowat-Wilson syndrome factor ZEB2

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Neural crest cells contribute to a large array of cell types throughout the body and are associated with numerous human pathologies. Mowat-Wilson syndrome is caused by de novo heterozygous mutations in the transcription factor ZEB2, with patients exhibiting characteristics indicative of neural crest defects including distinct craniofacial anomalies, heart defects, and Hirschsprung's disease. Using a model of human neural crest cells based on human pluripotent stem cells, we examined how mutations in ZEB2 affect the human neural crest cell lineage. Transcriptomic and chromatin accessibility analyses reveal

dynamic perturbations in the neural crest gene regulatory network and epigenetic landscape, and support a primary function for ZEB2 as a transcriptional repressor during human NC cell formation. Crucially, our work supports a role for ZEB2 in modulating the proper levels and transcriptional output of BMP signaling. Together, our findings elaborate on the molecular defects underlying Mowat-Wilson syndrome. This work is funded by K99DE029878 to R.M.C.

Program Abstract #213

Zebrafish models of Immunodeficiency Centromere Instability and Facial Anomalies Syndrome (ICF)

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DNA methylation plays a crucial role in vertebrate development as an essential epigenetic modification. It is particularly enriched at the repetitive satellite sequences that flank chromosome centromeres. Loss of methylation at these pericentromeric repeats is common in cancer and is a hallmark of the rare disease Immunodeficiency, Centromeric Instability, and Facial Anomalies (ICF) Syndrome. Patients with ICF syndrome typically also have low serum immunoglobulin levels, facial dysmorphism, and stunted growth. However, the connection between pericentromeric hypomethylation and disease phenotypes remains unclear. This study introduces two zebrafish models of ICF syndrome, created through CRISPR/Cas9 deletion of the zebrafish orthologs of known human ICF causative genes *ZBTB24* and *CDCA7*. Using our zebrafish models, we find that homozygous loss of either gene results in extensive loss of DNA methylation at pericentromeric regions similar to that observed in human ICF patients. However, only deletion of zebrafish *zbtb24* mirrors other features of ICF syndrome. This suggests that many phenotypic hallmarks of ICF syndrome may not be caused by the observed methylation loss. Mechanistically, we find that Zbtb24 is required for the expression of *cdca7*, suggesting that Zbtb24 may primarily function as a transcription factor. We hypothesize that Zbtb24 regulation of additional genes may drive developmental phenotypes in ICF patients and zebrafish with mutations in this gene. These findings may help explain why ICF cases with underlying mutations in *CDCA7* are relatively rare (~ 4% of all cases), as these cases may escape clinical detection. This work was funded by American Cancer Society Grant RSG-17-007 to MG.

Program Abstract #215

The human ciliopathy protein RSG1/CPLANE2 links the CPLANE complex to transition zone assembly

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Cilia are microtubule-based projections essential for development and homeostasis of nearly every organ system in the vertebrate body. The dysfunction of cilia in mammals leads to various diseases, known as ciliopathies. Ciliogenesis, the process of cilia formation, is a strict spatiotemporal process that requires dozens of proteins for its regulation. During ciliogenesis, basal bodies apically emerge from the cytoplasm to dock at the plasma membrane and the transition zone is established. Previously, we discovered that the Ciliogenesis and Planar polarity Effector (CPLANE) protein complex is required for ciliogenesis. Disruption of the small GTPase Rsg1/CPLANE2, a subunit of the CPLANE complex, leads to failed ciliary formation and a basal body docking phenotype. In this study, we have identified the first human allelic variants of Rsg1/CPLANE2 associated with ciliopathies. The human patients present with similar orofacial anomalies and polydactyly previously reported in other subunits of CPLANE. Through *in vivo* cell biology we have identified the etiology of these allele variants. With a proteomic approach, we also identified novel Rsg1/CPLANE2 interactors, Chibby1, Dzip1, and Fam92a. While Chibby1 and Dzip1 are components of the transition zone and regulate basal body docking at the plasma membrane, little is known about the role of Fam92a in ciliogenesis. We delineate the role of Rsg1/CPLANE2 in the basal body recruitment of transition zone proteins further linking CPLANE in ciliary processes. This work will expand our understanding of the mechanisms involved in rare genetic diseases detrimental to human health. NIH: F31DE033290

Program Abstract #216

Ift81 loss impacts early-stage embryonic development in mice

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Ciliopathies are a class of multisystemic genetic disorders that are characterized by mutations in cilia-

related genes. While there is much phenotypic heterogeneity seen in ciliopathies, some common features include skeletal dysplasias, renal disease, heterotaxy, and cerebral anomalies. Defects in the cilia may arise due to problems within the intraflagellar transport (IFT) complex, which is made up of two subcomplexes, IFT-A and IFT-B. Mutations in the *IFT81* gene, which is a member of the IFT-B subcomplex, have been reported to cause skeletal dysplasias such as short-rib polydactyly syndrome (SRPS) in humans with varying degrees of disease severity having been observed, depending on the specific mutation. This study seeks to dissect the mechanism by which mutations in *IFT81* result in SRPS phenotypes. Preliminary studies using direct characterization of founder, or "F0", mouse embryos corroborate the role of *Ift81* in SRPS where embryos highly edited with frameshifting indels (null) alleles display SRPS-relevant phenotypes. Further investigation of germline null alleles of *Ift81* in mice revealed early embryonic lethality with multiple developmental defects including developmental delay, neural tube defects, and left-right asymmetry abnormalities such as reversed heart looping and embryonic turning. The aim of this study is to characterize the phenotypes observed in early embryos when *Ift81* expression is disrupted, and to investigate the effects of *Ift81* loss on normal cilia formation and function. These studies promise to shed light on *Ift81* in normal development and disease pathogenesis. This work is supported by JAX Center for Precision Genetics (U54 OD030187)

Program Abstract #217
***cebra* Controls Cilia Function**
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The emergence of complex three-dimensional body and organ morphologies through embryonic development and growth is not well understood. In zebrafish, the initially curved embryo detaches from the yolk and straightens as the body elongates. Axial straightening is an essential morphogenetic event that generates the linear body axis characteristic of vertebrates and is a tractable system for understanding how specific morphologies arise. The beating of motile cilia in the central canal of the neural tube is crucial for axial straightening: mutants lacking cilia motility show failure of straightening and instead exhibit a curved body axis called "curly tail down". Additionally, cilia motility mutants that survive to adult stages exhibit body morphologies similar to human idiopathic scoliosis. Moreover, motile cilia play roles in left-right patterning of the organs, kidney function and olfaction. We recently isolated a spontaneously-occurring zebrafish mutation with a recessive inheritance pattern that exhibits curly tail down. Because of the C-shaped body axis, we named this mutant cebra. We found that cebra mutants disrupt cilia motility in the central canal, explaining the curly tail down, in addition to disrupting olfactory cilia motility. However, other tissues harboring motile cilia were unaffected, including the cilia within Kupffer's vesicle that control left-right patterning. In agreement, organ asymmetry was normal in cebra mutants. Thus, the impact of cebra on cilia function is context-dependent. Using an RNA-sequencing-based mapping tool, I demonstrate that the cebra mutation maps to a region of Chromosome 5 which harbors several candidate genes that I am currently screening using CRISPR. Overall, my results show that the cebra controls cilia motility in a tissue-specific manner and establishes a new axial morphogenesis gene that normally ensures the linear body axis forms during development. Funding sources: 1T32GM149387 R35GM142949

Program Abstract #218
Assessing genes associated with heterotaxy phenotypes in *X. tropicalis*
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Heterotaxy is a congenital condition in which the development of the left-right axis is disrupted, leading to internal organs which are abnormally formed, arranged, or absent. It is associated with significant physiological problems, such as issues with the immune system, the stomach, the intestines, the lungs, and/or cardiac abnormalities. Heterotaxy is conservatively estimated to affect 1 in 10,000 people worldwide, and accounts for ~3% of congenital heart disease (CHD). Congenital heart disease is the leading cause of mortality from birth defects and is found in ~1% of live births. It is suspected that there is a genetic component to CHD, but there are few confirmed causal genes. To study the genetic causes of heterotaxy, a list of candidate genes was compiled by identifying patients with heterotaxy phenotypes

and novel mutations from studies performed by two programs, the Pediatric Cardiac Genomics Consortium (PCGC) and the Pediatric Genomics Discovery Program (PGDP). These genes were then targeted at multiple loci in X. tropicalis embryos using CRISPR-Cas9 microinjection, with embryos subsequently scored for abnormal heart-looping patterns. Thus far, 11% of the screened genes have exceeded our threshold for affecting left-right patterning (>10% affected), with an additional 16% of genes displaying moderate phenotypes (>6% affected). Embryos treated for genes that displayed a strong phenotype will undergo whole-mount in situ hybridization to determine the expression of pitx2, a transcription factor involved in the genetic cascade controlling left-right patterning. The results of this screen indicate that approximately 27% of genes from the candidate list are worth further investigation as to how their roles affect left-right patterning. Further characterization of these positive genes will allow for a more complete understanding of the mechanisms that drive left-right patterning. This work is supported by the National Institutes of Health (1R01HD102186).

Program Abstract #219

Genetic Insights into Heterotaxy: The Role of CAD in LR Patterning and Cardiac Dysfunction

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Heterotaxy (Htx) constitutes malformations characterized by abnormal left-right (LR) axial determination, resulting in compromised heart architecture and severe cardiac dysfunction. Despite its primarily genetic origin, the majority of causal genes of Htx remain elusive. Exome sequencing from the Pediatric Cardiac Genomics Consortium (PCGC) and the Pediatric Genomics Discovery Program (PGDP) revealed de novo mutations in CAD (Carbamoyl-phosphate synthetase 2, Aspartate transcarbamoylase, and Dihydroorotase) in three individuals with Htx and structural heart malformations. CAD, a multifunctional protein crucial for the initial stages of pyrimidine nucleotide synthesis, plays a pivotal role in the metabolic system essential for cell development and proliferation across species. We used CRISPR/Cas-9 and Morpholino (MO) gene knockdown to reduce CAD expression in Xenopus tropicalis tadpoles, resulting in abnormal left-to-right patterning. Notably, abnormal cardiac looping was observed, with CRISPR and MO depleted tadpoles exhibiting 7-14% defective heart looping. A marker of global left-right patterning, pitx2c, displayed 11% defects in CRISPR and MO depleted embryos. Additionally, epidermal cilia driven flow across the surface of CAD depleted tadpoles was disrupted, supporting an association between CAD, cardiac malformation, and Htx, potentially through ciliary defects. This integrated approach provides a comprehensive understanding of the molecular interplay between CAD, cilia, and LR patterning in cardiac malformations. This work is supported by NIH R01HD102186

Program Abstract #220

***Syntaxin-binding protein 5* (*STXBP5*) is a candidate gene for germ layer specification, left-right patterning, and heterotaxy**

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About 1% of live births involve congenital heart disease (CHD), which is among the leading causes of infant mortality. A major cause of CHD is improper patterning of the left-right axis, a condition known as heterotaxy. Heterotaxy patients often require corrective surgeries as children and organ transplants as adults. Unfortunately, the genetic basis of this disease in most patients is still unknown. A recent whole exome sequencing study identified *syntaxin-binding protein 5* (*STXBP5*) as a candidate gene for heterotaxy. *STXBP5* interacts with the SNARE complex, regulating membrane fusion and exocytosis in processes such as the platelet reaction and neurotransmitter release. However, little is known about its role in embryonic development. Here, we investigate the role of *STXBP5* in left-right patterning and heterotaxy using *Xenopus tropicalis*. Knockdown (KD) of *STXBP5* in embryos using CRISPR-Cas9 leads to a cardiac looping phenotype consistent with heterotaxy. Using *in situ* hybridization, we observed improper expression of two key genes involved in left-right patterning, *dand5* and *pitx2*, in *STXBP5* KD mutants. Abnormal *dand5* expression prior to symmetry-breaking events at the left-right organizer as well as gastrulation defects in mutants suggested that *STXBP5* was acting at an earlier time point. We observed improper expression of *brachyury* in KD mutants, indicating irregular specification of the mesoderm. Interestingly, mesodermal specification and gastrulation defects in *STXBP5* KD mutants can be rescued with *smad2* overexpression. Given the involvement of *smad2* in *nodal* signaling and the necessity of

nodal for proper mesodermal specification and left-right patterning, we hypothesize that *STXBP5* is regulating the secretion of *nodal* signaling molecules. Ultimately, our findings highlight *STXBP5* as a candidate gene for heterotaxy and reveal a novel requirement for its role in germ layer specification. This work is funded by NIH RO1HD102186 to MKK.

Program Abstract #221

Xenbase: latest support for genomics and disease models.

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Xenbase (www.xenbase.org) supports biomedical, developmental and cell biology research using *Xenopus laevis* and *Xenopus tropicalis*, the African and Western clawed frogs. Xenbase is the central repository for *Xenopus* genetics and genomics data and provides researchers with bioinformatic resources and tools for complex analysis. Our mission is to 1) provide the latest genomes linked to genes and orthologs; 2) curate published research/literature for disease models, experimental phenotypes, and gene expression; 3) annotate *Xenopus* genes with GO terms (molecular functions, biological processes and cellular components); and 4) collate diverse genomics data from high throughput sequencing in a central, searchable, database. *Xenopus* genomes and *Xenopus* gene-to-human gene ortholog mappings (frequently used in GO enrichment analysis) are available from our download site. The backbone of *Xenopus* gene expression curation is the *Xenopus* Anatomy Ontology (XAO) and phenotype curation uses the *Xenopus* Phenotype Ontology (XPO), linking *Xenopus* disease models to the Disease Ontology (DO) when appropriate. In addition, Xenbase has recently expanded our education resources including an anatomy atlas, normal tables of development, staging landmarks, marker genes, and a set of open access illustrations of embryonic development. Aggregating all of this information in an easy to use and free to access web portal, Xenbase effectively connects *Xenopus* genes and phenotypes to human genes and diseases via multiple data resources including Monarch and the Alliance of Genome Resources (AGR). Here we provide an overview of Xenbase resources, tools, curated Xenopus data, and data interconnectivity with a special focus on disease models and phenotypes. Xenbase is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH).

Program Abstract #222

Mutations in *wnt3a* result in caudal scoliosis-like phenotypes in zebrafish

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The spinal deformity, scoliosis, affects about 3% of the pediatric population worldwide. We are conducting a screen for ENU-generated adult-viable zebrafish recessive phenotypes that resemble scoliosis to develop models of congenital or adolescent idiopathic scoliosis. We found four classes of scoliosis-like mutations 1) multiple body bends 2) dwarfs (fish that are almost normal size but shorter from head to tail) 3) scoliosis that affects the anterior body and 4) scoliosis that affects the caudal region. We mapped a caudal-class mutant to Chromosome 2, carrying a nonsense allele of *wnt3a*^{stl321}. We created new alleles of *wnt3a* with frameshift/STOP mutations near the defect in *wnt3a*^{stl321} (*stl995*, *stl997*). Similar to published *wnt3a* morpholino phenotypes (Thorpe 2005, Shimizu 2005), *wnt3a* alleles (*stl321*, *stl995*, transheterozygote *stl321xstl997*) exhibited tail tip defects at 1-2dpf; the tail tip appeared pinched, notochord shortened, central canal swollen, and some had increased blood islands. Adult *wnt3a* mutants for all three alleles manifest caudal scoliosis with variable penetrance and expressivity. We conclude these *wnt3a* alleles are causative for larval caudal tail malformations and adult scoliosis. By 21dpf, mutant larvae display malformed caudal vertebrae. The *stl321* mutants have fewer caudal vertebrae, while *stl997* mutants have a wild-type number of caudal vertebrae. We are using qRT-PCR to assess *wnt3a* RNA levels of each allele, and Wnt3a target *sp5l*, (Thorpe et.al., 2005) to assess Wnt3a activity in the mutants. Tail defects may result from an early mispatterning of the tailbud, or may result from a continued requirement of Wnt3a during spine development. To test this, we injected morpholino against *wnt3a* into WT embryos causing the early tail phenotype. Morpholino will not persist until caudal

bones form, so we will grow them to adulthood to learn if later function of Wnt3a rescues or reduces tail phenotypes. Funding: PO1-HD084387, U2CNS132415 to LSK

Program Abstract #223
Role of the *bbs2* gene in development of a straight body axis
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Scoliosis is an atypical curvature of the spine, and it is the most common musculoskeletal disorder affecting children worldwide. From a forward genetic screen for scoliosis in zebrafish, we isolated an intronic splice blocking mutation, *stl438*, in the *bbs2* gene which causes late-onset whole body scoliosis. Bbs2 is a member of the BBSome multimeric complex, which participates in ciliogenesis and ciliary transport. Previous studies have shown that disruption of cilia function in the spinal canal and brain ventricles is associated with scoliosis in zebrafish. *bbs2*^{stl438} mutants show defects of spinal canal cilia beating including increased beat frequency with reduced amplitude. At the same time, we observe a reduction of cilia in the choroid plexus epithelium in the brain. The choroid plexus produces cerebrospinal fluid that flows through the brain-spinal canal axis. Previous studies show that loss of the spinal canal resident protein rich Reissner fiber correlates with the onset of body curvatures. However, in some *bbs2*^{stl438} mutants, we observe the onset of scoliosis which precedes Reissner fiber loss. The Reissner fiber moves continuously in a rostral to caudal direction from the brain through the spinal canal (70 ± 24 nm/sec). Interestingly, in *bbs2*^{stl438} mutants we observed the Reissner Fiber moving much faster (~257 nm/sec) and exhibit ectopic accumulation of Reissner fiber material in the spinal canal, suggesting that the dynamics and the production of Reissner fiber may be altered prior to scoliosis onset. In conclusion, this work reframes our understanding of Reissner fiber and how it regulates spine stability and implicates a role for Bbs2 dependent cilia function in choroid plexus physiology. This work was supported by the NIH-NIAMS: P01HD084387.

Program Abstract #224
Serotonergic Control of Zebrafish Spine Morphology
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Scoliosis is characterized by the development of three-dimensional spinal curves and many cases arise without a known cause. We use zebrafish to model potential causes of scoliosis and determine mechanisms through which the spine normally takes shape. Serotonin is a neurotransmitter and hormone with functions as diverse as behavior control, bone mass accrual and bowel movement. Previous studies using chickens, salmon, and bipedalized rats have linked the removal of the pineal gland, a major source of serotonin, to the development of scoliosis. Moreover, an association study in humans showed that some scoliosis patients have lower levels of serotonin. However, direct evidence for a role of serotonin in spine morphology is lacking. Tryptophan hydroxylase 2 (tph2) is an enzyme essential for serotonin production. We demonstrate that zebrafish with a *tph2* loss-of-function mutation develop significant spinal curves. Using micro-computed tomography imaging at various time points, we determined that these mutants develop spinal curves by late larval stages and have vertebral fusions at the site of curvature by 1 year old. Together, these results suggest that Tph2 is essential for zebrafish spine morphology and that mutants model a scoliosis-like condition. Overall, this demonstrates that serotonin does control spine shape and also provides a tractable system with which to discover the underlying mechanisms of serotonin function in spine morphology. The funding sources for this project are: 5T32HD007348, R35GM142949 and R00AR070905.

Program Abstract #225
Deletion of *Pax1* scoliosis-associated regulatory elements leads to a female-biased tail abnormality
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Adolescent idiopathic scoliosis (AIS), a sideways curvature of the spine, is sexually dimorphic, with increased incidence in females. A prior GWAS identified a female-specific AIS susceptibility locus near the *PAX1* gene. Here, we used mouse enhancer assays, three mouse enhancer knockouts and subsequent

phenotypic analyses to characterize this region. Using mouse enhancer assays, we characterized a sequence, PEC7, that overlaps the AIS-associated variant, and found it to be active in the tail tip and intervertebral disc. Removal of PEC7 or Xe1, a known sclerotome enhancer nearby, and deletion of both sequences led to a kinky phenotype only in the Xe1 and combined (Xe1+PEC7) knockouts, with only the latter showing a female sex dimorphic phenotype. Extensive phenotypic characterization of these mouse lines implicated several differentially expressed genes and estrogen signaling in the sex dimorphic bias. In summary, our work functionally characterizes an AIS-associated locus and dissects the mechanism for its sexual dimorphism. This work was supported in part by the National Institute of Child Health and Human Development (NICHD) grant number 1P01HD084387.

Program Abstract #226

The evolutionarily conserved protein interaction network, *BrainMAP*, identifies novel interactors of INPPL1 important for skeletal development and disorders.

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Opsismodysplasia (OPS) is a rare endochondral bone disorder characterized by congenital dwarfism, growth plate defects, and bone malformations. Recessive mutations in *inositol polyphosphate phosphatase-like 1* (*INPPL1*) cause OPS; however, the molecular mechanisms underlying this disorder are largely uncharacterized. We previously identified adult-viable, recessive mutations in zebrafish *inppl1a* that truncate the catalytic domain of the protein, modeling several of the known OPS-causing mutations and phenotypes. Importantly, mutations in *INPPL1* only explain ~60% of overall OPS cases, suggesting that other genes may contribute to the disorder. In addition to the catalytic domain, INPPL1 also contains several protein-protein interaction domains, including SH2, proline-rich, and SAM domains. We hypothesize that INPPL1 participates in protein complexes that are important for its function in endochondral bone development. To identify candidate interactors of INPPL1, we turned to our collaborators who have developed a network of the evolutionarily conserved protein-protein interactions in the vertebrate brain, *BrainMAP*. Here, we report a systematic reverse-genetic approach to functionally validate protein interactors of INPPL1 that were first observed in *BrainMAP* and were also independently supported by AlphaFold Multimer structure validation. Specifically, we used CRISPR-Cas9 to induce deficiencies or indels in candidate genes and screened for skeletal defects in F0 crispants and F2 homozygous mutants, in which at least 3 of 5 candidates displayed phenotypes. In conclusion, this work supports the validity of *BrainMAP* for identifying protein-protein interactions important for cellular processes outside of the brain and may also lead to a deeper understanding of genes important for endochondral bone development and disorders, like OPS. This study was supported by NIH-NIAMS R01AR072009, P01HD084387 (RSG), NIH-NIGMS R35GM122480-08 (EM), and NIH-NICHD F31HD114419-01 (BV).

Program Abstract #227

***twist1* genes play overlapping roles in promoting forelimb outgrowth in zebrafish**

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Twist1, a basic helix-loop-helix (bHLH) transcription factor, is essential for normal limb development. Mutations in the human *TWIST1* gene cause Saethre-Chotzen syndrome, which can include limb defects as well as craniosynostosis, and mouse embryos lacking *Twist1* display stunted forelimb growth together with failure of cranial neural tube closure and defects in head mesenchyme. Previous studies in zebrafish have examined the roles of *twist1* genes in cranial suture and cranial neural crest development but have not uncovered roles of *twist1* in pectoral fin formation. Here, using transcriptless mutant alleles of *twist1a* and *twist1b*, we find stunted pectoral fins in *twist1a* mutants and no pectoral fin outgrowth in *twist1a;twist1b* double mutants. These phenotypes may reflect defects in the specification of the pectoral fin field, since we observe less *tbx5*-expressing fin mesenchyme in both *twist1a* mutants and *twist1a;twist1b* double mutants. In addition, in both *twist1a* mutants and *twist1a;twist1b* double mutants, the pectoral fin mesenchyme fails to aggregate normally into a compact and defined fin bud. We also observe that *twist1a* mutants have reduced expression of apical ectodermal ridge (AER) markers, while *twist1a;twist1b* mutants exhibit more dramatic reduction or even loss of AER marker expression.

Accordingly, we note that *twist1a;twist1b* mutants lack morphologically distinct AERs, while *twist1a* mutants have abnormally thin AERs. These AER defects could be attributed to a disruption in FGF10-mediated signalling in the underlying fin mesenchyme: we find that *twist1a* mutants have reduced *fgf10* expression, and that *fgf10* expression is more severely reduced in *twist1a;twist1b* mutants. Together, our data reveal novel phenotypes in *twist1* mutants and demonstrate that zebrafish *twist1* genes play overlapping roles that influence both the mesenchyme and AER of the developing forelimb.

Program Abstract #228

Uncovering Mechanisms Underlying Capillary Maldevelopment Caused By *FOXF1* Mutations Using A 3D In Vitro Vascular Model System

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Foxhead box F1 (FOXF1) is a transcription factor that is indispensable for proper pulmonary alveologenesis. Mutations in the FOXF1 gene cause a rare and severe congenital lung disorder called Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV). ACDMPV is characterized by vasculo-pulmonary growth defects including immature lobular development and a sizeable decrease in the number of capillaries in alveolar walls. Individuals with ACDMPV typically die within the first few weeks of life due to respiratory failure. At present, it is unclear how mutations in FOXF1 cause alveolar capillary malformations that ultimately lead to abnormal alveologenesis in humans. The lack of therapeutic or pharmaceutical interventions for ACDMPV is perpetuated by this gap in knowledge. Here, we report the derivation of vessel organoids (VOs) with iPSCs generated from ACDMPV patients with different FOXF1 mutations (ACD-VOs). Differentiating ACD-VOs exhibited clear developmental aberrations and altered FOXF1 expression. Also, ACD-VOs displayed distinct vascular defects such as a significant reduction in endothelial cell population, poorly formed vascular networks that lack lumen structures, and heightened levels of hypoxia, akin to phenotypes observed in ACDMPV patients. 3D gastruloids formed from ACD-iPSCs were less elongated compared to controls suggesting impaired symmetry breakage and expression of key mesodermal markers was significantly reduced. Hence, FOXF1 mutation clearly resulted in dysregulated mesodermal differentiation, thereby disrupting proper cellular patterning. By employing FOXF1 CUT&RUN at various stages of VO differentiation, we were able to discern molecular mechanisms underlying capillary maldevelopment caused by mutant FOXF1 proteins. Overall, we present a strategic approach to carefully decipher the role of FOXF1 in capillary development. N.P. is funded by the American Heart Association Pre-doctoral Fellowship.

Program Abstract #229

Regulation of endocytic trafficking and VEGFR2 receptor availability by a component of the microtubule motor dynein

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Dynein cytoplasmic 1 light intermediate chain 1 (LIC1, *DYNC1LI1*) is a core subunit of the dynein motor

complex. The LIC1 subunit also interacts with various cargo adaptors to regulate Rab-mediated endosomal recycling and lysosomal degradation. Defects in this gene are predicted to alter dynein motor function, Rab binding capabilities, and cytoplasmic cargo trafficking. Here, we have identified a *dync1li1* zebrafish mutant, harboring a premature stop codon at the exon 12/13 splice acceptor site, that displays increased angiogenesis. *In vitro*, LIC1-deficient human endothelial cells display increases in cell surface levels of the pro-angiogenic receptor VEGFR2, SRC phosphorylation, and Rab11-mediated endosomal recycling. *In vivo*, endothelial-specific expression of constitutively active *Rab11a* leads to excessive angiogenesis, similar to the *dync1li1* mutants. Increased angiogenesis is also evident in zebrafish harboring mutations in *rilpl1/2*, the adaptor proteins that promote Rab docking to Lic1 to mediate lysosomal targeting. These findings suggest that LIC1 and the Rab-adaptor proteins RILPL1 and 2 restrict angiogenesis by promoting degradation of VEGFR2-containing recycling endosomes. Disruption of LIC1- and RILPL1/2-mediated lysosomal targeting increases Rab11-mediated recycling endosome activity, promoting excessive SRC signaling and angiogenesis. Funding: NIH/NIGMS R35GM137976 (A.N.S.); American Heart Association Predoctoral Fellowship (D.J.)

Program Abstract #230

Myofibril size is proportionate to Mylpf dosage in zebrafish fast-twitch muscle

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Muscle growth requires the assembly of cellular components into contractile chains called myofibrils. While several factors are known to increase muscle cell size, little is known about how myofibril size is controlled. Our present work indicates that Myosin Light Chain Phosphorylatable Fast (Mylpf) regulates myofibril assembly and growth in zebrafish fast-twitch muscle, which is the sole cell type that expresses the zebrafish orthologs (*mylpfa* and *mylpfb*). Mylpf function is not only necessary and sufficient for myofibril growth, but myofibril growth rate is also proportionate to the amount of Mylpf protein. In the zebrafish *mylpfa* mutant, myofibrils are narrowed even though sarcomeric proteins are produced at normal levels and slow-twitch fibers are spared. In *mylpfa;mylpfb* double mutant, fast-twitch myofibrils and sarcomere structures are completely absent. In the double mutant, myosin heavy chain (MyHC) localizes inappropriately to the central cytoplasm, while F-Actin is correctly localized to the site of myofibril formation at the cell's periphery, suggesting that Mylpf may control myofibril growth via MyHC localization. We made a *mylpfa:mylpfa-GFP* transgene and show that Mylpfa-GFP expression is localized to myofibrils and linearly correlated with myofibril growth. This transgene restores myofibril width in the *mylpfa* mutant and increases the width in the wild-type sibling. Transgenic expression of human MYLPF-GFP also causes a similar myofibril increase, indicating functional conservation. However, a Distal Arthrogryposis (DA) associated allele of human MYLPF (c.470G>T, p.C157F) localizes poorly to the zebrafish myofibril, impairs myofibril growth in the wild-type sibling, and only partially restores myofibrils in the *mylpfa* mutant. These findings offer insights into why the MYLPF variant causes DA and how fast-twitch skeletal muscle cells control myofibril growth. Supported by NIH grants R15AR081019 and COBRE 1P20GM144265-01A1.

Program Abstract #231

Using *Drosophila* to investigate interactions between mammalian regulators of apoptosis, Bax and Bcl-xL, and the mitochondrial porin, VDAC

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Major regulators of apoptosis in mammalian cells are the Bcl-2 family proteins. These include the pro-apoptotic Bax and the anti-apoptotic Bcl-2 and Bcl-xL proteins. When activated, Bax forms complexes on the mitochondrial outer membrane, causing the release of Cytochrome c, triggering apoptosis. Bcl-2 and Bcl-xL inhibit apoptosis by binding to Bax and blocking its activity. The activities of Bax and other Bcl-2 family proteins also influenced by the mitochondrial porin called Voltage-dependent ion channel (VDAC) found in the mitochondrial outer membrane; however, the nature of their interactions is poorly understood. Murine Bax is a potent inducer of cell death when expressed in *Drosophila*. Expression of Bax in wing or eye induces cell death, causing loss of wing tissue and rough eye phenotype. We are

developing *Drosophila* as a model system for investigating interactions of mammalian Bcl-2 family members and VDAC. We show that co-expression human Bcl-xL with Bax completely blocks Bax activity, restoring normal wing development. We constructed transgenic lines expressing mouse *Vdac1* and *Vdac2*. We observe that *Vdac1* can rescue the lethal phenotype of mutant *Drosophila* VDAC ortholog *porin*. Using these phenotypes as an output we are investigating the interactions between Bax, Bcl-xL, and VDAC. We are currently testing whether observed similarities in amino acid sequence between BH3 domains in Bcl-2 family proteins and the N-terminal domain of VDAC are sites of protein interactions that regulate apoptosis. This research was funded by NIH Grant 1R15GM134456-01A1

Program Abstract #232

Elucidating the molecular mechanism regulating *Alx1* expression and function during craniofacial development

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Disruption of *Aristaless-Like Homeobox-1* (*ALX1*), encoding a homeodomain-containing transcription factor highly specifically expressed in cranial neural crest cells (CNCCs) populating the frontonasal prominence, causes frontonasal dysplasia syndrome-3 (FND3) characterized by severe midface hypoplasia and orofacial clefting. We have shown that *Alx1*$^{-/-}$ mice recapitulate the craniofacial malformations in FND3 patients, and discovered the role of *ALX1* in determining the frontonasal identity of CNCCs, indicating *Alx1* is a critical node in the gene regulatory network controlling craniofacial development. However, the molecular mechanisms regulating *Alx1* expression during development are largely unknown. In this study, we identified a candidate distal enhancer (DE1) in the *Alx1* cis-regulatory region bycombinatorial analyses of the chromatin structure and epigenomic profiles.
The *DE1* genomic region contains four evolutionarily conserved subregions (ECRs 1-4). Through enhancer-reporter assays, we found that the ECR4 domain, a highly enriched Histone H3K27ac peak in mouse embryonic frontonasal tissues, exhibited strong enhancer activity. We generated mice lacking the ECR4 domain by CRISPR/Cas9 genome editing and found the ECR4 deletion reduced *Alx1* mRNA expression in the developing frontonasal mesenchyme but the *Alx1*$^{\text{?ECR4/?ECR4}}$ mice exhibited no detectable morphological abnormality in craniofacial development postnatally. We further generated mice lacking the entire enhancer and found that *Alx1*$^{\text{?DE1/?DE1}}$ mice partly recapitulated craniofacial defects in *Alx1*$^{-/-}$ mice. These data identify *DE1* as a crucial enhancer regulating endogenous *Alx1* expression during frontonasal development. Ongoing studies are testing whether and how the ECRs work together to control *Alx1* expression and which specific transcription factor binding sites mediate the spatiotemporal regulation of *Alx1* expression during craniofacial development. This work was supported by NIH/NIDCR grant R01DE029417 to RJ.

Program Abstract #233

Uncovering Sage's Collaborative Role in Salivary Gland Gene Regulation

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Transcription factor (TF) networks are complex intricate systems that require coordination between multiple TFs to drive organogenesis and tissue-specific gene expression programs. Within the model system of the Drosophila embryonic/larval salivary gland, the basic helix-loop-helix TF known as Sage interacts with two other TFs - Fork head (Fkh) and Senseless (Sens) - to regulate expression of genes encoding secretory cargo proteins, the enzymes that modify these cargos, pro-apoptotic factors, and, likely, factors regulating overall organismal metabolism. To elucidate the mechanisms of TF interactions and gene regulation, we are utilizing the embryonic salivary gland to uncover how these three TFs interact with each other and with their endogenous targets to establish and maintain salivary gland-specific functions. First, we are identifying the full suite of genes that are bound by each factor in the embryonic salivary gland using tissue-specific Chromatin Immunoprecipitation and Sequencing (ChIP-Seq). Second, we are identifying SG-expressed genes that are affected by the loss of each TF using microarrays and single cell RNA sequencing (scRNASeq) to reveal not only how binding and gene expression are related but to also learn the range of functions under the control of each TF. By studying the correlations between TF binding site configurations and TF-dependent gene expression, we will develop and test models for coordinate gene regulation in the context of a functional organ.

Understanding the molecular details of how Sage, Fkh and Sens coordinate binding and gene regulation will illuminate how tissue-specific gene expression is achieved in this system and will provide paradigms for how transcription factors network in more complex organisms. We are additionally interested in learning if Sage plays a more direct role in controlling metabolic functions and, if so, identifying its relevant downstream targets.

Program Abstract #234

PBRM-1/PBAF function in maternal germline to regulate chromatin dynamics and embryonic developmental transitions in *C. elegans*

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During metazoan development, the transition from parental germline to embryo requires resetting the life cycle during a time of transcriptional quiescence. How the germline primes gene expression for early embryogenesis is not well understood. Nucleosome placement and remodeling play pivotal roles in establishing both closed and open chromatin landscapes across the genome, yet remain largely unexplored in *C. elegans* germ cells. We investigated the role of the nucleosome remodeling complex PBAF, a subcomplex of SWI/SNF, in maternal germline function by employing the auxin-inducible degron system to specifically disrupt the expression of PBRM-1, a core PBAF component, in germ cells. We identified the chromatin-targeting role of PBRM-1 in the germline, observing its binding at selected germline-expressed genes crucial for embryonic function. Given that PBAF can evict nucleosomes, we hypothesized that PBRM-1/PBAF establishes open chromatin at these genes. RNA-seq analysis of first-generation embryos with disrupted PBRM-1 expression revealed downregulation of genes crucial for early embryogenesis and upregulation of those pertinent to later developmental stages. Our findings suggest that PBRM-1 is essential for maintaining the chromatin state necessary for early embryonic gene expression while also modulating pathways crucial for later developmental stages. In future experiments, we will conduct transcriptional and chromatin state profiling in germ cells using IGN. With its multiple bromodomains, PBRM-1 is expected to interact with histone acetylation marks, particularly H3K4ac and H3K14ac. Together, profiling these marks and their overlap with PBRM-1 targets, their regulation and open chromatin, will provide deep mechanistic insights into how the PBAF nucleosome remodeling complex regulates chromatin states to establish a successful oocyte-to-embryo transition.

Program Abstract #235

Systematic exploration of transcription factor function in maize

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Transcription factors (TFs) have various functions in plant development. In a model crop like Maize, there are thousands of TFs and due to the sheer amount of them, we don't know the function of many. I have developed a system to systematically explore TF function with the use of high throughput ectopic expression of individual TFs. Ectopic expression of TFs induces the native pathways and with that information we can begin to tease apart the native function of those TFs. We have established methods for high-throughput transformation of leaf protoplasts in 384-well plates. Median transformation efficiencies are >70% and consistent between days and across the plates. We have used this to express 164 individual TFs in duplicate and measure the full transcriptome-wide response by RNA sequencing. Replicate samples consistently show a higher Pearson's correlation than random sample pairs. 30% of TFs cluster immediately next to their replicate, indicating that many TFs induce reproducible responses and the responses are sufficiently distinct to drive clustering. We also find evidence supporting that at least some of the genes induced are likely direct TF targets. For example, the knotted1 (kn1) motif is enriched in the promoters of genes induced by kn1 overexpression. In the next steps, we will continue the established workflow through the remainder of the cloned maize TFome, producing a resource for the community.
Funding Acknowledgements: NSF IOS-2315723 and NIH 1T32GM142624

Program Abstract #236

Protein profiling unmasks a new regulatory layer during zebrafish early embryogenesis

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The maternal-to-zygotic transition is crucial in embryonic development, marked by the degradation of maternally provided mRNAs and initiation of zygotic gene expression. While this process is widely studied at the transcript level, a systematic and global analysis on protein regulation has yet to be conducted in vertebrate models. Here, we conducted protein profiling throughout zebrafish embryogenesis using quantitative mass spectrometry, integrating transcriptomics and translatomics datasets. Our data shows that unlike RNA changes, protein changes are less dynamic over early developmental timepoints. Further, increases in protein levels correlate with mRNA translation, whereas declines in protein levels do not, suggesting active protein degradation processes. Surprisingly, genes with mRNA detected only after genome activation are present as protein since fertilization, challenging the classification of "pure-zygotic" genes based on RNA expression alone. Most of the "pure-zygotic" gene's products detected before genome activation can be correlated to mRNA expression from oocytes, and unexpectedly, from spermatozoa. As a proof of concept, we utilized CRISPR-Cas13d to target znf281b mRNA, a gene whose protein significantly accumulates within the first two hours post fertilization, demonstrating its crucial role in development. In conclusion, these results highlight the importance of looking beyond transcriptional regulation in cell fate transitions and embryonic development, providing a more comprehensive understanding of the process, and when coupled with CRISPR-Cas13d offers a new approach to unravel maternal factors function during embryonic development. Research was funded by Stowers Institute for Medical Research and NIH grants GM136849 and R21OD034161.

Program Abstract #237
Exploring the Role of Repetitive Sequences in Nuclear Domain Formation
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Nuclear domains are sharply outlined compartments that selectively accumulate nuclear proteins and have poorly understood regulation. B-body is a specific nuclear domain that includes the long non-coding RNA *Hsr-omega* and the RNA-binding protein Bruno (Bru); B-bodies exist in developing flight muscles of *Drosophila*. The *Hsr-omega* genetic locus produces multiple RNA isoforms, greatly ranging in size from ~1 kb to over 25 kb. The long *Hsr-omega* isoforms feature a 10-kb repetitive region (RR) containing tandemly repeated sequences. We set to investigate the role of the RR in B-body formation and interaction with Bru. Using the UAS/Gal4 system, we expressed in developing flight muscles the 277-bp long repetitive core element (RCE), that is overrepresented in the RR. The construct was tagged with a unique sequence that enabled construct tracking by *in situ* hybridization. Using fluorescence microscopy, we observed that ectopically expressed RCE was exported from the nucleus and showed no affinity toward B-bodies. Bru was not attracted to the transcriptional sites of ectopic RCE expression, suggesting a lack of direct interactions between B-body protein residents and this part of *Hsr-omega* sequence. Our results demonstrate that Bru binding is mediated by sequences located outside the RR. Future work employing a precise RR genomic excision should clarify the role of the RR in B-body formation. The research is funded by NSF, grant # 2042814.

Program Abstract #238
miR-31 modulates local translation of Fascin at the mitotic spindle to ensure proper mitosis in early embryogenesis
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microRNA-31 (miR-31) is a highly conserved microRNA that plays roles in cell proliferation, migration, and differentiation. We discovered miR-31 and some of its validated target transcripts to have a dynamic localization during mitosis in the early cleavage stage of the sea urchin embryo. miR-31 and target transcripts Fascin, Gesolin and Rab35 are enriched on the mitotic spindle of the dividing sea urchin embryo when mitosis is rapid and highly coordinated. We found that miR-31 inhibition led to developmental delay correlated with increased cytoskeleton and chromosomal defects. Fascin is a known cytoskeletal binding protein, which binds to and regulates microtubules and F-Actin, and de novo translation of Fascin occurs at the mitotic spindle of sea urchin embryo. Loss of Fascin leads to chromosomal abnormalities, developmental delay, and cytoskeletal defects. Importantly, miR-31

inhibition leads to significant increase of newly translated Fascin at the spindle of dividing sea urchin embryos. Forced ectopic localization and translation of Fascin transcripts at the cell periphery led to significant developmental and chromosomal segregation defects, leading to our hypothesis that miR-31 regulates local translation of its targets at the mitotic spindle to ensure proper cell division. Further, Fascin knockdown led to decreased tubulin and F-actin, suggesting its function in regulating the cytoskeleton to potentially mediate mitosis. miR-31-mediated post-transcriptional regulation at the mitotic spindle may be an evolutionarily conserved regulatory paradigm of mitosis, as we observe miR-31 and Fascin localize to mitotic spindle of mammalian cells. Overall, this work highlights the novel and potentially evolutionarily conserved role of miR-31 in regulating local translation of cytoskeletal transcripts that impact mitosis and early development. This work is funded by NSF MCB to JLS.

Program Abstract #239

Profiling of Active Enhancers in Human Embryonic Palatal Mesenchyme Cells

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Cleft palate is due to abnormal development of the secondary palate and has a frequency of approximately 1 per 2,500 births. Knowledge of the molecular interaction pathways that contribute to human palate development and are disrupted in cleft palate is incomplete. To identify genomic enhancer regions potentially involved in regulating gene expression in the developing palate, genome-wide profiling of the binding of eight histone proteins (H3K4me1, H3K4me3, H3K9me3, H3K27ac, H3K27me3, and H3K36me3, H4K20me1, and H3K79me2) to chromatin in a human embryonic palatal mesenchyme (HEPM) cell line was performed using CUT&RUN and ChIP-seq assays. Profiles were analyzed using the ChromHMM algorithm, which integrates genome-wide data on histone binding to partition the genome into chromatin states that represent functional regulatory elements. Eighteen chromatin states that could be broadly categorized into enhancers (n = 207,849), transcription start sites and flanking sites (n = 46,689), transcription regions (n = 43,396), and repressed regions (n = 220,297) were identified. To identify cell-specific, active enhancers, the subset of enhancers that were annotated as active (n = 66,213) were compared for overlap with 827,518 enhancers that were previously reported in a combined analysis of chromatin state data from over 100 cell and tissue types and are therefore active in a broad range of cell types. This comparison identified 17,599 active enhancers specific to HEPM cells. The identification of HEPM cell-specific enhancers facilitates further investigation to identify transcription factors that bind the enhancers to regulate gene expression during palate development and to uncover the mechanisms through which disruption of enhancer regions by genetic variants contribute to cleft palate. Funding for this project was provided by Marshfield Clinic Research Institute.

Program Abstract #240

Multi-omic analysis of the Hand2-dependent gene regulatory network active within the developing mandible

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The proper patterning of the mandible requires instructive cues from multiple signaling pathways as well as complex interactions between tissue specific transcription factors including homeobox and basic helix-loop-helix (bHLH) family members. Of these, *Hand2*, a bHLH family member, is required for maintaining distal identity, with loss of *Hand2* activity within cranial neural crest cells resulting in micrognathia and aglossia. Our prior work revealed a previously unappreciated role for Hand2 in Gli-mediated transcription during mandibulogenesis, in which robust Gli3 transcriptional activity during skeletal and glossal development required interaction with Hand2. Despite this finding, several questions remain regarding the mechanism of how Hand2 may influence chromatin accessibility to coordinate signaling input during mandibular development given that pioneer activity has not been ascribed to Gli3. To address this question and elucidate the molecular mechanisms underlying the morphological defects observed in *Hand2*-mutant mandibles, we utilized single-cell joint profiling to simultaneously measure both chromatin accessibility (snATAC-seq) and gene expression (snRNA-seq). Cluster analysis comparing *Hand2*-mutant versus wild-type mandibles revealed striking changes in cell populations at both the genomic and transcriptomic-levels highlighting key transcription factors required for normal development. Collectively, our studies suggest a novel role for Hand2 in orchestrating mandible development via the regulation of

chromatin accessibility and inform on the downstream gene regulatory network. This work is currently funded by NIH-NIDCR R01DE031750 (K.A.P. and S.A.B)

Program Abstract #241

Molecular mechanisms of alcohol-induced cognitive impairment in *Drosophila melanogaster* larvae

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Alcohol-induced cognitive impairment poses a substantial public health concern, emphasizing the critical need to comprehend its inherent molecular mechanisms for the development of effective interventions. This study is dedicated to unraveling the molecular underpinnings of alcohol-induced cognitive deficits, employing the Drosophila melanogaster larvae model. Employing a comprehensive approach that integrates behavioral assays, genetic manipulations, and molecular analyses, we scrutinize the impact of both acute and chronic alcohol exposure on learning and memory in Drosophila larvae. Notably, alcohol-induced alterations elicit homeostatic adaptations within the organism, characterized by a progressive escalation in alcohol tolerance and the onset of physiological dependence. Our findings unequivocally demonstrate that acute alcohol exposure triggers pronounced cognitive impairment in larvae, as evidenced by disruptions in associative learning and memory formation. Furthermore, chronic alcohol exposure instigates neural adaptations in Drosophila larvae, culminating in cognitive ethanol dependence. Recent investigations have indicated a myriad of transcriptional changes in the brain following chronic alcohol exposure, potentially elucidating the underlying cognitive adaptations. This study seeks to examine whether alcohol-induced transcriptional changes mediate specific alterations in larval brain synaptic activity, thereby steering cognitive adaptation. To this end, candidate genes implicated in this process will undergo scrutiny through Larval learning assays and the UAS-GAL4 transgenic system. The application of RNA interference (RNAi) will enable the targeted knockdown of these genes, facilitating the exploration of their individual roles in cognitive adaptation. Funding Support: Puerto Rico Louis Strokes Alliance for Minority Participation (PR-LSAMP) HRD-2008186,

Program Abstract #243

Identifying the Repression Complex Mediating Transcriptional Repression of Hedgehog Signaling

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The Hedgehog (HH) signaling pathway plays an essential role in regulating a diverse spectrum of developmental processes such as embryonic patterning, organogenesis, and growth control. Accordingly, dysregulation of HH signaling causes cancer and severe genetic birth defects, such as craniofacial anomalies and polydactyly. HH signaling is mediated by GLI transcription factors, which in the absence of pathway activation are converted into transcriptional repressors. While a few target genes require GLI transcriptional activation, the majority of HH target genes are activated solely by the loss of GLI repressor (GLI-R) activity, termed GLI de-repression. GLI transcriptional repression is associated with reductions in the active enhancer marker, H3K27ac, and chromatin accessibility at HH-responsive GLI-bound enhancers. This result suggests that GLI represses target genes by inactivating enhancers with HDACs and chromatin remodelers. I established an in vivo system for identifying proteins interacting with chromatin-bound GLI3 in mouse limb buds and identified multiple interacting proteins. In addition to pulling down some known GLI interacting proteins, we identified several proteins that are components of the NuRD (nucleosome-remodeling and deacetylase) complex. I am currently testing the hypothesis that GLI3 transcriptional repression is mediated by the recruitment of the NuRD complex. This work is funded by the NIH RO1 HD073151.

Program Abstract #244

Investigating Cdx1a function in zebrafish trunk and tail development

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Trunk and tail development in vertebrates critically depends on the activity of the three members of the Cdx family of transcription factors. In zebrafish, loss of Cdx4 function results in a reduction in the spinal

cord territory, a temporal delay in hox gene induction that results in a caudal shift in their transcriptional domain, and a misalignment of axial identities between neural and mesodermal tissues, among other phenotypes. In contrast, little is known about the function of the zebrafish Cdx4 paralogues, Cdx1a and Cdx1b. To investigate the function of zebrafish Cdx1a, we have generated several cdx1a mutant alleles using CRISPR gene editing approaches. Using these mutants, we have mapped a number of critical residues to the Cdx1a protein required for activity and are investigating its function in trunk and tail tissue specification and patterning. Significantly, loss of Cdx1a activity does not result in overt phenotypic changes unless it is in conjunction with loss of Cdx4. Preliminary data shows that Cdx4 regulates Cdx1a but not the other way around. Our analysis suggests that trunk and tail specification and patterning in zebrafish critically depend on unique and redundant Cdx1a and Cdx4 activities. (This work was supported by NSF IOS-1755386, The Virginia Foundation of Independent Colleges, and the University of Richmond School of Arts and Sciences.)

Program Abstract #245

Cooperative action of the pancreas transcription factors Pdx-1 and OC1 regulates the transition from multipotent progenitor cells to endocrine cells

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Diabetes is characterized by dysfunction of insulin-secreting ß cells. Thus, investigating ß cells and its regulatory elements like islet enhancers, super enhancers, and active promoters that organize into 3D hubs orchestrate the transcriptional networks crucial for the appropriate growth, differentiation, and division is critical. The homeodomain transcription factor Pdx1 plays a major role in early pancreatic specification, organ size regulation, and ß-cell formation, proliferation, and identity at various stages. The Pdx1 C-terminus interacts with the one cut homeodomain transcription factor Oc1 and these two factors cooperate to activate the endocrine gene program. Pdx1/Oc1 double heterozygous embryos have reduced endocrine differentiation, although single heterozygotes show no developmental phenotype. Interestingly, despite only being transiently co-expressed in multipotent pancreatic progenitor cells (MPCs), haploinsufficiency for both factors have long-term effects on postnatal islet function and ß-cell compensation in response to metabolic and proliferative stimuli. Thus, we hypothesize that Pdx1/Oc1 cooperativity changes the epigenetic landscape in MPCs or endocrine progenitors, allowing for endocrine fate specification and ß-cell compensatory adaptation. The ongoing study will examine how Pdx1 and Oc1 cooperate to modulate the epigenetic landscape to promote endocrine differentiation and will identify key epigenetic marks that regulate endocrine specification at different stages of pancreas development in single and double heterozygous and overexpression mouse model. The use of small molecule epigenetic modulators will establish a new paradigm in promoting endocrine differentiation and releasing the repressive epigenetic brake for the use in non-endocrine-derived ß cell differentiation and regeneration.(Funding: R01DK135032).

Program Abstract #246

Embryonic neural primordium fate specification at the maternal to zygotic transition *in vivo*

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Embryonic development engenders cell fate determination through a complex hierarchy of intermediate specifications. Traditionally, these cell fate vectors have been anticipated to originate at the onset of gastrulation concurrent with the differentiation of totipotent embryonic stem cells into the germ layers. However, the advent of modern -omics technologies has revealed broad transcriptional diversity congruent with the onset of zygotic genome activation. We have demonstrated a pregastrulation spatiotemporal niche of co-regulation of two transcription factors (TFs), Odd-paired (Opa)/(ZIC3) and Ocelliless (Oc)/OTX1/2, with putative zygotic genome activation capability, pioneer factors, spatially confined to the early embryonic region of the future brain and occupying distal elements proximal to a profusion of brain and early neurogenic genes. Here we show that cell fate diversification at these early stages rapidly shifts from maternal to zygotic control by interrogating the dynamic effects of repressor Su(H) and have developed *in vivo*, optogenetic and real-time transcription observation tools to modulate expression of Opa and Oc pregastrulation to interrogate the primordial fate determining events which

specify the earliest brain lineages. We propose an innovative developmental model elucidating the dynamic regulation of pregastrulation cell specification engendering this diverse cell population during gastrulation and introduce cutting-edge optogenetic transgenic control tools, utilizing LEXY and LANS, designed to control endogenous and exogenous Opa and Oc TFs to enable precise modulation of real-time transcriptional reporters for brain gene expression *in vivo*, providing spatiotemporal precision throughout pre- and post-gastrulation stages. This research is vital for unraveling the intricate mechanisms underlying neurodevelopmental cell fate determinations, which intricately orchestrate the cellular diversity observed in mature animals. Funding: UTA STARS

Program Abstract #247
Transcriptional Regulation of Neonatal Nutrition and Immunity
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The neonatal period of life is a critical time of rapid development. To fuel this growth, the neonatal intestine must selectively absorb nutrients while establishing an immune defense against pathogens. These two core functions are intimately linked, as impaired nutrition early in life impairs systemic immune function. Neonates utilize a unique mode of nutrient absorption: macromolecules are absorbed in bulk and subsequently degraded intracellularly. Our lab has identified three transcription factors crucial to this process: MAFB, cMAF, and BLIMP1 (protein product of the *Prdm1* gene). Conditionally deleting these genes from intestinal epithelial cells disrupts macromolecular uptake, leading to neonatal malnutrition. However, the mechanisms linking MAFB/cMAF/BLIMP1 function and neonatal nutrition remain poorly understood. Our data indicate that co-expression of *MafB*, *cMaf*, and *Prdm1* is necessary to maintain a neonatal enterocyte functional state, and is required for the uptake of amino acids. MAFB, cMAF, and BLIMP1 control this process through a shared endolysosomal transcriptional program. Given the intimate link between malnutrition and immunity, we analyzed immune infiltration in our mouse models of neonatal malnutrition. Epithelial deletion of *MafB*/*cMaf* or *Prdm1* reduces neonatal CD4+ T cell infiltration, and disrupts intraepithelial CD4+ T cell localization in adults. Intraepithelial T cells can act as a "first responders" against intestinal pathogens, and this deficit could therefore leave these mice susceptible to infection. Together these data indicate that MAFB, cMAF, and BLIMP1 control a coordinated neonatal nutritional program. This program is required for amino acid uptake in neonatal enterocytes, and has both short- and long-term consequences on intestinal immunity. This work has been supported by a Hartwell Foundation Postdoctoral Fellowship and an NIH T32 DK007568.

Program Abstract #248
Characterizing Minor Spliceosome snRNA Expression During Cold Stress in *Arabidopsis thaliana*
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Plants are sessile organisms and cannot escape any adverse conditions that arise in their environment. Therefore, it is imperative that they possess the molecular tools to respond and adapt to various stresses, both biotic and abiotic. Cold stress due to low and/or freezing temperatures can severely impact growth and development, as well as yield in agricultural settings. Plants sense low/freezing temperatures through plasma membrane rigidification as well as structural and lipid composition changes. In combination with these alterations, receptor-like kinases in the membrane activate calcium ion channels. The increase in cytoplasmic calcium activates calcium-binding proteins and associated downstream signaling cascades, with reactive oxygen species and nitrous oxide also playing a role, leading to the activation of transcription factors which regulate the transcription of cold acclimation genes. Through expression of cold response (COR) genes and accumulation of cryoprotectant molecules, plants become cold acclimated. To understand how this complex process affects the activity of the minor spliceosome, which has been previously shown to be required for proper development and uniquely affected by cold stress in *Arabidopsis thaliana* and *Zea mays*, we exposed A. thaliana to 4? for different lengths of time and at different stages of development and assessed gene expression via RT-PCR. We hypothesized that minor snRNAs would be downregulated upon cold shock due to previously observed increased in minor intron retention due to cold shock. However, we found that U4atac is notably upregulated by 24h of cold shock in 3 week old plants, and all snRNAs exhibited expression changes after 24h of cold shock in 4 day old seedlings. Funding/support for this project was generously provided by the Society of Developmental

Biology Choose Development! Program and by the UConn Maximizing Access to Research Careers program.

Program Abstract #249
Epigenetic marks correlated with differential zygotic activation of parental genomes in plant embryogenesis
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After fertilization in animals, maternal mRNAs and proteins regulate development until the onset of zygotic transcription. In plants, the extent of maternal regulation of early embryo development has been less clear: hybrid rice zygotes showed an overwhelming maternal transcript bias, while Arabidopsis Columbia (Col)/Cape Verde Islands (Cvi) hybrid embryos had essentially equal maternal and paternal contributions, and Col/Landsberg erecta (L*er*) hybrid zygotes showed asymmetric activation of maternal and paternal genomes. Using functional assays, we recently demonstrated the Arabidopsis Col/Tsu hybrid to be a faithful proxy for understanding parent-of-origin behavior in the reference ecotype Col. Here we analyze transcriptomes of Col/Tsu embryos from zygote-1cell to mature stages, finding a strong maternal transcript bias in zygote-1cell and octant embryos. Quantification of intron reads and comparison of transcript levels in the egg and embryo suggested that the observed maternal bias was due to preferential transcription of maternal alleles in the zygote and early embryo, rather than carry-over from the egg cell. Comparison of maternally or paternally biased genes in all Arabidopsis hybrid embryos with DNA and histone methylation data revealed a correlation between different degrees of parental bias and various epigenomic contexts. Analysis of rice parent-of-origin transcriptome data and epigenomic data also showed strong correlations between parentally biased gene expression and certain epigenomic marks. The transcriptomic data presented here further supports the idea that parent-of-origin contributions to early embryogenesis differ between hybrids of Arabidopsis. Correlations between parent-of-origin biases and epigenetic marks in Arabidopsis and rice embryos point the way to future mechanistic studies. Funding: Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCyT) Mexico; NRC-GHI and ACRD Canada.

Program Abstract #250
Quantitative proteome dynamics across embryogenesis in a model chordate
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The evolution of gene expression programs underlying the development of vertebrates remains poorly characterized. Here, we present a comprehensive proteome atlas of the model chordate *Ciona*, covering eight developmental stages and ~7,000 translated genes, accompanied by a multi-omics analysis of co-evolution with the vertebrate *Xenopus*. Quantitative proteome comparisons argue against the widely held hourglass model, based solely on transcriptomic profiles, whereby peak conservation is observed during mid-developmental stages. Our analysis reveals maximal divergence at these stages, particularly gastrulation and neurulation. Together, our work provides a valuable resource for evaluating conservation and divergence of multi-omics profiles underlying the diversification of vertebrates. This study was funded by NIH grant (T32GM007388) to Princeton University, NIH grant (NS076542) to M.S.L., NIH grant (R35GM128813) to MW, Eric and Wendy Schmidt Transformative Technology Fund to M.W., Diekman collaboration fund to M.S.L. and M.W. and Princeton Catalysis Initiative to M.S..L and M.W.

Program Abstract #251
Understanding the role of *ebony* in tapeworm reproductive development
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Tapeworms are prolific parasites, who continuously generate new tissue, including the ability to constantly produce germ cells. These flatworms consist of a head, a neck, and a strobilized body made up of thousands of segments (proglottids). The neck is the only regeneration-competent tissue from which new proglottids containing complete male and female reproductive systems regenerate. Putative *nanos+* germ cells are detectable in the neck, indicating that factors within this region could be acting as a germ

cell niche. We ask: what cells and signals make up this germ cell niche in the neck? Previous work implicated the gene *ebony* in the sexual maturation of female schistosomes (Chen et al. 2022), and germ cell regulation in planarians (Issigonis et al. 2023). We hypothesize that *ebony*, along with its products, modulate the germ cell niche and are involved in the maintenance and development of germ cells themselves. Using the rat intestinal tapeworm, *Hymenolepis diminuta*, we observe *ebony* expression in the most anterior of the neck around a subset of nuclei that make up osmoregulatory canals. This region of the canals flanks undifferentiated germ cells. Thus, *ebony*+ cells are poised to act as a germ cell niche. We find *ebony* expression influences germ cell development, as knockdown of *ebony* by RNA interference results in a reduction of germ cells in the neck. Tapeworm tissue contains two *ebony* products: β-alanyl-tryptamine (BATT) and β-alanyl-serotonin (BAST) detectable by mass spectrophotometry. Consistent with our model, BATT supplementation increases production of undifferentiated germ cells. Given these data, *ebony* and BATT play an important role in regulating germ cell specification/maintenance and are likely critical for maintaining the germ cell niche. Additional functions of BATT and BAST in germ cell differentiation or germline stem cell maintenance are presently being investigated. Funding is provided by NIH/NIAID DP2: AI 154416-01.

Program Abstract #252

A Y-linked duplication of anti-Mullerian hormone is the master sex determination gene in threespine stickleback fish (*Gasterosteus aculeatus*)

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Master sex determination genes have evolved independently in many taxa. While a wide variety of genes are used across vertebrates to initiate the critical developmental process of sex determination, the gene anti-mullerian hormone (*amh*) has convergently evolved as a sex determination gene in multiple species. Ancestrally, *amh* is dispensable for male determination; thus, how *amh* initiates testis development and how it evolves this novel function is not known. In threespine stickleback (*Gasterosteus aculeatus*), *amh* was duplicated onto the Y chromosome (*amhy*) approximately 20 million years ago. To determine if the Y-linked *amhy* is the sex determination gene in this species, we have knocked out *amhy* using CRISPR/Cas9. XY *amhy*-KO fish show male to female sex reversal and develop functional ovaries with viable oocytes that can transmit an X or Y chromosome. We have also introduced a transgene with amhy and its native regulatory elements into XX fish. XX *Tg(amhy)* fish show female to male sex reversal, developing testes rather than ovaries. XX males are severely subfertile, suggesting additional Y linked genes are important for spermatogenesis. These findings show *amhy* is both necessary and sufficient for male determination, demonstrating that it is the master sex determination gene in threespine stickleback. We hypothesize that regulatory evolution of *amhy* generated novel expression patterns key to the evolution of novel function in sex determination. We are performing time-course RNA-seq across early development to characterize expression divergence between *amhy* and its autosomal paralog, *amh08*, as well as the timing and transcriptional landscape of early sex differentiation in threespine stickleback. Our findings establish threespine stickleback as a premier model to investigate how *amh* regulates gonadal development and how this gene repeatedly evolves novel function as a master sex determination gene. Funding: NSF MCB1943283, NIH 1R01GM147312, NIH 1T32GM142623

Program Abstract #253

Ontogeny of the Non-Senescent, Highly Proliferative Echinoderm Ovary

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Canonically studied female organisms typically experience diminished reproductive potential well prior to their expected lifespan. Echinoderms, however, show no such declination and instead often show increased fecundity with age. To understand the origin and maintenance of the unique reproductive features of the echinoderm ovary, we conducted RNA-seq analyses of all adult tissues, and performed tissue enrichment analyses across their developmental trajectory to identify genes selectively involved in gonad development. We present our results testing the genetic basis of sex determination in these

animals, hypothesizing a multigenic contribution. We identified genes involved in patterning (FoxL2, Six4), male sex determination (Dmrt, SoxH), meiosis (SYCP3, MeioB, Rec8), and development of the hormone axis (Dax1). Using CRISPR-Cas9 KO in *Lytechnius variegatus*, we identified the phenotypes of sea urchins deficient in meiotic and gonadogenic candidates. We identified hallmark gene expression stages of the ovarian cycle: I - IV, which correspond to seasonal fluctuations in fecundity. We found that Stage I ovaries express low levels of both male- and female- sex associated transcripts. Initiation of oogenesis was marked by expression of key gene products involved in both oocyte and somatic cell transitions at Stage II. Further, we developed cell culture methods to investigate the response to signaling pathways in ovaries using Activin, Wnt, and Estrogen. We identified a homeostatic mechanism for steroidogenesis in the echinoderm ovary, and are using these findings to test the molecular conservation of gonad development and maintenance in other echinoderms. By integrating mechanisms of sex determination, RNA seq, histology, and CRISPR-Cas9 gene KO with animal husbandry and cell culture, we present a model for understanding the retention of reproductive potential in a specialized ovary of high fecundity. (NIH R35GM140897; NSF IOS-1923445)

Program Abstract #254
Meiotic checkpoint effect on synaptonemal complex mutants.
Clayton Parker, Katherine Billmyre
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One of the most important stages of development is the production of gametes to pass genetic information from one generation to the next. Improper chromosome segregation, or aneuploidy, can lead to fertility issues or birth defects. There are many mechanisms that contribute to the proper segregation of chromosomes during meiosis, including the distribution of double-stranded breaks (DSB) and crossovers (CO). A large meiotic protein structure called the synaptonemal complex (SC) is known to be important in DSB and CO placement. In *Drosophila melanogaster* the central region of the SC contains a filament protein, C(3)*G*. A small deletion within the coiled-coil region of C(3)*G* produces fragmented SC, along with reduced chromosome pairing, and differences in recombination between chromosomes. In this background, the X chromosome has significantly reduced recombination when compared to wild type, while chromosome 3 has an increase in recombination in the centromere-proximal region. The reduction of recombination on one chromosome alongside an increase on another is similar to the interchromosomal effect, a phenomenon that occurs during Drosophila meiosis. The increased recombination that occurs with the interchromosomal effect can be rescued by removing Pch2, a meiotic checkpoint protein. To test if the variation in recombination seen in c(3)G^{del3} flies is due to the interchromosomal effect, we are examining recombination on the X chromosome and the autosomes in pch2^{null}, c(3)G^{del3} double mutants. If the double mutants show a reduction in the centromere-proximal crossing over down to wild-type levels, the autosomal phenotype seen in c(3)G^{del3} flies may be due to the interchromosomal effect. In the future, we will use this system to further investigate the mechanism(s) regulating crossover positioning in Drosophila meiosis. Funded by R00GM138759-03 from NIGMS

Program Abstract #255
Chromosome-specific differences between autosomes and the *X* chromosome
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Errors in meiosis are a leading cause of birth defects and infertility in humans. There is a lot of evidence from work in humans and model systems that chromosome-specific errors occur during meiosis. A key meiotic structure is the synaptonemal complex (SC), which is necessary for proper double-strand break (DSB) formation and crossing over. In *D. melanogaster*, partial loss-of-function mutations in a key SC protein exhibit chromosome-specific changes in crossing over with a decrease in crossovers (COs) on the *X* chromosome and a shift in COs on autosomes. These previous results suggest there is an inherent difference between how the *X* chromosome and autosomes undergo meiosis. To better understand the timing of early meiotic events on individual chromosomes, I am taking an imaging approach to visualize events such as replication, synapsis, and DSB formation on specific chromosomes. Currently, I am establishing an imaging system using LacO/LacI to mark specific chromosomes in combination with BrdU to identify newly replicated chromosomes. We can further use this system to examine timing of synapsis,

and DSBs formation/repair antibodies against SC and DSB markers. With these methods we will be able to identify differences in early meiotic events on the autosomes and *X* chromosome. Once we have an understanding of wild-type chromosome behaviors we can apply our approach to mutants with chromosome-specific errors to identify the underlying cause of those errors. All research is supported by R00GM138759-03 grant from NIGMS.

Program Abstract #256
Identifying Potential Phosphorylation Sites within the Synaptonemal Complex
Ava Murphy, Katherine Billmyre
University of Georgia, USA
Meiosis, an essential process for sexual reproduction, allows for the generation of genetic diversity through a process called recombination and chromosome segregation. The synaptonemal complex (SC) is a critical structure for the process of recombination. SC assembly and disassembly is regulated by post-translational modifications, where specific sites become phosphorylated to influence the structural integrity and functionality of the SC. These specific phosphorylation sites are unknown in Drosophila. Cyclin-dependent kinase (CDK) and its associated Cyclin regulatory subunits are key components in the regulation of phosphorylation. To delve deeper into the regulatory role of CDK and Cyclins in controlling the SC during meiosis, we employed RNA interference (RNAi) to downregulate the expression levels of CDK and Cyclins within the Drosophila ovary. By perturbing the levels of these key regulatory molecules, we sought to understand their specific contributions to SC regulation. This work is funded by a R00GM138759-03 from NIGMS to K. Billmyre.

Program Abstract #257
Soma to Germ: Germline Regeneration in *Parhyale hawaiensis*
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During embryonic development, somatic cells – which age and die – are separated from an 'immortal' cell lineage – the germline. The germline is passed on from generation to generation in sexually reproducing organisms, making it essential for the survival and the evolution of a species. In many well-studied bilaterians, the germline once ablated cannot be restored, resulting in infertile adults. However, *Parhyale hawaiensis*, a marine amphipod crustacean, has the ability to regenerate its germline despite being incapable of total body plan regeneration. Combining blastomere ablation and lineage tracking techniques, we have generated a population of germline-ablated hatchlings and shown that the germline is regenerated from a mesodermally-derived somatic source. The experimental ablation of the progenitor germ cell (at the embryonic 8-cell stage) results in an absence of embryonic and post-embryonic primordial germ cells, assayed via the loss of several germline markers. Nevertheless, Vasa expression (used as a proxy for germ cells), reappears post-embryonically (2-3 weeks post hatching) in germline-ablated hatchlings – yielding fertile adults. We hypothesize that germ cell fate is acquired by mesodermally-derived somatic cells via inductive signals from an empty germline stem cell niche present within the gonads. To this end, we present preliminary morphological and molecular data to characterize the mesodermal and germline components of the gonads and the location of potential stem cell niches, and outline an approach for understanding the mechanisms that allow for germline replacement in this species.

Program Abstract #258
The role of *nanos* in germ cell regulation and regeneration in *Hymenolepis diminuta*
Cierra Gladfelter, Tania Rozario
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Tapeworms are parasitic flatworms that cause disease worldwide in humans and livestock. They regenerate thousands of hermaphroditic segments called proglottids from their neck, and each proglottid can be fertilized. Commitment to germ cell fate may occur in the neck, but it is unknown how germ cells are regulated and the role they play in regeneration. We wish to molecularly characterize germ cell development in the rat tapeworm, *Hymenolepis diminuta*, by uncovering genes necessary for growth and reproduction. *Nanos*, a conserved germ cell regulator, is expressed in germ cells and somatic

stem cell populations in other worms like schistosomes and acoels. Tapeworms only have one homolog of *nanos* and our goal is to examine its role in germ cell and/or stem cell regulation. We performed a descriptive analysis of *nanos* expression at progressive stages of reproductive development using whole mount in situ hybridization. We find that *nanos* is expressed throughout the whole neck, genital anlagen, and gonads. To ascertain if *nanos*+ cells in the neck bear hallmarks of germ cells, we performed double fluorescent in situ hybridization with putative germ cell markers known to express in the gonads. All gonadal markers examined co-express with *nanos* in the neck, suggesting *nanos* expression marks early germ cells. Interestingly, one gene (*protocadherina*) expresses in a subset of *nanos*+ cells indicating heterogeneity within the *nanos*+ population. The double positive cells are confined to the most anterior part of the neck, where regenerative ability is most pronounced. It is unclear whether the double positive population represents germ cells or stem cells. Our next steps will be to silence *nanos* using RNA interference to examine its role in germ cells, the stem cell population, and the propensity of the tapeworm to regenerate. Our study will resolve the role(s) of *nanos* and help demystify how germ cells influence tapeworm regeneration. Funded by NIH/NIAID DP2: AI 154416-01.

Program Abstract #259

Spindle orientation in *C. elegans* mitotic germ cells is regulated by Dynein and LIN-5/NuMA

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Mitotic spindle orientation sets the cell division plane in response to cell-intrinsic and -extrinsic cues, and is thus critical for maintaining tissue organization during development. *C. elegans* germ cells, like those in most animals, are organized as a syncytium, which is essential for fertility. In *C. elegans*, germ cells are found in a circumferential monolayer around a shared core of cytoplasm, the rachis, and each germ cell maintains a connection to the rachis via a stable cytoplasmic bridge. How this tissue organization arises and is maintained as germ cells extensively proliferate during development is unclear, particularly as these cells lack canonical cell-cell junctions and are not known to undergo oriented cell divisions. We have found that germ cell spindles orient along two distinct axes: within the tissue plane and thus parallel to the rachis surface, and along the gonad's proximal-distal axis, which is also the axis of tissue elongation. Acute depletion of the microtubule force generator dynein and its conserved regulator LIN-5/NuMA disrupted spindle orientation in both axes, and longer depletions led to a severe disorganization of the germline tissue. Analysis of LIN-5 localization revealed that it is largely excluded from the rachis surface relative to the basolateral cortices during interphase. In mitosis, LIN-5 cortical association was found to be dynamic, with localized enrichment on lateral cortices adjacent to the spindle poles. Interestingly, spindle orientation biases were maintained in cultured gonad explants, indicating tissue- and/or cell-autonomous regulation. We propose that strict exclusion of LIN-5 from the rachis surface, in combination with its dynamic association with lateral cortices during mitosis, orients germ cell spindles to maintain *C. elegans* germline tissue architecture through cell division. Funded by a grant from the Canadian Institutes of Health Research (PJT-153283) to JCL and ARG.

Program Abstract #260

Characterization of larval temporal factors throughout embryonic CNS development

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Neural progenitors give rise to distinct populations of neurons throughout development. Drosophila larval neural progenitors, neuroblasts (NBs), express temporal gradients of transcription factors and RNA-binding proteins that establish neuronal diversity throughout larval development. The function of temporal transcription factors (TTFs) is well-studied in larval neural development. Several factors are expressed early in larval development, including Imp and Chinmo, while other factors are expressed later, including Syp, Mamo, and Broad. While the gene regulatory network of these factors has been thoroughly characterized in larvae, little is known about their role in embryonic CNS development. It is unknown whether the larval gradients of early and late factors are consistent in embryonic development, whether the embryo serves as an extension of the early larval factors, or if the embryo has a differing gene regulatory network all together. Further, the relationship between the embryonic expression of these "larval" factors and the embryonic TTF cascade (Hb, Kr, Pdm, Cas) is unknown. The embryonic TTF

cascade has been well characterized and expression of specific TTFs in this cascade are known to be required for establishing neuronal identity. However, the interactions between larval TTFs and embryonic TTFs in embryonic development has not been explored. Here, we characterize the expression patterns of "larval" temporal factors in the embryonic CNS; investigate the cross-regulation between embryonic and larval temporal factors; and test the function of larval temporal factors in embryonic CNS development. Results to date will be presented. This work is supported by the NIH (F31HD108945 to KHF and R37HD27056 to CQD) and the Howard Hughes Medical Institute.

Program Abstract #261
Constitutively Active Ras Leads to Increases in Dendritic Complexity in *Drosophila melanogaster* Sensory Neurons
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Ras signaling pathway is involved in many processes in the cell that are crucial for differentiation, development, and survival. Although the role of Ras in these processes has been studied extensively, its role in neuronal dendritic development isn't fully elucidated. Earlier studies show that constitutively active (C.A.) Ras, which is always in its active state, lead to complex dendritic profiles. However, later studies have either failed to replicate these results, or shown that C.A. versions of Ras activating genes don't give the same results, albeit increasing activated Ras levels. It is crucial to understand the regulatory role of Ras and its downstream effectors in dendritic development as these genes are implicated in rare genetic disorders. These disorders, namely Rasopathies, lead to growth abnormalities, developmental delays, and intellectual disabilities. There are no cures, and the treatments are limited, as the mechanisms through which mutated Ras pathway genes affect the cells isn't clear. Through our research, we aim to determine the role of Ras in dendritic development. We use the peripheral sensory neurons of the *Drosophila melanogaster* larvae. We show that C.A. versions of two paralogs of Ras (Ras85D & Ras64B) lead to increases in dendritic complexities in two neuron classes *in vivo*. In addition, expressing dominant negative versions or total knockout of these genes reduces dendritic complexity. Interestingly, the effects of Ras85D are more severe than Ras64B. To characterize downstream partners of Ras required for its role in dendritic development, we are conducting genetic interaction experiments, introducing C.A. Ras and knockdown of candidate downstream partners in our neurons. Our research is funded by NIH grants NS086082 & NS115209 and a Brains & Behavior Seed Grant.

Program Abstract #262
Adjacent Neuronal Fascicle Guides Motoneuron 24 Dendritic Branching and Axonal Routing Decisions through Dscam1 Signaling
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The formation and precise positioning of axons and dendrites are crucial for the development of neural circuits. Although juxtracrine signaling via cell-cell contact is known to influence these processes, the specific structures and mechanisms regulating neuronal process positioning within the central nervous system (CNS) remain to be fully identified. Our study investigates motoneuron 24 (MN24) in the *Drosophila* embryonic CNS, which is characterized by a complex yet stereotyped axon projection pattern, known as 'axonal routing.' In this motoneuron, the primary dendritic branches project laterally toward the midline, specifically emerging at the sites where axons turn. We observed that Scp2-positive neurons contribute to the lateral fascicle structure in the ventral nerve cord (VNC) near MN24 dendrites. Notably, the knockout of the Down syndrome cell adhesion molecule (*dscam1*) results in the loss of dendrites and disruption of proper axonal routing in MN24, while not affecting the formation of the fascicle structure. Through cell-type specific knockdown and rescue experiments of dscam1, we have determined that the interaction between MN24 and Scp2-positive fascicle, mediated by Dscam1, promotes the development of both dendrites and axonal routing. We uncover a key neuronal structure serving as a guiding reference for neural circuitry within the *Drosophila* embryonic CNS, highlighting the essential role of an adjacent axonal fascicle in precisely coordinating axon and dendrite positioning in motoneuron 24 (MN24) through Dscam1-mediated inter-neuronal communication. This enhances our understanding of the molecular underpinnings of motoneuron morphogenesis in *Drosophila*. Given the occurrence of analogous axon fascicle formations within the vertebrate spinal cord, such structures may

play a conserved role in the morphogenesis of motoneurons via Dscam1 across phyla. This work was funded by the NIH grant (R01NS107558) to DK.

Program Abstract #263

Pioneer and follower neurons are transcriptionally distinct, and their axon targeting is regulated by retinoic acid signaling

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During nervous system development, pioneer neurons are the first to explore a tissue environment by extending their axons toward new targets. Through this initial extension, pioneer axons act as guidepost and scaffolding for follower neurons. Despite years of study, whether pioneer neurons are a molecularly distinct population is unknown. To overcome this challenge, we evaluated the transcriptional profile of peripheral sensory neurons of the zebrafish posterior lateral line (pLL) ganglion during axon growth. Single-cell RNA sequencing (scRNA-seq) of pLL neurons during axon extension revealed two distinct neuronal populations, one of which expressed the neurotrophic factor receptor Ret. We previously showed that Ret was enriched in pLL pioneer neurons. Indeed, marking *ret+* cells with a transgenic marker confirmed their pioneer identity. Transcriptional profiling of differentiating pLL neurons showed that many follower-specific markers are expressed in early pLL progenitors, defining the pLL ground state. It also suggested that a "pioneer" is a later developmental state derived from follower progenitors. Differential expression analysis of the scRNA-seq data set revealed active retinoic acid (RA) signaling in followers, but not in pioneers. Inhibition of RA biased pLL neurons towards pioneer targets, whereas activating RA biased neurons towards follower targets. We discovered that RA downregulation in pioneers is necessary for expression of neurotrophic factor receptor *ret,* which is responsible for correct targeting. This study provides insights into the molecular identity of pioneer neurons and reveals the regulatory role of RA signaling in their development. Funded by NS111419 and NS112795 to AVN.

Program Abstract #264

Inhibition of FGF Signaling Disrupts Development of the Enteric Nervous System

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Development of the enteric nervous system (ENS) starts with colonization of the gut tube by posteriorly migrating enteric neural crest cells (ENCCs). As ENCCs migrate through the gut they are exposed to different signals from surrounding tissues, and from each other, which are crucial for appropriate proliferation, migration and differentiation. By understanding the complex and dynamic signals involved in ENS formation we will have the knowledge to interrogate what happens when irregular patterning of the ENS occurs. Mining our lab's published single-cell RNA sequencing dataset of neural crest derivatives, we discovered that Fibroblast Growth Factor (FGF) signaling pathway components are strongly expressed in the enteric neuron lineage in zebrafish larvae. Specifically, based on unbiased bioinformatic analysis, we found a distinct transcriptional change in FGF pathway prevalence between early neural crest cells and later differentiated enteric neurons, suggesting functional roles for FGF during early enteric neurogenesis. Using zebrafish larvae, we define spatiotemporal gene expression patterns of FGF signaling pathway components using Whole-mount Immuno-Coupled Hybridization Chain Reaction (WICHCR). We also investigate if FGF signaling pathway is functionally active during early ENS development by treating FGF reporter transgenic lines with FGF inhibitor, and capture ENCC-enteric neuron developmental dynamics using in vivo time-lapse confocal microscopy. Furthermore, drug treated embryos were fixed for WICHCR analysis to assay changes in FGF signaling component gene expression levels. Together, these experiments revealed that enteric neurons display strong FGF pathway signaling component expression and that FGF inhibition during early colonization phases stunted ENCC migration, decreased ENCC numbers, and severely abrogated ENS formation, thereby implicating FGF signaling in playing functional role during ENCC development. Funding provided by NSF 1942019.

Program Abstract #265

Identification of transcriptional regulators downstream of Evx1 and Evx2 in zebrafish V0v neurons.

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During development, precise formation of neural circuits is important to elicit relevant behaviors. One important aspect of neuronal specification is the choice and use of neurotransmitter, as it determines whether neurons inhibit or excite other cells within neural circuits. Evidence suggests that neurotransmitter specification occurs during neuronal differentiation through the action of specific postmitotic transcription factors. The zebrafish spinal cord serves as a valuable model system for investigating the specification of functional properties in diverse neuron types. Notably, Evx1 and Evx2 transcription factors (TFs) play a crucial role in determining the excitatory neurotransmitter phenotype of V0v neurons within the vertebrate spinal cord. These TFs are selectively expressed in V0v neurons within the zebrafish spinal cord. To elucidate the regulatory network governing neurotransmitter properties in V0v neurons, we conducted expression profiling of these neurons in zebrafish in wild type and *evx1;evx2* double mutants. Our study uncovered the presence of two molecularly distinct subtypes of zebrafish V0v spinal interneurons at 48h. By this stage, neurons lacking both *evx1* and *evx2* genes transfate into either inhibitory interneurons or motoneurons. Furthermore, our results reveal 25 transcriptional regulator genes whose expression in V0v interneurons is dependent on Evx1/2 and an additional 11 transcriptional regulator genes repressed by Evx1/2 in V0v neurons. Taken together, our findings signify a notable advancement in understanding the gene regulatory networks specifying V0v spinal interneurons. This study was funded by a collaborative NSF grants to SB (IOS:1755340) and KEL (IOS:1755354)

Program Abstract #266

Spatiotemporal regulation of axon specification in the developing zebrafish vagus nerve

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Nervous system function relies on the precise organization of neurons and their axon targets. Neural networks are often organized into topographic maps such that the position of cell bodies corresponds to the position of their axons within target tissues. To study topographic maps during development, we focus on the vagus nerve, a vital component of the parasympathetic nervous system, which is topographically organized along the anterior-posterior (A-P) axis. We previously observed temporal differences in the axon target selection of anterior and posterior vagus neurons, suggesting a potential importance of temporal cues. Using timelapse microscopy, we observed that anterior neurons make axons earlier than posterior neurons. Importantly, delaying outgrowth of anterior neurons disrupts targeting, suggesting that timing of axogenesis influences target selection. While differences in axogenesis timing have previously been attributed to differences in cell birth time, we found that vagus axogenesis timing is independent of birth time. The observation that neurons of the same age can make axons at different times suggests a novel pathway to regulate neuronal differentiation. Rather than being regulated at the level of birth time, we hypothesize that the A-P axogenesis pattern is regulated by extrinsic spatial cues. Heterotopic neuron transplants between anterior and posterior positions showed that neuron position influences the timing of axonal outgrowth, suggesting the presence of a spatial axogenesis cue. Using spatially-resolved RNAseq, we identified candidate axogenesis regulators that are differentially expressed along the A-P axis. We are currently using pharmacological and transgenic approaches to identify the extrinsic signals that regulate the pattern of vagus axogenesis. Together, these findings reveal a novel patterning mechanism in which axogenesis is extrinsically regulated to promote proper innervation patterning. Funding: R00 NS121595 (AJI)

Program Abstract #267

Vagus nerve development: a transcription factor code for viscerotopy?

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During development, the central nervous system establishes precise connections with the body to coordinate organ function. A crucial component of communication between the brain and body is the vagus nerve (cranial nerve X), which innervates multiple organ systems including the heart, lungs and digestive tract to regulate blood pressure, heart rate, respiration and digestion. Despite this important role, the molecular mechanisms guiding the vagus nerve to these organ targets during development remain unknown. We have developed the zebrafish embryo as a powerful model for interrogating vagus nerve development, taking advantage of its optical clarity and genetic accessibility. Using a novel photoconversion-based retrograde axon tracing approach we show that vagal motor neurons (mXns) that project to different organs (e.g. gallbladder, stomach, intestines) are spatially segregated within the hindbrain vagus nucleus. We hypothesize that these distinct mXn "target groups" have distinct molecular identities that guide axon targeting. To test this hypothesis we have generated a developmental scRNAseq atlas focused on cranial motor neurons and have validated the spatially restricted expression of transcription factors and cell-surface molecules within the vagus motor nucleus. We are generating genetic tools to correlate gene expression with target groups, and performing a reverse mutagenesis screen to test the role of these candidates in topographic map formation, revealing preliminary mXn identity phenotypes. We have also observed that mXn axons contact specific subsets of enteric neurons (ENS) during motor axon pathfinding and have begun testing the role of these contacts in guiding topographic motor targeting. This work will reveal how a major pathway of communication between the brain and organs is established during development. Supported by NICHD F32, NINDS and the Koss Family Foundation.

Program Abstract #268

A cell-state switch establishes regenerative competency in the zebrafish vagus nerve

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Peripheral nerve damage caused by injury, disease, or aging is widespread and can often be debilitating. Humans possess a limited capacity to regenerate injured neurons, and the misdirection of regrowing axons is a major barrier to functional circuit reformation and recovery. Thus, a greater understanding of the underlying mechanisms is essential for the development of regenerative therapies. Many factors regulating regenerative growth cone formation and axon outgrowth have been characterized, but the regulation of proper axonal target selection is still poorly understood. The zebrafish is a powerful model for addressing these questions, as it is highly regenerative, genetically tractable, and has optically clear embryos allowing for live imaging. Motor neurons of the conserved vagus nerve extend five primary axon branches into the pharyngeal arches and viscera in a stereotyped and topographically organized manner, making it an excellent model for studying axon decision making. We have developed a novel larval neuron transplantation approach to examine single-cell behavior in regeneration. Using this method to track regenerating axons in different environments, we find that vagus motor axons regrow robustly and with high topographic accuracy. Notably, although Hgf-Met signaling is required for developmental vagus motor axon guidance, we find that it is dispensable for regenerative axon guidance, indicating a regeneration-specific guidance cue. Next, we demonstrate that early embryonic neurons cannot respond to these regenerative cues, showing that regenerative competency is gained during vagus development. Finally, we identify a cell-state switch in which, upon completion of developmental axon guidance, neurons turn off responsiveness to developmental signals, establish topographic identity, and activate regenerative competency. This work reveals novel insights into the processes underlying the acquisition of regenerative capabilities. Funding: R00 NS121595 (AJI).

Program Abstract #269

A novel mediator of Fgf8 signaling in development of vertebrate telencephalon.

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A genome-wide expression screen of metabolism-related genes (MRGs) of chick embryos by whole-mount RNA *in situ* hybridization (ISH), revealed Phactr1 (Phosphatase and actin regulator 1), to be expressed in the ventral telencephalon. Its expression domain was interesting since it coincided with that of the signaling molecule Fgf8, a known regulator of telencephalon patterning. A double RNA *in situ*

hybridization of Phactr1 and Fgf8, on brain sections of the chick embryo at HH23 confirmed that their domains of expression overlap significantly. For further analysis, we performed a spatiotemporal expression profiling of Phactr1 and Fgf8 in the chick telencephalon from stages HH14- HH26. Both Phactr1 and Fgf8 were expressed in the antero-ventral midline of the telencephalon. Existing transcriptomic data also suggests that Phactr1 is a downstream target of FGF signaling in another context, thus, we hypothesized that the expression of Phactr1 might be regulated by FGF signaling in the antero-ventral forebrain. To verify this, we ectopically expressed Fgf8 through *in ovo* electroporation in the chicken brain and observed upregulation of expression of Phactr1. Additionally, ectopic Fgf8 expression also downregulated transcription factors such as Nkx2.1 and Dlx1, which are originally expressed in domains adjoining the ventral midline of the telencephalon. Further, we observed that ectopic Fgf8 expression also induced loss of proliferation. While it is not known what mediates these effects on transcription factor expression and proliferation, our data strongly suggests that Phactr1 is a possible mediator for these effects downstream of FGF signaling. Therefore, now we are performing Phactr1 gain- and loss-of-function experiments to understand the role of Phactr1 in telencephalon patterning. Taken together, unravelling the Fgf8-Phactr1 relation would establish a new paradigm towards understanding vertebrate telencephalon development. Graduate research funded by MHRD India.

Program Abstract #270

Regulation of neural progenitor cells in the developing ventral telencephalon

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Research on neural progenitor cells (NPCs) in the developing brain has been primarily focused on those residing in the dorsal telencephalon. As a result, little is known about mechanisms controlling the developmental progression of NPCs in the ventral telencephalon. Although ventral and dorsal telencephalic NPCs have overlapping properties, they must possess unique features that underlie their distinct cellular outputs. For example, in the mouse dorsal telencephalon most NPCs are found in the ventricular zone (VZ), whereas the subventricular zone (SVZ) is the main site of cell proliferation in the ventral telencephalon. We find that the Hippo pathway components YAP/TAZ and TEAD1/2 have different expression patterns in the developing ventral telencephalon that suggest overlapping and nonoverlapping roles. By utilizing Nestin-cre mediated gene deletion, we find that losses of Yap;Taz and Tead1;2 produce distinct phenotypes in telencephalon development. Loss of Yap;Taz results in premature progression of NPCs to a later developmental state, whereas loss of Tead1;2 results in the stalling of NPC developmental progression and an increase in the early progenitor population. Loss of INSM1, a zinc-finger transcription factor that interacts with TEAD, also expands early-state NPCs in the ventral telencephalon, suggesting that TEAD and INSM1 functionally interact in ventral telencephalic NPCs. Using single-cell RNA-seq, we find that Notch pathway components are upregulated in Tead1;2 knockout ventral telencephalon, which may contribute to the expansion of the early progenitor population. Overall, our findings demonstrate the overlapping and nonoverlapping regulatory functions of YAP/TAZ and TEAD1/2 in the developmental progression of NPCs in the ventral telencephalon. Funding for this study was provided by the National Institute of Health.

Program Abstract #271

Role of Nr2f2 in retinotopic mapping in vivo

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Precise development of visual circuits is essential for vertebrates to adaptively respond to their environment. Visual information is transferred from the retina to the brain by retinal ganglion cells (RGCs), whose axons project in a topographic manner to several brain regions including their primary target, the optic tectum (OT) in fish, chick and amphibians or superior colliculus in mammals. While research over previous decades has identified important molecules regulating topographic map formation, we still lack a full picture of the signaling mechanisms at play. Through whole genome sequencing, our lab has linked defects in retinal axon mapping observed in *who-cares* (*woe*) zebrafish mutants to a loss-of-function mutation in the nuclear receptor subfamily 2, group F, member 2 (*nr2f2*) gene. Using molecular and *in vivo* approaches, we aim to decipher how Nr2f2 regulates the formation of retinotopic maps in vertebrates. Expression of *nr2f2* was observed in the dorsal retina and midbrain of developing embryos by

in situ hybridization, suggesting that *nr2f2* might act cell-autonomously in RGCs or in the axonal environment to control retinotectal mapping. Taking advantage of the Cre-loxP system to express TagRFP selectively in a subpopulation of dorsal RGCs, we are now characterizing the retinotectal defects observed along the dorso-ventral axis in *woe* mutants. Confocal imaging of retinal axons at 3 days post-fertilization revealed that dorsal axons ectopically terminate in the medial OT in *woe* mutants, a region normally occupied by ventral axons. These results demonstrate a previously unreported role for Nr2f2 in governing retinotectal mapping in the vertebrate visual system. Future work will focus on characterizing the retinotectal map along the antero-posterior axis in *woe* and defining which transcriptional programs are regulated by Nr2f2 in RGCs. Funding support provided by the National Institutes of Health (R21NS124542).

Program Abstract #272
Left-Right Asymmetry of Neuroanatomy in the Adult Mouse Brain
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Left-right asymmetry of the human brain is widespread through its anatomy and function. However, limited microscopic understanding of it exists, particularly for anatomical asymmetry where there are few well-established animal models. In humans, the brain shows a subtle, brain-wide series of local asymmetries in cortical thickness or surface area, in addition to a macro-scale twisting in which the right hemisphere is shifted anteriorly relative to the left – the cerebral petalia. While a brain-wide signature of anatomical asymmetry is apparent in chimpanzees, other model species (zebrafish, Drosophila, chickens) show only a single instance, raising questions about whether these isolated examples are distinct from the subtle, brain-wide pattern seen in humans and chimpanzees. Here, we utilize mice to investigate anatomical brain asymmetry, using 6 different cohorts of animals from 5 different research groups. We found an anterior-posterior pattern of volume asymmetry in mice, where anterior regions are larger on the right while posterior regions are larger on the left. This pattern appears driven by a similar trend in surface area asymmetry and is supported by a concordant pattern of positional asymmetries. The cerebral hemisphere as a whole appears shifted anteriorly on the right while the brainstem and cerebellum appears shifted anteriorly on the left. These results together indicate a small torsion of the brain is present in mice, similar to the cerebral petalia in humans. By establishing mice as an animal model for this question, we aim to provide a foundation for future studies to probe the cellular, genetic, and developmental underpinnings of anatomical brain asymmetry in humans. We speculate that our observations reflect molecular chirality of the cytoskeleton, given emerging evidence for its role in humans and its well-established role in asymmetry of invertebrates. This work received support from the Harvard Brain Science Bipolar Disorder Seed Program.

Program Abstract #273
Spatiotemporal Organization of the Developing Mouse Vestibular Ganglion
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The vestibular system of the inner ear, comprised of five sensory end organs as well as their innervating neurons, is derived from a common primordium, the otic placode. During early otic development, neuronal fate determination is orchestrated by a cascade of proneural basic helix-loop-helix (bHLH) gene expression beginning with Neurogenin1 (Neurog1). Transient expression of Neurog1 in a small subset of otic precursors leads to commitment towards the neuronal lineage and subsequent delamination from the otic epithelium to form the cochleovestibular ganglion (CVG). Developmental patterning leads to populations within the CVG adopting either a vestibular or auditory fate and the eventual rise of two anatomically distinct ganglia. Unlike the auditory system where the first order neurons have well-defined topographic maps, the organization of neurons within the vestibular ganglion (VG) does not appear to be spatially organized based on their peripheral or central targets. The mechanism underlying vestibular ganglion neuron (VGN) identity and organization is unknown. To address this, our study investigates

whether sequential and spatial delamination of neuroblasts from the otic epithelium determines the fate and position of individual VGNs. Using Neurog1-creERT2 transgenic mice to lineage-trace the neuronal-fated cells and EdU labeling to determine neuronal birthdates, we uncover distinct temporally regulated neuronal lineages within the VG. Our results indicate that the temporal sequence of neuron production regulates the organization of VGNs, with the earliest born neurons occupying restricted regions within the VG. Additionally, temporally distinct VGN populations appear to innervate specific regions within the vestibular end organs. Currently, we are working to determine the molecular signatures of spatially distinct VGN populations using scRNA-seq and investigating the underlying mechanism regulating VGN identity. Funding: NIDCD Intramural program (1ZIADC000021)

Program Abstract #274

Unraveling how BMP signaling essays diverse roles during cortical development

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The mammalian cerebral cortex, essential for higher brain functions such as cognition and perception, is a complex structure comprising of neurons arranged in six layers. It originates from a single telencephalic vesicle in the neural tube, where precursor cells undergo divisions to generate neurons. These neurons then migrate outward, guided by radial glial cells, and settle into distinct layers, eventually giving rise to the laminar cortex. Any disruptions/aberration in this meticulously regulated process often results in malformation of the brain and has been linked to various neurological disorders. A plethora of molecules have been reported to play important roles in cortical development. One key player in this process is bone morphogenetic protein (BMP) signaling, as demonstrated in an earlier study conducted in our laboratory by Saxena M. et al. (2018). Through this study, bone morphogenetic protein (BMP) signaling has been implicated to play a key role in the regulation of dendritogenesis, polarity establishment and radial migration of upper-layer neurons in the mouse cortex. To gain mechanistic insight into how BMP signaling assays these diverse roles in the developing cortex we have conducted a study to systematically identify specific downstream targets of BMP signaling involved in regulating radial migration and polarity in cortical neurons. For this purpose, we have employed a combination of techniques including neural progenitor cell cultures, bulk mRNA sequencing, spatial transcriptomics and in utero electroporation. Our results reveal promising candidate genes and pathways which are likely to be involved in guiding the radial migration and polarity establishment of newly formed upper-layer cortical neurons. In future, this ongoing study is expected to reveal the complex regulatory gene networks governed by BMP signaling and their contribution to the remarkably coordinated process of cortical neurogenesis. Funding: Department of Biotechnology, India.

Program Abstract #275

EMX2 regulates planar cell polarity by blocking STK32A-mediated regulation of GPR156 in mouse inner ear hair cells

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Hair cells in the vestibular maculae are divided into two groups with polarized stereociliary bundles oriented in opposite directions enabling detection of a broad range of motion. Both groups are aligned along a polarity axis established by core Planar Cell Polarity (PCP) proteins but each interprets that axis differently to generate characteristic bundle orientations. EMX2, GPR156, and STK32A have been shown to have a role in how these two groups of cells integrate PCP signals. We tested the interactions of these factors through genetic epistasis experiments in which hair cells were evaluated in mice with mutations in *Gpr156* and *Stk32a, and Emx2* and *Stk32a*. We found that in the *Gpr156*;*Stk32a* double knockout (dKO) utricle, hair cells were uniformly oriented in a single direction, phenocopying the *Gpr156* KO, and rescuing the misoriented bundle phenotype seen in *Stk32a* KOs. Moreover, hair cell misorientation in *Stk32a* KOs coincided with ectopic redistribution of GPR156, and protein interaction assays confirmed the binding of STK32A and GPR156. Together these results suggest that STK32A negatively regulates the function of GPR156 in one group of hair cells. Previously we showed that EMX2 blocked *Stk32a* transcription in the second group and thatthe *Emx2* mutant phenotype included upregulation of *Stk32a*. Consistent with this, in the *Emx2*;*Stk32a* dKO, the *Emx2* mutant phenotype was transiently rescued, presumably because without STK32A, the GPR156 function is restored. The resulting phenotypes support a functional

relationship between these factors in which hair cells expressing EMX2 negatively regulate the expression of *Stk32a*, thereby allowing GPR156 accumulation at apical cell boundaries where it reorients stereociliary bundles to form a line of polarity reversal. In the absence of *Emx2*, all hair cells express STK32A, the GPR156 function is blocked, and all stereociliary bundles orient in the opposite direction by default. This work was supported by NIH R01DC013066.

Program Abstract #276
Uncovering the role of Importin9 in Hedgehog Signaling
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In vertebrates, proper Hedgehog (Hh) signaling requires the cilium. Components of Hh signaling, such as the Hh transducer SMO and the GLI transcription factors, localize dynamically to cilia in the presence of Hh ligand. Protein entry and exit are regulated by several mechanisms including transport carriers that facilitate the translocation of ciliary proteins. Precise control of the Hh pathway is critical as abnormal activation or suppression of Hh signaling causes cancer or birth defects. We identified a recessive, ENU-induced mouse mutant in *Importin9* (*Ipo9*) that exhibits embryonic lethality and morphological defects consistent with abnormal Hh signaling. IPO9 is a karyopherin with established roles in nuclear transport, yet we found IPO9 localized to the base of cilia. Another karyopherin, importin-β2, transports proteins into the cilium in a RAN GTP-dependent manner, suggesting that in addition to nuclear transport, importins are involved in ciliary transport. An independent, unbiased genome-wide CRISPR screen identified IPO9 as a novel regulator of Hh signaling. We generated *Ipo9* mutant cells and observed normal ciliation rates and cilia length. We found a reduced *Gli1* expression in *Ipo9* mutant cells after stimulation with either Hh ligand or SMO agonist, SAG, indicating reduced Hh response. As SAG directly activates SMO, this argues that IPO9 regulates the Hh response at a step downstream of SMO. IPO9 interacts with ARL13B, a ciliary GTPase that regulates Hh signaling downstream of SMO, where GLI proteins function. GLIs are either cleaved into transcriptional repressors (GLIR) or processed into activators (GLIA) and their relative amounts regulate the transcriptional output of the Hh pathway. While cilia regulate both GLIA and GLIR, ARL13B regulates GLIA and not GLIR. Together, these data support a model where IPO9 regulates the Hh response, potentially as an effector of ARL13B in regulating GLIA. This research is funded by NIH grant # 5R35GM148416.

Program Abstract #277
Un-LOX-ing the roles of Loxl1 in zebrafish meningeal development
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The meninges are membranous tissue layers that surround, support, and protect the central nervous system, yet there is little known about their development and the various cells that populate them. Fluorescent granular perithelial cells (FGPs) are a perivascular cell type that has recently been identified in the zebrafish leptomeninges based on their expression of known lymphatic endothelial cell markers. However, there is a current lack of unique molecular markers for these cells. To this end, we conducted single-cell RNA-sequencing (scRNA-seq) of adult zebrafish meninges and identified*lysyl oxidase-like 1* (*loxl1*) as specifically expressed by FGPs in the leptomeninges. In situ hybridization chain reaction (HCR) confirmed *loxl1* expression in FGPs of both zebrafish larvae and adults. Loxl1 is a secreted extracellular matrix (ECM) cross-linking protein that is required to stabilize elastin and collagen fibers and can be chemically inhibited with the pan-lysyl oxidase (LOX) inhibitor BAPN. BAPN treatment of zebrafish embryos resulted in significantly reduced FGP number in the optic tecta as well as smaller meningeal vein diameter in larvae at 5 days post-fertilization, suggesting LOX activity is required for FGP and blood vessel development. These data are consistent with previously reported roles of LOXL1 in cancer cell development in vitro via promotion of cell proliferation, migration, and survival, as well as angiogenesis. Zebrafish FGP and meningeal blood vessel development thus present an ideal model in which to study the roles of *loxl1* in cell development in vivo. This work is supported by an NICHD R00 Pathway to Independence Award to M.V.G. (R00HD098273), and by the University of Utah T32 Genetics Training Program (T32GM141848) to M.H.

Program Abstract #278

Leptomeningeal-neural organoid (LMNO) fusions as models for studying neurodevelopment.

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Neural organoids derived from human pluripotent stem cells are useful models that have been key to advancing knowledge of human CNS development. However, neural organoid platforms lack non-neuronal cell types that produce factors critical for proper CNS development such as the meninges, the fibrous tri-layer structure that encases the CNS. Most meninges-neuron signaling studies are conducted using rodent models, or in cell culture models that fail to recapitulate the 3D architecture of the meninges-brain interface. We established a model for studying meningeal-neuronal interactions during development, called Leptomeningeal-Neural Organoid (LMNO) fusions. LMNOs are generated by co-culturing *Col1a1-GFP*+ transgenic mouse leptomeninges (meningeal fibroblasts are GFP+) with day 15 human iPSC-derived neural organoids. The leptomeninges and organoids fuse within 6 days in culture and remain fused throughout culture duration (30 to 60 days). Neural organoid and meningeal compartments retain key cell-type characteristics; meningeal cell types like arachnoid barrier cells and leptomeningeal border-associated macrophages were observed at day 30 and 60 in culture. Using activated Caspase-3 to assess cell viability, we found the meninges were neuroprotective at 30 days. We also observed REELIN+ organoid-derived cells in the meningeal compartment of LMNOs. *In vivo*, REELIN+ Cajal-Retzius cells migrate from in response to meningeal fibroblast-derived Cxcl12. The meningeal part of the LMNOs expressed *Cxcl12*, suggesting the REELIN+ Cajal-Retzius-like cells derived from the organoid migrated to the meningeal compartment in response to Cxcl12, mimicking *in vivo* events. The LMNO system is a novel framework for the culturing neural organoids with meninges, and recapitulates key features of tissue development and composition. This system will be useful for future studies interrogating meninges-neuronal signaling during brain development and homeostasis. Funding: 1RF1MH123971, F31NS125875.

Program Abstract #279

Molecular mechanism regulating the fate and function of the *Drosophila* sleep-wake circuit

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Behaviors conserved across species rely on neural circuits comprising diverse cell types. How neural stem cells produce diverse neural cell types is an open question in neurobiology. We use the *Drosophila* central complex as a model to investigate the neural stem cell-specific genetic, and developmental programs that specify diverse cell types and circuits. Currently, my work focuses on the development of a sleep-wake circuit, which includes sleep-promoting dorsal fan-shaped body neurons that inhibit downstream helicon cells to promote sleep. Previous results from our lab have identified unique Type II neural stem cells that generate the dFB neurons. These Type II neural stem cells express IGF2 mRNA-binding protein (Imp). Preliminary data indicate that the number of sleep-wake neurons is reduced upon Type II NSC knockdown of Imp. I will present the role of Imp in specifying cell types of the sleep-wake circuit. The National Science Foundation, the Sloan Foundation, and the Brain & Behaviour Research Foundation supported this research.

Program Abstract #280

Characterizing the mechanism of action of two genetic regulators of dopaminergic neuron regeneration in the planarian brain

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Although the ability to regenerate tissue after injury exists in many species across the animal kingdom, the ability to regenerate a brain is rare. One organism that can regenerate a brain de novo is the planarian. Planarians are flatworms with robust regenerative capability and a complex nervous system consisting of a brain, ventral nerve cords, and a peripheral nervous system (PNS). Our lab uses the asexual planarian Schmidtea mediterranea to elucidate how robust regeneration occurs in nature. To parse the mechanisms underlying complex neural regeneration, we began with dopaminergic neurons. Dopamine has been linked to locomotive behaviors as well as neurogenesis in many organisms, making these

neurons an attractive candidate with which to study the journey from stem cell to mature neuron during regenerative neurogenesis. In planarians, dopamine is synthesized through an evolutionarily conserved pathway involving the enzyme tyrosine hydroxylase (th) which we can use as a marker for dopaminergic neurons. Using single-cell transcriptomic data, we identified 73 candidate genes with enriched expression in th+ cells. Next, we knocked down candidate genes and then assessed the impact on dopaminergic neuron regeneration. We identified seven key genes required for th+ cells in the brain or PNS. Knocking down two genes, amyloid-beta protein precursor or lim domain only 3, significantly decreased the number of th+ cells in the brain. We are currently discovering whether these genes are specific to dopaminergic neurons or play roles in the regeneration of other neurons. Our work will uncover cellular and molecular mechanisms by which genes govern dopaminergic neuron regeneration, which will provide a framework for elucidating the genetic pathway for the regenerative neurogenesis of other neurons in planarians. This work was supported by funding from the NIH/NINDS, Alfred P. Sloan Foundation, McKnight Foundation, ARCS Foundation, and a T32 from NIH/NIGMS.

Program Abstract #281

What can a single embryo tell you? Discovering the Embryonic Dopaminergic Neural Development and How it Influences Adult Social Behavior

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In recent years, researchers have been diligently working to uncover the profound details of the dynamic, intricate relationship between neural circuits and social behavior. Dopamine stands out as a key player, known to regulate emotion, pair bonding, and mood disorders. A fundamental aspect of social behavior observed in various species, including humans, is pair bonding, the formation of long-lasting social bonds between mating partners. In mammals, oxytocin and vasopressin have been extensively studied for their role in coupling. However, the role of neurotransmitters such as dopamine in pair bonding is poorly studied in Convict cichlid fish species. *Amatitlania nigrofasciata*, known as having strong pair bonding and commitment to biparental care, is a fascinating model for studying monogamy. These fish form a long-lasting partnership with males and females, actively protecting nests, rearing eggs, and caring for the fry. In contrast, *Mchenga conophorus* and *Danio rerio* are chosen as an alternative model for non-monogamous (polygamous) behavior. This provides valuable comparative data for understanding the neurobiological aspects of social attachment between partners. We use a multidisciplinary approach to investigate the role of dopamine signaling in defining monogamous behavior for fish. Tyrosine Hydroxylase (TH) is the rate-limiting enzyme in synthesizing dopamine production and is commonly used as a marker to identify dopaminergic neurons. Results have shown an increased expression of TH in monogamous fish via RNA-ISH was observed in brain regions associated with social motivation in fish. In this study, *Amatitlania nigrofasciata* has a gradual ontogeny compared to polygamous fish, potentially influencing the development of monogamous behavior through increased TH levels in the early stages of development. We would like to thank to the Department of Biology and The Graduate School at Georgia State University for their support throughout this research.

Program Abstract #282

Identifying and Characterizing Multisensory Integrating Neurons in the Zebrafish Optic Tectum

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The superior colliculus (SC) is a mammalian midbrain structure involved in multimodal sensory integration and is implicated to have a role in neurodevelopmental disorders. Although the presence of multisensory integrating neurons (MINs) in the SC has been well documented by electrophysiology techniques, little is known about their morphological or molecular characterization. To identify and study MINs, we utilized SC's non-mammalian homologous structure–the optic tectum (OT)–in the genetically tractable model organism zebrafish. In this process, we generated transgenic lines that allowed for fluorescent detection of neuronal activity by expressing the genetically engineered calcium indicators: cytoRGECO and H2B-jRGECO1a, respectively in mechanosensory hair cells of the ear and OT. To activate the vestibular sensory pads in 7 days-post-fertilization (dpf) larvae, we used a piezoelectric actuator probe. We found that upon the application of this vestibular stimulus in the posterior cristae (PC), some OT neurons showed time-

locked activation. This indicates that OT circuitry in 7dpf larvae is mature enough to receive vestibular stimuli. Furthermore, when 7dpf larvae were sequentially and simultaneously exposed to vestibular and visual stimulations (1 second pulse of 488nm light), we found OT neurons that responded to concurrent stimuli and showed characteristics of multisensory integration. Identification of MINs in the zebrafish OT sets up the stage for better morphological and molecular characterization and their role in neurodevelopmental disorders using a genetically tractable model. Funding by BYU Gerontology Program

Program Abstract #283

Valproic Acid Treatment Alters Cellular Composition within the Optic Tectum of Larval Zebrafish

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The integration of multi-modal sensory inputs (e.g., visual, auditory) into a cohesive framework is critical for guiding an organism's interactions with its environment. In mammals, this function is partially executed by the superior colliculus (SC), a midbrain structure which utilizes multisensory integration to guide involuntarily behaviors toward or away from relevant environmental stimuli, ultimately influencing social behavior. Accumulating evidence has implicated the SC in neurodevelopmental disorders such as autism spectrum disorder (ASD), highlighting the need to understand its developmental mechanisms. One such mechanism involves the regulation of neuronal differentiation through the redox state of the cell. Proper regulation of redox states is crucial as it leads to protein modifications, which fundamentally alters protein function within the cell and the tissue as a whole. Interestingly, valproic acid (VPA)–an antiepileptic drug–has been shown to both affect the redox state of differentiating neurons in culture and is associated with higher incidence of ASD in children following fetal exposure. To better understand the connection between redox stress, VPA, and regions of multisensory integration like the SC, we treated developing zebrafish with VPA and characterized the development of the optic tectum (OT)–the homologous non-mammalian structure of the SC. We found a delay in neuronal specification and the absence of at least one neuronal subtype within the OT following VPA treatment. Furthermore, single-cell RNA sequencing in treated vs untreated larvae reveals changes in neuronal composition and depletion of certain glial populations within the OT. Together, these data provide strong evidence that VPA affects development of the OT and, by association, might influence human neurodevelopment and the possible molecular changes leading to ASD. Funding: NSF GRFP 2023350047 & NINDS: R15NS131998-01A1

Program Abstract #284

Investigating a tumour-eliminating mechanism in the *Drosophila* central nervous system

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Dedifferentiation has great potential for delivering tissue regeneration; however, uncontrolled dedifferentiation can cause tumorigenesis. To elucidate how these two outcomes are controlled, we take advantage of the *Drosophila* central nervous system (CNS), where neurogenesis and tumorigenesis have been well characterised. We and others have previously shown that in the *Drosophila* CNS, the loss-of-functions of transcription factors such as Nervous finger 1 (Nerfin-1) or Longitudinals lacking (Lola) can induce neuronal dedifferentiation, which results in the formation of ectopic neural stem cells. We found that whether dedifferentiated cells become malignant is dependent on their positional identity. This mirrors what has been observed in human brain cancers, where stem cells in different brain regions display different susceptibility to oncogenic insults. Using genetic manipulations and RNA-sequencing, we demonstrate that the selective vulnerability to tumour malignancy in different regions of the *Drosophila* brain is governed by the differential expression of the oncogenic factor Chronologically inappropriate morphogenesis (Chinmo). Furthermore, we found that dedifferentiated cells in different parts of the brain exhibited distinct responses to extrinsic factors such as the steroid hormone ecdysone. Hence, manipulations of these factors are sufficient to alter tumour malignancy. Together, our work shed light on how regionalised patterning in the developing CNS can determine the malignant competency of dedifferentiated-induced tumours. Funding sources: The University of Melbourne.

Program Abstract #285
The inflammation-mediating cyclooxygenase pathway regulates embryonic development
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Over 30 million people take non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen daily. However, taking these drugs during pregnancy is linked to a greater risk of birth defects. A subset of the defects resulting from NSAID use can be characterized as neural tube defects that affect the central nervous system or neurocristopathies that affect the 30 tissue and cell types derived from neural crest cells. Though we know NSAIDs inhibit cyclooxygenase (COX) isoenzymes, the role of the COX pathway is understudied in early embryonic development. Using avian embryos, we have characterized the earliest onset and spatiotemporal expression and localization of genes and proteins in the COX pathway including enzymes, effectors, and receptors. To uncover the role of COX pathway signaling in neural tube and neural crest formation, we performed gain and loss of function experiments in *Gallus gallus* (chick) embryos during key neural tube and crest developmental stages. We saw that loss of COX1, and a downstream receptor EP_3, reduced the number of neural crest cells, inhibited neural crest cell migration, and prevented closure of the neural tube. In contrast, neural crest cells were unaffected after loss of COX2. Increasing COX1 and COX2 inflammatory signaling by overexpression led to apoptosis-induced cellular extrusion into forming neural tubes. My research demonstrates a previously uncharacterized role of the inflammation-mediating COX pathway in embryonic neural tube and neural crest development. T.A.L. was supported by the NSF GRFP award #2036201.

Program Abstract #286
BRWD3 is a novel polymicrogyria gene
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Polymicrogyria is a neurodevelopmental condition defined by excessive folding of the neocortex due to disruptions in neural migration which lead to malformed sulci and gyri. The condition has an array of clinical presentations including impaired intellectual development, seizures, and cerebral palsy. Whole-genome sequencing of a patient with polymicrogyria revealed a likely pathogenic variant in the X-linked gene Bromodomain and WD-repeat domain containing 3 (*BRWD3*). Bromodomains are regions on histone acetyltransferases that are involved in the epigenetic regulation and gene activation. Malformations in the human neocortex such as macrocephaly and microcephaly have previously been associated with pathogenic variants in bromodomain coding regions within the genome. Here, we created a mouse model of *Brwd3* recapitulating the human variant (S1320L). In situ RNA hybridization revealed *Brwd3* is differentially expressed in the neocortex at various points in embryonic development, as well as the basal ganglia, hippocampus, and neural tube. *Brwd3* S1320L mosiac and hemizygous male mice survive at normal rates. Immunohistochemistry revealed an abnormal distribution of known neocortical layer markers, CTIP2 and SATB2, in S1320L mice at P90. Neuronal migration was more directly observed through tracking proliferating neurons in the neocortex through an EdU pulse chase experiment suggesting compromised migration. We are now using RNA-sequencing and ATAC-seq to determine the genomic consequences for this variant in *Brwd3*. Thus, we have established a new mouse model for polymicrogyria based on a novel human variant. This work is funded by the Society for Developmental Biology's Choose Development! Summer Fellowship (BC) , The Ohio State University's Undergraduate Research Scholarship (BC), as well as the recruitment funds from the Steve and Cindy Rasmussen Institute for Genomic Medicine at Nationwide Children's Hospital (RWS).

Program Abstract #287
Temporal Modulation of COE Transcription Factor Modes in Neural Development
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Across species, Collier/Olf1/EBF (COE) transcription factors (TFs) are involved in neural development. In humans, COE mutations lead to a neurodevelopmental syndrome involving severe intellectual disability and ataxia. In *C. elegans*, COE TFs act as terminal selectors (mode 1). Terminal selectors are TFs

maintained over the life of neurons and maintain features important for their life-long function, such as the expression of neurotransmitters and cell surface proteins. But, in more complex CNSs, COE TFs can operate in a different mode (mode 2). For example, in the spinal cord, various COE TFs are transiently expressed in neurons. Here, COE TFs stabilize commitment to a neural cell fate and suppress reversion to stemness. It is likely that both modes can operate in complex CNSs. However, little is known about how neurons choose among different COE modes. There are multiple interacting variables--progenitor domains (space) and the time of a neuron's birth during neurogenesis (time). Here, I use neuronal progeny from a single progenitor in the embryonic Drosophila nerve cord to deconvolve these variables. Specifically, I analyze the sole Drosophila COE TF, Collier, in the NB3-3 lineage (constant space), which generates EL neurons at different times (variable time). In ELs, I find both COE modes. In early-born ELs, COE expression is maintained and required for the EL subtype marker, Acj6. Whereas in late-born ELs COE expression is transient and required to establish the expression of the EL identity gene, Eve, which is not dependent on COE expression in early-born ELs. Further, I find COE expression in early- and late-born ELs is regulated through separate enhancers. In summary, my data show that within a single lineage, time can modulate COE mode: Early-born ELs tend to be in mode 2, and later-born ELs in mode 1. This provides insights for COE TF regulation in neural development and potential mechanisms of pathogenesis. This work was supported by NIH T32HD055164.

Program Abstract #288

A novel hyaluronidase is expressed by oligodendrocyte progenitors and generates hyaluronan fragments that regulate oligodendrocyte differentiation and myelination

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Oligodendrocytes (OLs) generate the myelin of the central nervous system (CNS) that is critical for the survival and high-speed conduction velocities of neurons. OLs develop from OL progenitor cells (OPCs) that arise from ventricular germinal zones of the embryonic neural tube. OPCs proliferate and migrate in developing white and gray matter before differentiating into OLs that myelinate growing axons. We found that digestion products of the glycosaminoglycan hyaluronan (HA) generated by hyaluronidase activity in white matter blocked OPC maturation and remyelination. This activity is linked to a specific hyaluronidase, the Cell Migration Inducing and hyaluronan binding Protein (CEMIP), that is expressed by early embryonic OPCs. Using qPCR, we found that CEMIP expression is developmentally regulated, with transcript levels increasing in the brains of mouse embryos with increasing age, coincident with Olig2 transcript levels. Immunofluorescence revealed that OPCs express CEMIP in a variety of areas throughout the developing and adult brain, including the median eminence and the arcuate nucleus. Embryo-derived OPC cultures expressed CEMIP, and this expression decreased over time as the cells differentiated into myelinating OLs. Increased CEMIP expression and HA fragments generated by CEMIP delay OPC differentiation into OLs through a toll-like receptor 4-dependent pathway. Furthermore, CEMIP-derived HA fragments block functional remyelination in vivo. These findings support the hypothesis that CEMIP is a critical regulator of the onset and progression of myelination, which acts by generating digestion products of HA that block OL differentiation during early embryogenesis. CEMIP expression is then repressed in the perinatal period to promote myelination at appropriate developmental stages. Funding: Grant MS160144 from the Congressionally Directed Medical Research Programs, RG4843A5/1 from the National Multiple Sclerosis Society, and NIH grant P51OD011092.

Program Abstract #289

The BMP gradient formation depends on cell contraction and the interaction between Frazzled and E-cadherin

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A long-standing problem in developmental biology is how a dynamic field of moving cells acquire positional information while being displaced in space. We previously showed that the DPP/BMP-4 gradient organizes stereotyped cell movements in the Drosophila blastoderm stage by inducing cell constriction in the dorsal midline that pulls lateral cells to the dorsal region. This cell constriction depends on the epithelial polarity genes *frazzled* (*fra*), *guk-holder* (*gukh*) and the cell junction protein E-cadherin (ECAD). These cell movements result in DV patterns of cell density packing that allow the BMP gradient to

achieve its peak and establish precise cell fates. Thus, cell movements enable precision of morphogen response. It is unknown how FRA, ECAD and cell contraction enhances the BMP signaling. Previous research show that FRA stabilizes ECAD at the membrane and ECAD binds to BMP receptors. We hypothesize that FRA/ECAD are targets of Presinilin (PSN) and FRA protects ECAD from excessive cleavage by competition. We confirm that loss of PSN increases the levels of FRA and ECAD, and generates the expected effects of increased BMP activity and cell constriction. Using super-resolution microscopy, we show that ECAD and the BMP receptor TKV indeed co-localize in the dorsal region of the embryo. Cells with peak BMP signaling have fewer ECAD-TKV complexes than adjacent cells with lower BMP levels. This is reminiscent of the developing wing disk, where TKV levels are lower within peak regions of BMP and higher in low BMP regions. We are testing whether ECAD-TKV form high vs. low density clusters in the regions of peak and low BMP signaling, and whether the internalization of TKV is more efficient in the dorsal midline. Our results bring a previously unexplored regulatory component in the DPP signaling and provides a novel mechanism that explains how an environment with high BMP signaling can avoid the disruption of cell junctions and subsequent cell invasion as seen in cancer.

Program Abstract #290

Functional analysis of insect cuticle components using ectopic expression systems.

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The insect cuticle is an extracellular matrix secreted by epidermal cells that serves as the exoskeleton. Its main components are chitin, a polysaccharide, and hundreds of cuticular proteins. Mutants of chitin biosynthetic pathways and cuticular proteins exhibit body shape abnormalities. We have shown that several *Drosophila* cuticular proteins are required for the formation of specific cuticle structures that control the body shape via physical properties of the cuticle. Then, how do cuticular proteins and chitin produce structures in the cuticle? Coexistence of many cuticular proteins with chitin in the native cuticle makes it difficult to analyze directly the specific functions of individual molecules. Therefore, we decided to induce chitin biosynthesis and expression of cuticular proteins in Drosophila organs that do not intrinsically produce cuticle. First, we induced chitin synthesis in the larval wing imaginal disc and the salivary gland by simultaneous expression of *krotzkopf verkehrt* (*kkv*), encoding a chitin synthase, and *rebuf* (*reb*), encoding a protein with an MH2 domain. Chitin was detected in the lumens of both organs. In the wing disc, chitin formed a fibrous, planar sheet, while chitin synthesized in the salivary gland appeared punctate. This may be due to differences in organ geometry or cell properties.. Next, *obstructor-E* (*obst-E*), which encodes a cuticular protein with three chitin-binding domains that is required for three-dimensional folding of the larval cuticle, was co-expressed in the wing disc with *kkv* and *reb*. A matrix containing both chitin and Obst-E was formed. This matrix showed three-dimensional protrusions, in contrast to the planar structure formed by chitin alone. The change may reflect the endogenousfunction of Obst-E in regulating cuticle structure. Funding: KAKENHI 20H05945, Suntory Rising Stars Encouragement Program in Life Sciences, Toray Science and Technology Grant 22-6305 and JST FOREST Program JPMJFR224W.

Program Abstract #291

Serotonergic signaling through 5-HT-2A, B and C receptors is required for morphogenesis of neural crest derived tissues in chicken and frog embryos.

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Serotonergic signaling through 5-HT2B receptor impacts development of cardiac, craniofacial and eye tissues in mouse and frog embryos. However, questions remain regarding the mechanisms of the 5-HT2B, and of the 5-HT2A and C receptors, in the morphogenesis of neural crest derivatives. Here we studied the influence of 5-HT2A, B and C signaling on cardiac neural crest development in chicken embryos, and on craniofacial and pigment cell morphogenesis in frog embryos. Chicken embryos at pre-migratory crest stage (HH8) were treated with 20μM 1-methylpsilocin (1-MP), an inverse agonist for 5-HT2B and agonist for 5-HT2C. To focus on the phenotypic effects of 5-HT2B in the 1-MP-treated embryos, we applied SB242084 (10μM), a 5-HT2C antagonist, before 1-MP treatment. Chicken embryos were collected at HH14, and at HH32/36 to assess for defects in cardiac neural crest migration, and in structural heart development,

respectively. *Xenopus laevis* frog embryos were treated at early tailbud stages with 10 µM SB242084 or 25CN-NBOH, an agonist for 5-HT2A receptor. These embryos developed to late tailbud stage when they were examined for defects in craniofacial, eye and pigment cell morphogenesis. In chicken embryos, application of 1-MP (together with pre-treatment with SB-242084) led to altered migration patterns in cardiac neural crest cells at HH14, and to defects in the aorticopulmonary and interventricular septa, cardiac crest derivatives, at HH32 and HH36. In frog embryos, treatment with SB242084 resulted in significantly smaller heads, malformed and smaller eyes, and more pale and smaller bands of pigment cells, whereas application of 25CN-NBOH resulted in larger eyes and smaller pigment cell bands, compared to controls. These results suggest that serotonergic signaling through the 5-HT-2A, B and C receptors are required for morphogenesis of neural crest derivatives in both chicken and frog embryos. Funding NIH R151R15DA058203; Kadner Pitts.

Program Abstract #292

Role of *Six1* in the development of cranial bone and suture in the mouse

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Craniofacial anomalies, such as those affecting the development of the cranial bones and sutures, constitute one-third of all congenital birth-defects. Branchio-oto-renal (BOR) syndrome is such a birth-defect that presents with hearing loss and craniofacial and kidney defects. *SIX1* or *EYA1* mutations are found in 50% of BOR patients, while the cause of the remaining 50% is still unknown. SIX1 is a transcription factor, and EYA1 is its co-activator. The mutation in *SIX1* was recently linked to craniosynostosis, where the cranial bones fuse early and hinder brain development in young children. In addition, previous studies showed that SIX1 function is co-factor dependent and required for craniofacial osteogenesis. Based on these data, we hypothesize that SIX1 regulates cranial bone development, and this function is co-factor dependent. To test this hypothesis, we are characterizing three novel co-factors of SIX1, SOBP, MCRS1, and PA2G4 in mice recently identified in *Xenopus*. Our findings indicate that while SOBP, MCRS1, and PA2G4 are expressed with SIX1 in the developing face of mice, only SOBP and MCRS1 are bona fide SIX1 co-factors. Next, we are using the *Six1*-KO mouse line, neural crest cell (NCC), and pre-osteoblast cell (OPC) lines to characterize the role of *Six1* in the development of the cranial bones, particularly frontal and parietal bones. Mutant mouse embryos present defects in these bones and associated sutures, which demonstrates that SIX1 is needed for the development of these structures. Altogether, our work is generating new knowledge on the mechanism of action of SIX1 in cranial bone and suture development to find novel therapeutic targets. Funding: NIH R03 DE028964 and K01 DE031783-01A1 to Andre L.P. Tavares, The George Washington University School of Medicine & Health Sciences, The Delaware Center for Musculoskeletal Research, and The University of Delaware College of Arts & Sciences.

Program Abstract #293

Potential interaction at the protein level between Hey factors and Six1

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Approximately 35% of birth defects result in craniofacial anomalies, such as those derived from mispatterning of neural crest cells (NCCs) and placodes that give rise to structural and sensory elements of the face and skull. Prior studies show that the transcription factor Six1 is required for proper patterning of the NCCs and placodes. To function, Six1 requires co-factor binding, such as Eya1 co-activator. Mutations in the genes encoding these two proteins lead to Branchio-otorenal (BOR) syndrome, characterized by craniofacial anomalies, hearing loss, and kidney defects. Currently, genetic screening for BOR mutations is performed primarily for *six1* and *eya1*. However, mutations in these genes account for only ~45% of BOR cases. This suggests there are other causative genes yet to be identified. Yeast-2-Hybrid analysis using Xenopus larvae suggest that Hey1 and Hey2 may be putative binding partners of Six1. Available expression data in *Xenopus* show that these two genes are expressed with Six1 in NCCs and otic placode. In addition, in mouse, Six1 and Notch signaling interact during patterning of the mandibular arch NCCs. Altogether, these data support the hypothesis Hey1 and Hey2 act as Six1 co-factors during craniofacial development and could be responsible for defects observed in BOR patients. To test this hypothesis, I am performing *in situ* hybridization to examine the spatial and temporal expression of *hey1* and *hey2*, co-immunoprecipitation assays to determine whether they bind Six1, and

luciferase assays to determine if they modulate Six1 transcriptional activity and/or Notch activity. Then, loss- and gainof- function experiments will be performed in *Xenopus* using CRISPR/Cas9 and morpholino injection or introducing exogenous mRNA to verify if they, along with Six1, regulate patterning of NCCs within the branchial arches and the otic vesicle. Funding Sources: University of Delaware College of Arts & Sciences

Program Abstract #294
Exploring the role of Claudin-3 in chick neural tube mechanics
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The neural tube, the embryonic precursor to the brain and spinal cord, begins as a flat sheet of epithelial cells whose edges, the neural folds, elevate upwards and fuse along the dorsal midline of the embryo. Failure to fuse the neural tube causes neural tube defects, such as anencephaly or spina bifida. We found that depletion of a tight junction protein, Claudin-3 (Cldn3) in chicken embryos, causes spinal neural tube defects due to failure in neural fold fusion. The apical cell surface morphology of Cldn3-depleted cells is altered; there is increased membrane blebbing at cell-cell contacts and smaller apical surfaces. F-actin is reduced at apical bicellular junctions. Our live imaging analyses identified differences in how the neural folds of chick embryos come together in the spinal vs. cranial region. We observed that the neural folds in the future spinal region of chick embryos make contact at distinct points and then the regions between these contact points fuse. This is distinct from the progressive anterior to posterior zippering from the first point of fusion that is observed in the cranial region. Given that depletion of Cldn3 causes spinal neural tube defects, and the loss of F-actin at the apical-lateral cell membrane, we hypothesize that Cldn3 plays a role regulating cell movement and shape changes required for the biomechanical process of spinal neural fold fusion. To test this hypothesis, we are using live imaging to characterize spinal neural fold fusion in control and Cldn3-depleted embryos to quantify apical cell shapes, movements, and junctional rearrangements. Future work will test mechanical properties of cells during spinal neural fold fusion in control and Cldn3-depleted embryos. This research is working towards a better understanding of the morphogenetic events of neural fold fusion and the specific mechanisms controlled by Cldn3. Funding for this project is provided by an NSERC Discovery Grant.

Program Abstract #295
Analysis of strain-specific developmental tempos and rates of branching morphogenesis
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Development occurs as a highly coordinated sequence of events, whose order and timing are critical for the generation of tissue form. Branching morphogenesis generates functional form across several organs, including the lung, kidney, pancreas, and salivary gland. Synchronized timing of proliferation and differentiation are essential for building properly branched epithelia within each organ. This timing must also be regulated across the embryo to ensure functional maturity by the time of birth. To interrogate developmental tempos in mice, we took advantage of mouse strains with different gestational lengths. We evaluated the tempo of embryonic development of each strain using morphological measurements of crown-rump length, webbing of forelimb and hindlimb, intranasal distance, and length of long bones. In parallel, we evaluated the rates of branching morphogenesis in different organs in each strain by quantifying the number of epithelial branch tips. We found a strong correlation between gestational length and developmental tempo, with shortest gestational length correlating with the fastest tempo and rate of branching morphogenesis. These data reveal that embryos from different strains of mice display developmental allochrony, which provide insight into how the embryo keeps time. This work is supported through NIH Director's Pioneer Award (5DP1HD111539-02).

Program Abstract #296
Local changes in epithelial tension differentially regulate specific branching modes during early lung development
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In the developing lung, the embryonic airways originate as a wish-boned shaped tube, which undergoes a series of branching events to build the bronchial tree. This process involves two distinct branching modes: lateral branching, wherein daughter branches emerge along the length of a parent branch, and bifurcations, where the tip of a parent branch splits to form two daughter branches. The embryonic airways are fluid-filled, and recent studies have suggested that altered luminal pressure can influence rates of airway branching morphogenesis. Still, it is not clear how local changes in epithelial tension impact the cellular behaviors that underlie the formation of new epithelial buds, nor whether individual branching modes are differentially affected by increased fluid pressure. Here, we use microinjected fluid droplets to locally perturb epithelial tension along the developing airway in cultured embryonic mouse lungs. These injections were localized to regions of the bronchial tree that primarily undergo either lateral branching or bifurcations. Local dilations of the embryonic airway suppressed the formation of new lateral branches, perturbed the patterns of cell displacements that sculpt epithelial buds, and led to increased smooth muscle wrapping along the airway epithelium. However, microinjected droplets in bifurcation-forming regions did not alter the number of new buds, but instead caused the branching bronchial tree to skip specific generations of branches. These changes in the local branching pattern were also accompanied by altered smooth muscle wrapping in the neighborhood of the injected droplets. Taken together, these data highlight the importance of mechanical forces during embryonic lung development and suggest that individual branching modes are differentially regulated by changes in tissue mechanics. This work is supported by the NIH grant R01HL145147.

Program Abstract #297

TGFβ signaling determines spacing between branches in the embryonic chicken lung

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The branched structure of the lungs provides a high surface area-to-volume ratio, which enables efficient diffusion-driven gas exchange. Generating this structure requires that the epithelial branches avoid contact with each other during development. Although several studies have suggested that the lung epithelium shows self-avoidance across several species, the underlying mechanism remains elusive. Using the embryonic chicken lung as a model system, we investigated how epithelial branches sense each other and avoid collision during morphogenesis. Immunofluorescence analysis revealed a decrease in proliferation in epithelial cells located in close proximity to an adjacent branch, while physically removing the neighboring branch prevents this decrease. Blocking signaling through the TGFβ receptor increases the number of proliferative cells and eventually leads to branch-branch contact. These data reveal that the spacing between branches in the embryonic chicken lung is tuned by inhibitory morphogen signaling. This work was supported in part by the National Science Foundation.

Program Abstract #298

Ndrg1b, a novel regulator of N-cadherin during tissue morphogenesis

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Cadherin-based cell adhesion is essential for many aspects of morphogenesis, and has been extensively studied owing to its essential role in epithelial integrity and development. The Brewster lab has a longstanding interest in the role of *N-cadherins(N-cad)* in tissue morphogenesis, and identified an essential role for this adhesion molecule in shaping the neural tube, the precursor of the brain and spinal cord. In this study we report on the role of *N-myc Downstream regulated gene 1b (ndrg1b)* as a novel regulator of N-cad trafficking during myogenesis of the zebrafish embryo. *Ndrg1b* is a member of the NDRG subfamily of adapter proteins, several members of which have been shown to mediate the endocytosis or recycling of transmembrane proteins, including the glucose transporter, the low density lipoprotein and E-cadherin members. We show here that ndrg1b is ubiquitously expressed during gastrula and segmentation stages, with higher expression in the developing nervous system and somites. Ndrg1b knockdown using morpholinos results in developmental defects that are remarkably similar to those observed in *N-cad* mutants, especially with regards to impaired myogenesis. In zebrafish, slow muscle precursor cells originate from the adaxial cells next to the notochord that express *N-cad.* These precursor cells undergo lateral migration to reach their final destination in the flank of the embryo, where they differentiate into slow muscle fibers. In *ndrg1b*-depleted embryos, slow muscle precursors do not

complete their migration and N-cad appears misclocalized intracellularly. Based on these observations, we hypothesize that *N-cad* turnover is essential for slow muscle precursor migration and that *Ndrg1b* mediates *N-cad* trafficking in this cell population. Data supporting this model will be presented. This work was funded by National Institute of Health/NICHD (R21HD089476).

Program Abstract #299

A stem cell zoo to study interspecies differences in developmental tempo

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During embryogenesis, different mammalian species present differences in their developmental speed. These controlled differences in the tempo or duration of developmental processes are known to influence animal size and morphology, being an important mechanism of evolutionary change. However, the mechanisms regulating developmental tempo have remained elusive due to difficulties in performing direct interspecies comparisons. In this study, we used in vitro differentiation of pluripotent stem cells to recapitulate the segmentation clocks of diverse mammalian species varying in body weight and taxa: mouse, marmoset, rabbit, human, cattle, and rhinoceros. Quantification of the segmentation clock oscillations across species revealed that their period did not scale with the animal body weight, but with the embryogenesis length. The biochemical kinetics of the core clock gene HES7 displayed clear scaling with the species-specific segmentation clock period. However, the cellular metabolic rates did not show an evident correlation. Instead, genes involving biochemical reactions showed an expression pattern that scales with the segmentation clock period, providing evidence of the potential transcriptional regulation of developmental tempo. We are now using gain-of-function screens to characterize the transcriptional signature of developmental tempo, aiming to accelerate the human segmentation clock. Investigating the genetic control of developmental tempo will help us understand how the species-specific phenotypes are determined at the cellular level and how they changed during evolution. Funding: EMBL, ERC, BIF fonds PhD fellowship.

Program Abstract #300

Intercalation dynamics driving endoderm morphogenesis during mouse gastrulation

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The mouse gut endoderm is the precursor of the digestive and respiratory tracts. It is a mosaic tissue formed from two cell populations of different developmental origins. These being the visceral endoderm (VE) that arises at embryonic day (E)3.5 in the preimplantation blastocyst, and the definitive endoderm (DE) which emerges at E7.0 during gastrulation from the pluripotent epiblast. The morphogenesis of the endoderm during gastrulation occurs as DE cells intercalate into the existing VE layer resulting in dispersal of the VE cells. Very little is known about the mechanisms controlling cell intercalation into an existing epithelium. Using live imaging of different genetically engineered mouse reporters, we are dissecting the mechanisms driving the dynamic DE intercalation process. 3D time-lapse imaging of a ZO1-GFP fusion reporter (tight junctions), coupled with membrane segmentation, reveals that DE cells intercalate into the VE layer preferentially at high-fold vertices. Analysis of ZO1-GFP reporter and an E-Cadherin-GFP fusion reporter (adherens junctions), reveals that junctions are remodeled as DE cells intercalate. Tracking intercalation sites in combination with a VE lineage-specific reporter (Rhox5H2B-Cherry) reveals that DE cells integrate preferentially near VE cells, suggesting that DE and VE cells may have different properties and could guide sites of intercalation. To address whether mechanical forces play a role in intercalation, we have established a protocol for laser ablation in the mouse post-implantation embryos, using a two-photon laser for precise (3D defined ROI) ablation of the actomyosin network, combined with laser point scanning confocal time-lapse imaging. Our ablation experiments reveal that high-fold vertices retain higher tension than tricellular junctions, which we propose facilitates the opening of vertices, promoting the intercalation of DE cells. Study supported by the NIH (R01HD094868, R01DK127821, R01HD086478, and P30CA008748).

Program Abstract #301

Tendon heterogeneity in the zebrafish embryo: looking to the eye for differences in tissue-specific attachments

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Tendons attach muscles to multiple tissue types to accommodate diverse structural and functional needs. Vertebrate tendon fibroblasts (tenocytes) are distinguished by their expression of scleraxis (scx), an early tenocyte fate determinant transcription factor, but differences in the genetic programs between tenocytes of varying attachment types remain largely unexplored. We have investigated this heterogeneity through a transcriptomic analysis of craniofacial tendons in zebrafish. The extraocular muscles (EOM) that control eye movements exemplify functional tissue heterogeneity, since they feature both hard tissue attachments to the skull as well as unique soft-tissue tendon attachments to the eye. Single-cell RNA sequencing using sorted mCherry positive cells taken from heads of *Tg(scxa:mCherry)* embryos at 72 hpf, coupled with quantitative in-situ hybridization chain reaction in whole embryos, identified novel EOM-associated tendon subpopulations. These tenocyte clusters differ in their extracellular matrix components associated with distinct mechanical properties, and express key retinoic acid (RA) synthesis genes (*aldh1a2* and *rdh10a*) and RA signaling effectors (*pitx2*), implicating RA signaling in their development. Consistent with this hypothesis, blocking RA signaling pharmacologically with DEAB uniquely prevents the formation of EOM tendon insertions on the eye, without affecting their skull attachments. Conversely, elevated RA levels cause expansion of tenocyte number at EOM-tendon insertions. These results suggest differences in gene regulatory networks involved in EOM tenocyte cell specification at hard versus soft tissue attachments. Studies are ongoing to investigate how RA establishes the pattern of EOM tendon attachments. We are also integrating our scRNA-seq data over multiple stages to identify transitional subpopulations during the development of these understudied components of the musculoskeletal system. This work is supported by NIAMS [R01AR67797].

Program Abstract #302
Reconfiguration of epidermal morphogenesis for asymmetric mechanical environment
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Early embryos are known to exhibit various postures, such as bending, folding, twisting, and curling, within the spatial constraints of the egg membrane and the uterus. However, the impact of posture-dependent mechanical stress on embryogenesis remains largely unexplored. We address this question using the *Xenopus laevis* embryo, an ideal model in which both posture and morphogenesis can be directly perturbed and their effects comprehensively analyzed. *Xenopus* embryos bend their bodies laterally to elongate anteroposteriorly within the confines of the spherical vitelline membrane from early tailbud stage until just before hatching. We focused on how the epidermis continues to tightly cover the left and right sides of the body, which have different surface areas resulting from bending. *Xenopus* embryos are thought to bend passively, as they grow in a straight posture after the early removal of the vitelline membrane. However, the epidermis on the inside of the bend contracts even more than that on the outside after surgical separation, suggesting that the epidermis is not an elastic passive tissue, but an active tissue that responds to bending. We hypothesized that apical constriction regulates epithelial length, but found no signal for phosphorylated myosin on the apical surface inside the bend. Instead, we observed that at the hinge point, the basement membrane extends deep into the body and the epidermal cells adopt a multilayered-like structure. This specific structure was maintained after 10 minutes of artificial straightening with a coverslip and did not form after 10 minutes of artificial bending of the early vitelline removal embryo with a 3D-printed mold, suggesting that this structure is stable and is formed by a biological process such as cell rearrangement and ECM remodeling. We will discuss the processes of formation and loss, and the potential biological functions of this unique epidermal structure. Funding: JSPS, The Japan Science Society.

Program Abstract #303
Determining the requirements of proper N-linked glycosylation during placental morphogenesis via examination of *Alg3* and *Stt3b* knockout mouse lines.
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Alg3 knockout (KO) mutation and *Stt3b* KO result in mid-gestation lethality. While KO embryos are properly patterned at E9.5, there are severe placental defects and embryos do not survive past E10.5.

Alg3 and *Stt3b* encode enzymes responsible for building and transferring N-glycans, respectively, to proteins. These glycans serve as signals for quality control, mediate trafficking through the secretory pathway, and ultimately influence glycoprotein function. We aim to further understand how proper N-linked glycosylation is required during embryogenesis by examining the mechanisms driving the observed placental defects and determining if they cause the mid-gestation lethality. Given the role of N-linked glycans in assisting with protein quality control, we hypothesize that perturbations may result in activation of the unfolded protein response (UPR), leading to intracellular damage. We are performing RT-PCR on *Alg3* and *Stt3b* KO placentas to examine factors activated during the UPR. Additionally, perturbed glycosylation may impair glycoproteins that traverse the secretory pathway. We are conducting lectin histochemistry on *Alg3* and *Stt3b* KO placentas to examine the abundance, localization, and glycan modifications of glycoproteins. Finally, we hypothesize that the placental defects drive mid-gestation lethality and will perform a conditional knockout of *Stt3b* in embryonic tissues. We expect a rescue of mid-gestation lethality. We have not observed strong activation of the UPR in *Alg3* and *Stt3b* KO placentas and aim to continue analyzing the effects on glycoproteins. We hypothesize aberrant glycoprotein signals that normally coordinate placental morphogenesis is the key mechanism driving the observed defects. Examining how fundamental cellular processes such as N-linked glycosylation are required during placental morphogenesis is critical to advance our understanding of placental biology. Funding by R01HD096073 to KDT.

Program Abstract #304

A newly identified role for canonical WNT/β-catenin signaling in human early post-implantation development.

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Around 60% of human conceptions are lost before and during implantation due to reasons that are poorly understood. Within a week of fertilization, the epiblast transforms from an apolar mass of cells into an organized and polarized epithelium with a central lumen. The precise cell signals orchestrating morphogenesis during the remodeling of human epiblast tissue at implantation are not clearly defined. Here, we present a novel bioengineered 3D model system that uses pluripotent stem cells without the use of hydrogel or other ECM substitutes to mimic human epiblast development from days 6-8 post fertilization. Through modulation of WNT/β-catenin signaling followed by immunofluorescence analysis with high-resolution confocal microscopy in both our 3D human epiblast model and *in vivo* human embryos, we unveil a crucial role for this signaling pathway in orchestrating epiblast tissue remodeling during early implantation. Through RNA sequencing and mechanical analyses, we further identify potential mechanisms underlying the downstream effects of canonical WNT signaling on this phenotype. Our findings contribute a novel model for studying human epiblast development and uncover a previously unrecognized function of WNT/β-catenin signaling in regulating epiblast remodeling during the early stages of implantation. Funding: Richard and Susan Smith Family Foundation, Yale Stem Cell Center Chen Innovation Award and Repro Grants

Program Abstract #305

Investigating the Role of WNT Signaling Modulation in the Evolution of the Developing Human Cerebral Cortex

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The cerebral cortex performs many cognitive functions unique to humans including reasoning, language processing, and consciousness. The intricate patterning of the cortex during embryonic development is foundational to its cellular, molecular, and genetic organization. Precise embryonic patterning is highly dependent on cell regulation by the WNT signaling pathway. In the cortex, this highly conserved pathway has been shown to direct cell fate, polarity, and migration. Specifically, during neurogenesis, WNT signaling promotes the balance of neural progenitor cell (NPC) proliferation and differentiation. Our lab previously identified *HARE5*, a Human Accelerated Region (HAR) that acts as an enhancer of *FZD8*, a WNT pathway receptor. HARs are genomic loci that have accumulated mutations in the human lineage despite being located within regions of high conservation among vertebrates. Our studies revealed that *HARE5* is associated with accelerated NPC cell cycle and increased cortex size suggesting HAR

regulation of WNT pathway members may promote human specific features of corticogenesis. Through *in silico* analysis, I identified 9 HARs, in addition to *HARE5*, that are likely to act as enhancers of genes encoding WNT pathway members during human brain development. I hypothesize that synergistic regulation of WNT signaling by these HARs resulted in human specific modulation of corticogenesis. Through preliminary studies utilizing luciferase assays in human NPCs, I have confirmed that many of these HARs exhibit differential enhancer activity when compared to its chimpanzee ortholog. After further characterization, I will prioritize HARs to manipulate in combinations with *HARE5* to observe synergistic effects using cultured human NPCs and cortical organoids. This study of HAR involvement in WNT signaling during corticogenesis will aid in unraveling evolutionary mechanisms that promote features of the human cerebral cortex. Funding: NIH R01MH132089, NRSA T32HD040372, and NSF GRFP

Program Abstract #306

Role of cWnt in the specification of the ventral nerve cord in *Capitella teleta*

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The origin of the centralized nervous system has been highly debated as molecular mechanisms underlying its formation are well-studied in just a few species. In vertebrates and insects, a gradient of bone morphogenetic protein (BMP) and activation of the MAPK cascade is necessary for the formation of the dorsal-ventral axis and the specification of neuroectoderm. The canonical Wnt signaling pathway (cWnt), on the other hand, has been known to be involved in the specification of the anterior-posterior axis with variable roles in neuroectoderm specification. Nonetheless, In Spiralia, there is little evidence that BMP is involved in this process, and the role of cWnt is unknown. Our lab studies the annelid *Capitella teleta* partly due to its stereotypical program of cell division known as spiral cleavage. Our project aims to elucidate the molecular mechanisms underlying the specification of the ventral nerve cord (VNC) in *Capitella teleta*. Previous studies in the lab showed that the micromere 2d, fated to form the VNC, can form neural tissue in isolation. However, it fails to do so in the presence of the other animal micromeres. This suggests a combination of neural determinants and external signals involved in the specification of this organ. In a pilot experiment, we detected several differentially expressed genes, including Spiralia-specific TALE genes (SPILE) and components of the cWnt pathway. We characterized the expression pattern of some cWnt components via *in-situ* hybridization. Furthermore, we perturbed cWnt signaling by treating embryos with the agonist azakenpaullone and the antagonist iCRT-14, assaying VNC formation via *in-situ* hybridization with the neural marker *Ct-elav1*. For future directions, we will isolate additional blastomeres to dissect the molecular mechanism underlying VNC specification of *C. teleta.* We will also block the expression of selected SPILE genes via CRISPR/Cas9. This work has been funded by NSF (#1656378) and Clark University.

Program Abstract #307

The evolutionary modifications of the GoLoco motif in the AGS protein facilitate micromere formation in the sea urchin embryo

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Micromeres of sea urchin embryos, arising from asymmetric cell division (ACD) at the 16-cell stage, function as organizers, which is a distinctive feature exclusive among echinoderm embryos. Our previous research highlighted the pivotal role of Activator G-protein Signaling (AGS), a polarity factor, in micromere formation. However, AGS and its associated ACD factors are broadly present across taxa, leaving a question about the evolutionary modifications of AGS or its molecular milieu that led to the acquisition of micromeres only in sea urchins. AGS comprises TPR domains at the N-terminus and GoLoco motifs at the C-terminus. While protein sequences of echinoderm AGS orthologs vary in the C-termini, the N-termini are almost identical. With a hypothesis that evolutionary modifications of GoLoco motifs contributed to the micromere formation in sea urchins, we tested a series of AGS-GoLoco motif mutants. In this study, we identified the critical roles of GoLoco motifs in micromere formation, and their modifications facilitate the functional diversity among AGS orthologs. Furthermore, AGS-knockdown blocked the localization of other ACD factors (Insc and NuMA) and fate determinant (β-catenin) at the micromere-side of the cortex during ACD, suggesting that sea urchin AGS plays a critical role in

micromere lineage segregation. Indeed, the endomesodermal genes regulated by micromeres (*foxa*, *blimp1b*, *endo16* and *wnt8*), showed decreased expression territories in AGS-knockdown embryos. Lastly, we introduced sea urchin AGS into pencil urchins, ancestral types of sea urchins, which randomly forms 0-4 micromere-like cells. Sea urchin AGS facilitated micromere-like cell formation and expedited the enrichment of another fate determinant, Vasa, in the resultant pencil urchin embryos. Taken together, these results support the contention that evolutionary modifications of sea urchin AGS protein played a critical role in the emergence of micromeres during sea urchin diversification.

Program Abstract #308

Understanding the genetics and development of red phenotypes in *Anolis sagrei*

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Pigmentation plays a crucial role in species survival, serving functions from camouflage to thermal regulation. In vertebrates, the diversity of pigments is produced by specialized cells known as chromatophores. There are three major types of chromatophores: melanophores, iridophores, and xanthophores. However, previous genetic studies on amniote pigmentation have primarily focused on mammals, which only contain melanophores. Our research aims to bridge this knowledge gap by studying *Anolis sagrei*, a model lizard that possesses all three chromatophore types. Notably, *A. sagrei* are typically brown headed (BH) but also have a naturally occurring redhead (RH) phenotypic variant. This study seeks to understand the biology behind the RH phenotype, with a particular focus on the xanthophore cell type, which produces red, orange, and yellow pigmentation. Our initial biochemical approach to understanding the RH phenotype involves measuring the abundance of pterin and carotenoid pigment types in RH vs BH head skin through high-performance liquid chromatography to determine which pigments are contributing to red coloration. To uncover the genetic basis of the RH phenotype, we have also performed a preliminary GWAS study using RAD-seq data generated from a recent study. This data set comprises several hundred male *A. sagrei* and includes BH and RH individuals. Through this GWAS, we identified a region on chromosome 5 that is very strongly associated with the RH phenotype. This region contains 30 genes, including three pigment-related genes of interest: *paics*, *ppat*, and *kit*. To further narrow the RH interval and help identify the causative gene for the RH phenotype, we plan to employ Pool sequencing (Pool-seq) and RNA-seq of RH and BH individuals. Through this study, we aim to understand the pigment molecules responsible for RH coloration, narrow the genomic region, and identify the causative gene for this phenotype. This work is supported by NHGRI R01HG013006.

Program Abstract #309

Rpl6y* as a candidate gene for primary sex determination in *Anolis sagrei

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The accurate and timely initiation of sex determination (SD) is fundamental to species survival and reproductive success. Despite its importance, genetic SD (GSD) mechanisms have only been carefully studied in a few vertebrate species, and our understanding of reptilian GSD systems remains limited. Our work aims to uncover the GSD signal cascade in the brown anole, *Anolis sagrei,* by clarifying the timing of embryonic gonad differentiation, tracing the evolution of sex chromosomes, and identifying the genetic basis of primary SD. The *Anolis* XX/XY sex chromosome system is estimated to have arisen 160 mya, suggesting the GSD system and underlying mechanisms are conserved across *Anolis* species and other related squamates. To identify candidate genes for primary SD, the genome of an XY brown anole was sequenced and transcriptome analyses were performed on XX and XY gonadal ridges during early gonad differentiation. From this data, we propose *rpl6y* as a candidate determinant of primary SD in *Anolis sagrei*. *Rpl6y* has robust expression during early XY gonad differentiation and is highly diverged from its X homolog, *rpl6x*. *Rpl6y*orthologs have been identified in the XY genome assemblies of four additional *Anolis* species and six of their close relatives. Our data has uncovered a potential regulatory mechanism in which *rpl6x* expression is downregulated in XX and XY gonads by the gonad-specific *Antisense Transcript for rpl6x in Anolis* (*astra*). We hypothesize that *astra* downregulates *rpl6x* expression in XX and XY gonads ahead of SD initiation and the upregulation of *rpl6y* in XY gonads. With the growing evidence for *rpl6y* as a primary SD gene, we are eager to tease apart this novel GSD mechanism. To functionally investigate the role of *rpl6y* in SD, we will determine

whether loss of function results in XY sex reversal using CRISPR/Cas9 gene editing. Funding Sources include NSF #1927156, NSF GRFP, ARCS Foundation, and UGA Graduate School.

Program Abstract #310

Morphoelastic models discriminate between different mechanisms of left-right asymmetric stomach morphogenesis

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The mechanisms by which the vertebrate stomach undergoes its evolutionarily conserved leftward bending remain incompletely understood. Although the left and right sides of the organ are known to possess different gene expression patterns and undergo distinct morphogenetic events, the physical mechanisms by which these differences generate morphological asymmetry remain unclear. Here, we develop a continuum model of asymmetric stomach morphogenesis. Using a morphoelastic framework, we investigate the morphogenetic implications of a variety of hypothetical, tissue-level growth differences between the left and right sides of a simplified tubular organ. Simulations reveal that, of the various differential growth mechanisms tested, only one category is consistent with the leftward stomach curvature observed in wild- type embryos: equal left and right volumetric growth rates, coupled with transversely isotropic tissue thinning on the left side. Simulating this mechanism in a defined region of the model over a sustained period of growth leads to mature stomach-like curvatures. Partial funding for this project was provided by the Center for Research in Scientific Computation at NCSU (ANN), Simons grant 524764 (SRL), and NIH R01 HD095937 (NNY).

Program Abstract #311

Gene Loss and the Evolution of the Snake Body Plan

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Relative to limbed species within Squamata, snakes have evolved many prominent morphological changes, including the evolution of limb loss. Recent comparative genomics research has shed light on potential limb development genes that are lost from the genomes of snakes. Among these lost genes are *hoxd12* and *twist2*, which, through previous work, have been shown to play roles in the developing limb buds of other model vertebrates, such as mice and chicks. The loss of these genes may have contributed to the evolution of limb loss in snakes or might have been lost due to relaxed selection. However, the function of these genes in reptiles has not been determined. Therefore, we are using CRISPR-Cas9 genome editing to disrupt the function of these genes in a limbed squamate, *Anolis sagrei*. Through these efforts, we have produced *hoxd12* mutant lizards, which we identified through Sanger sequencing of the targeted exon. We are now in the process of producing *twist2* mutants. Our initial plans are to perform high-resolution micro-CT scans to investigate morphological phenotypes in mutant and wild-type lizard hatchlings to determine whether the limbs or other body structures are altered when *hoxd12* or *twist2* gene function is lost. We will also examine the expression patterns of these genes across a series of embryonic developmental stages using in situ hybridization to identify major sites of expression. Together, these analyses will reveal the role of *hoxd12* and *twist2* in a limbed squamate reptile and may provide insights into why the loss of these deeply conserved genes occurred in the snake lineage. This work is funded by NHGRI R01HG013006.

Program Abstract #312

Exploring Reptile-Specific Hindlimb Enhancers to Understand the Evolution Of Shorter Hindlimbs In *Anolis* Ecomorphs

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Anolis lizards on different islands of the Caribbean have repeatedly and independently evolved the same sets of habitat specialists, termed 'ecomorphs'. Between ecomorphs, Anolis lizards show significant variation in relative hindlimb length, with some ecomorphs exhibiting long hindlimbs and others exhibiting short hindlimbs. Comparative analysis of several anole species identified independent deletions in the hindlimb enhancer B (HLEB) region of three different lineages of short-limbed anoles. HLEB is an enhancer

that regulates expression of *T-box Transcription Factor 4 (tbx4)*, a gene involved in vertebrate hindlimb development. However, it is unlikely that HLEB is the only factor regulating hindlimb length in *Anolis*. We hypothesize that there are additional enhancers regulating *tbx4* expression in anoles, and that some species of short-limbed anoles may have sequence alterations at these sites, resulting in reduced relative hindlimb length. To address this possibility, we are performing H3K27ac ChIP-seq on embryonic stage 5 and stage 7 *Anolis sagrei* forelimbs, hindlimbs, and eyes. This dataset will allow us to search for previously unknown active regulatory elements around the *tbx4* locus. To identify conserved enhancers, we will compare our A. sagrei H3K27ac ChIP-seq data with previously-generated A. carolinensis H3K27ac ChIP-seq data. With 50 million years of divergence between species, overlapping ChIP-seq peaks will aid in identifying highly conserved regulatory elements associated with hindlimb development. To determine if these putative enhancers are diverging in short-limbed anoles, we will perform sequence comparisons across a range of long and short-limbed anole species. If significant sequence divergence is found at putative enhancer loci in short-limbed anole species, functional validation using CRISPR-Cas9 mediated gene editing in *A. sagrei* will be performed. This work is funded by NSF grant 1927156 and NIH grant 1T32GM142623.

Program Abstract #313

Molecular characterization of the mechanosensory cells of the tunicate coronal organ

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The vertebrate inner ear is a complex sensory organ that detects balance and sound information using specialized mechanosensory cells called hair cells. Hair cells possess stereovilli and a single kinocilium that extend from the cell body and respond to mechanical stimuli by depolarizing the cell and releasing neurotransmitters to the neurons that innervate them. While the development and function of the vertebrate hair cell are well studied, the evolutionary origin of this cell type in chordates remains unknown. Tunicates, the closest living relatives to vertebrates, are sessile filter-feeders as adults and use the coronal organ, a continuous line of secondary mechanosensory "hair cells", to detect particle and water flow through their oral siphons. In this novel study, we have molecularly characterized these mechanosensory cells in *Ciona robusta* using single-nuclei RNA-seq (snRNA-seq) and hybridized chain reaction (HCR) staining. From the sequencing data, we have identified a putative hair cell population which expresses homologs of vertebrate hair cell-associated genes, including human deafness genes like *Otof* and *Cdh23*. Additionally, based on the snRNA-seq data, these cells express genes associated with vertebrate hair cell mechanotransduction, depolarization machinery, and synaptic activity. Our preliminary results indicate that the tunicate coronal organ sensory cells are molecularly homologous to vertebrate hair cells. Our future experiments will focus on functional and developmental testing to support this conclusion. Funding from NIH T32 GM139534-02 and the Vivian L. Smith Chair in Neuroscience.

Program Abstract #314

EBF is the Boss a' -Nova : A Conserved Regulatory Pathway for Acetylcholine Receptor Clustering at the Chordate Neuromuscular Junction

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The vertebrate body plan requires not only a complex nervous system to support it but also a staggering architecture of innumerable communication points between neurons, known as synapses. As complexity of an organism's nervous system increases, so does the need for synaptic communication, meaning that a single neuron can form thousands of synapses. Thus, the study of individual synapses makes for a labyrinthian neurodevelopmental puzzle. The Neuromuscular Junction (NMJ) formed between a motor neuron and muscle cell is the most accessible synaptic connection for study given its size. To form the mammalian NMJ, motor neurons rely on the protein Agrin which they secrete to interact with the muscle cell. The motor neurons secrete a neural-specific isoform that then activates LRP receptors on the muscle cell membrane and MuSK signaling to cluster Acetylcholine Receptors (AChR) on the muscle surface. The Neural-specific Agrin splice variant ("Z+") possesses "Z" exons, microexons that encode a short peptide that increases AChR clustering by 1,000 fold compared to Z-negative isoforms. We have found this

process is conserved in the tunicate *Ciona robusta*, where we have found cryptic Z exons encoding similarly short peptide motifs predicted to bind to LRP. Through CRISPR-Cas9 mediated knockouts, we have confirmed that Neural Z+ Agrin is required for AChR clustering at the *Ciona* larval NMJ and have further confirmed that the RNA splicing factor Nova and transcription factor Ebf/COE play important regulatory roles in the inclusion of Z exons specifically in motor neuron-expressed *Agrin*. This work aims to further untangle the regulatory pathway responsible for the development of neuromuscular junctions and expands our timeline of its evolution to at least the last common ancestor of vertebrates and tunicates. This work was funded through the following grants: NIH: R15GM119099 and NIH R01-HD104825.

Program Abstract #315
A change in cis-regulatory logic underlying obligate versus facultative muscle multinucleation in chordates
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Vertebrates and tunicates are sister groups that share a common fusogenic factor, Myomaker (Mymk), that drives myoblast fusion and muscle multinucleation. Yet they are divergent in when and where they express Mymk. In vertebrates, all developing skeletal muscles express Mymk and are obligately multinucleated. In tunicates, Mymk is only expressed in post-metamorphic multinucleated muscles, but is absent from mononucleated larval muscles. In this study, we demonstrate that *cis*-regulatory sequence differences in the promoter region of *Mymk* underlie the different spatiotemporal patterns of its transcriptional activation in tunicates and vertebrates. While in vertebrates Myogenic Regulatory Factors (MRFs) like MyoD1 alone are required and sufficient for *Mymk* transcription in all skeletal muscles, we show that transcription of *Mymk* in post-metamorphic muscles of the tunicate *Ciona* requires the combinatorial activity of MRF/MyoD and Early B-Cell Factor (Ebf). This macroevolutionary difference appears to be encoded in *cis*, likely due to the presence of a putative Ebf binding site adjacent to predicted MRF binding sites in the *Ciona Mymk* promoter. We further discuss how *Mymk* and myoblast fusion might have been regulated in the last common ancestor of tunicates and vertebrates, for which we propose two models.

Program Abstract #316
Neural Crest Lineage in the Proto-Vertebrate Model *Ciona*
Lauren Todorov[1,2], Laurence Lemaire[3], Michael Levine[1,2]
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Neural crest cells are multipotent progenitors that form at the border of the invaginating neural tube before migrating to give rise to a diverse array of cell types, including melanocytes, neurons, and glial cells. Impaired neural crest development and differentiation are implicated in one-third of all congenital birth defects, as well as in aggressive cancers like melanoma and neuroblastoma. The development of neural crest cells is unique to vertebrates and critical for the evolutionary transition from filter-feeding to active predation with the expansion of the cranium and the formation of both the jaw and paired sense organs (eyes, ears, and nose). *However, the origin of neural crest in the proto-vertebrate ancestors remains uncertain.* Previous studies identified two potential neural crest cell types in *Ciona*, sensory pigment cells and bipolar tail neurons (Abitua et al., 2012; Stolfi et al., 2015). A more recent study suggests that the tail neurons are homologous to cranial ganglia and therefore derived from placodes rather than neural crest (Papadogiannis et al., 2022). By contrast, we show that the pigment cell lineage also produces neural progenitor cells that differentiate into the nervous system of juveniles during metamorphosis. Neural progenitors are a major derivative of neural crest in vertebrates, suggesting that the last common ancestor of tunicates and vertebrates contained a multipotent progenitor population at the neural plate border. It would therefore appear that a key property of neural crest, multipotentiality, preceded the emergence of vertebrates. This project was supported by the National Institute of Neurological Disorders and Stroke (NINDS) of the National Institutes of Health (NIH; NS076542), the National Institute of General Medical Sciences (NIGMS) of the NIH (T32GM007388), and the Saint Louis University startup funds.

Program Abstract #317

Neural crest lineage in the proto-vertebrate model *Ciona*

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Neural crest cells are multipotent progenitors arising from the neural plate border and producing ectomesenchymal cell types such as melanocytes, most of the peripheral nervous system, and craniofacial bones. These cells are unique to vertebrates with no equivalent in invertebrates. However, their evolutionary origin remains elusive. To explore this question, we use the tunicate *Ciona* since this invertebrate chordate is among the closest living relatives to vertebrates. A previous study showed that one of the potential closest cell types to neural crest derivatives is the pigment cells of the central nervous system of *Ciona* larvae, which originate from the neural plate border. Here, we show that the pigment cell lineage also produces neural progenitor cells that differentiate into the nervous system of juveniles during metamorphosis. Neural progenitors are another major derivative of neural crest in vertebrates suggesting that the last common ancestor of tunicates and vertebrates contained a multipotent progenitor population at the neural plate border. It would therefore appear that a key property of neural crest, multipotentiality, preceded the emergence of vertebrates. This work is supported by a grant (NS076542) from the National Institute of Neurological Disorders and Stroke of the National Institutes of Health (NIH), the National Institute of General Medical Sciences of the NIH (grant number T32GM007388) and by Saint Louis University startup funds (G001654).

Program Abstract #319

Sweat gland development requires an eccrine dermal niche and couples two epidermal programs

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Eccrine sweat glands are indispensable for human thermoregulation and like other mammalian skin appendages form from multipotent epidermal progenitors. Limited understanding of how epidermal progenitors specialize to form these vital organs has precluded therapeutic efforts towards their regeneration. Herein, we applied single nucleus transcriptomics to compare the expression content of wildtype, eccrine-forming mouse skin to that of mice harboring a skin-specific disruption of *Engrailed 1 (En1)*, a transcription factor that promotes eccrine gland formation in humans and mice. We identify two concurrent, but disproportionate, epidermal transcriptomes in the earliest eccrine anlagen: one that is shared with hair follicles, and one that is *En1*-dependent and eccrine-specific. We demonstrate that eccrine development requires the induction of a dermal niche proximal to each developing gland in humans and mice. Our study defines the signatures of eccrine identity and uncovers the eccrine dermal niche, setting the stage for targeted regeneration and comprehensive skin repair. This research was supported by: NIAMS 5T32AR007465 (HD), NIAMS R01AR077690 (YK), NSF BCS-1847598 (YK), NIAMS P30-AR069589 (YK), NICHD R24HD000836 (IG, BDRL), NHGRI U01-HG012047 (HW)

Program Abstract #321

Changes to the mtDNA copy number during yeast culture growth

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Mitochondria are membrane-bound organelles that originated from an ancient symbiosis. Most originally mitochondrial genes have become integrated into the nuclear genome, but in almost all cases, independent mitochondrial DNA (mtDNA) persists within the mitochondria. The yeast Saccharomyces cerevisiae serves as an important model system for the investigation of mitochondrial biology, providing insight into the roles these organelles could potentially play in other organisms as well. While changes to mitochondrial morphology and function have been shown to accompany changes in physiological state, a substantial increase in mtDNA copy number over time in a growing culture had not been previously shown. We therefore sought to quantify the change in mtDNA in S. cerevisiae as a function of time by qPCR analysis, tracking it's nuclear and mitochondrial genome content as it shifts from

fermentation to the diauxic shift, into a fully respiring physiology—in a fermentable carbon source. Specifically, we demonstrate how the mtDNA copy number increase in growing S. cerevisiae cultures coincides with early stages of the diauxic shift and increases up to fourfold by the end of the respiratory phase. When they are grown on a non-fermentable carbon source, there is no diauxic shift and thus there is an attenuated copy number increase. While mtDNA copy number increase occurs both in haploid and diploid strains, it is to a lesser extent in wildtype diploids. We also demonstrate that the observable copy number increase in mtDNA is not driven by an increase in cell size. Therefore, we conclude that mtDNA copy number in yeast is a highly dynamic phenotype.

Program Abstract #322
Evidence of neocortex-like primitive laminar pallium in the avian embryo and its organization by microRNA-19b
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Complex cognitive functions in mammals have been linked to the presence of the layered neocortex. Recent findings on the similarities in cognition and the number of neurons between the avian and mammalian pallium suggest transitioning from an ancestral non-layered pallium to an advanced layered pallium. This study sheds light on some of the mechanisms through which the six-layered laminar neocortex of mammals may have evolved from the nonlaminar pallium found in birds and reptiles. The mammalian neocortex is characterized by a six-layered laminar structure with neurons following an "inside-out" pattern of birth and migration. The deeper layers of the cortex are formed by early-born neurons while late-born neurons give rise to more superficial layers during development. In contrast to mammals, the avian pallial neurons exhibit an "outside-in" birth pattern, whereby the first-born neurons are located atop, while subsequent neurons migrate beneath, resulting in spatially segregated nuclei instead of distinct laminar layers. Our expression analysis revealed the existence of a transient two-layer lamina marked by Fezf2 and Mefc2c in the developing chick pallium, mirroring the order of neocortical layers. Further, we have demonstrated a proliferation-dependent mode of specification that is partly regulated by microRNA-19b. This suggests a closer ontogenetic similarity between the avian pallium and the mammalian neocortex. This study provides greater insight into avian brain development and evolutionary transitions leading to neocortex-like organization in birds. Acknowledgment- SERB, Department of Science & Technology, Govt. of India

Program Abstract #323
Transition from juvenile to adult feathers: Exploring regional specific sexual dichromatism by analyses of zebra finch's red cheek
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Color traits provide visual signals for communication, sexual selection, and speciation, but the developmental mechanism is under-studied. Here we use zebra finch, *Taeniopygia guttata*, to explore molecules involved in the complex coloring of their craniofacial plumages. First, we describe the developmental process from juvenile to adult feathers, highlighting the transition of feather forms and colors. We then focus on the formation of male red cheek patch, which relies on multi-scales: skin domains, coloring mechanism, and sexes. Through comparative analyses of the cheek and scalp (i.e. crown) in both sexes and several genetic color variants, we made the following key findings. 1) A two-layered control is used for complex color patterning: the first is to set up a specific region with transcription factors such as *PITX1*, *PAX1*, and *PAX6* for the cheek, and the second is to express pigment-implementation genes, including male-biased expression of transporters associated with red pigment synthesis. 2) Unexpectedly, *ASIP* is expressed in both male and female cheeks, without specifically triggering pheomelanin expression. *PAX1* in cheek fibroblast may be upstream players of male-biased color patterns, and *PAX6*/*SOX10* in melanocytes may facilitate male-biased expression of *GRP143*, *SLC45A2*, and *TMEM163*, leading to higher pheomelanin production. 3) Sexual dichromatism can be affected by dosage on the sex chromosome (e.g., Z chromosome) as seen in *SLC45A2*. 4) Comparative genomic analyses imply craniofacial colors in passerines can be achieved by the convergently-evolved coloring mechanisms. The study reveals several unexpected findings of coloring control and sets up the

stage for further investigation. This research was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR60306), Dragon Gate Grant from National Science of Technology Council, Taiwan (105-2911-I-002-520) and Taipei Medical University, Taiwan (111-5400-002-400_1664162799641000).

Program Abstract #324

Tell-tail signs: Investigating the developmental mechanisms of vertebral proportion

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The axial skeleton is a defining feature of all vertebrate animals, but despite its functional importance, little is known about how vertebrae elongate. The abundance of natural size diversity and relative geometric simplicity of the rodent tail skeleton makes it a powerful system for studying how individual vertebrae acquire the information to become specific lengths. We examined the extreme tail differences between the closely related mouse (*Mus musculus*) and the bipedal lesser Egyptian jerboa (*Jaculus jaculus*). The jerboa tail, normalized to body length, is approximately 1.5x longer than the mouse yet has four fewer vertebrae. Using comparative µCT imaging and tissue histology of mouse and jerboa tail skeletons, we identified temporal and cellular parameters of vertebral growth cartilages that establish specific skeletal proportions in the tail during development. We found that the mid-tail region of both species undergoes a more rapid rate of growth to achieve the greatest lengths at the 6-7th vertebrae. Strikingly, this accelerated growth rate in jerboa leads to a much greater elongation of the longest tail vertebrae, resulting in a dramatically longer adult tail. To uncover the genetic drivers of disproportionate growth in the axial skeleton we performed bulk RNA-seq of mouse and jerboa tail vertebral growth cartilages with similar and different rates of elongation. We assess differential expression of the genetic programs driving chondrocyte proliferation and hypertrophy underlying intra- and interspecific differences in tail vertebral elongation. We also explore the involvement of natriuretic peptide receptors in tail crescendo-decrescendo patterns by altering chondrocyte proliferation and hypertrophy. These data inform our understanding of fundamental mechanisms underlying skeletal proportion and vertebral diversity. 5R01AR075415-02, 1F32AR079923-01, Wu Tsai Human Performance Alliance.

Program Abstract #325

The functions of heterogeneous embryonic origins in shoulder girdle development and evolution

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The morphological transformation of the pectoral/shoulder girdle is fundamental to the water-to-land transition in vertebrate evolution. Although previous studies have resolved the embryonic origins of tetrapod shoulder girdles, those of fish pectoral girdles remain uncharacterized, creating a gap of understanding in girdle transformation from fish to tetrapods. Here, we extensively identified the embryonic origins of the pectoral girdle of zebrafish (Danio rerio), including the cleithrum—an ancestral pectoral girdle element lost in extant tetrapods. Our combinational approach of photoconversion and genetic cell lineage tracing revealed that the cleithrum develops from three adjoining embryonic populations: head mesenchyme (cranial neural crest cells) and lateral plate mesoderm-derived cells (cardiopharyngeal mesoderm and trunk lateral plate mesoderm). Subsequent molecular staining corroborated that each cell population differentiates into osteoblasts in the cleithrum. Intriguingly, our single-cell genomics demonstrated that the gene expression profiles and chromatin structures of osteoblasts are highly diverse depending on embryonic origins. Thus, the pectoral girdle is a composite bone originating from diverse cell lineages with different genetic underpinnings. Also, the cell populations that contribute to the pectoral/shoulder girdle conspicuously differ between aquatic fish and terrestrial vertebrates. The changes in the heterogeneity of embryonic origins might cause the girdle morphological evolution, enabling vertebrates to adapt to terrestrial habitats. This research was supported by the National Science Foundation under Grant IOS 2210072 and the Institutional support provided by the Rutgers University School of Arts and Sciences and the Human Genetics Institute of New Jersey.

Program Abstract #326

Tgfbr1 regulates lateral plate mesoderm and endoderm reorganization during the trunk to tail transition

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During the trunk to tail transition the mammalian embryo builds the outlets for the intestinal and urogenital tracts, lays down the primordia for the hindlimb and external genitalia, and switches from the epiblast/primitive streak to the tailbud as the driver of axial extension. Genetic and molecular data indicate that Tgfbr1 is a key regulator of the trunk to tail transition. Tgfbr1 has been shown to control the switch of the neuro mesodermal-competent cells from the epiblast to the chordo-neural hinge to generate the tail bud. We show that Tgfbr1 signaling also controls the remodeling of the lateral plate mesoderm (LPM) and of the embryonic endoderm associated with the trunk to tail transition. In the absence of Tgfbr1 the two LPM layers fail to converge at the end of the trunk, extending as separate layers until the caudal embryonic extremity, and lacking markers of primordia for the hindlimb or for external genitalia. The vascular remodeling involving the dorsal aorta and the umbilical artery leading to the connection between embryonic and extraembryonic circulation was also affected in the Tgfbr1 mutant embryos. Similar alterations in the LPM and vascular system were also observed in Isl1 null mutants, indicating that this factor acts in the regulatory cascade downstream of Tgfbr1 in LPM-derived tissues. In addition, in the absence of Tgfbr1 the embryonic endoderm fails to expand the endodermal cloaca and to extend posteriorly to generate the tail gut. Our data, together with previously reported observations, place Tgfbr1 at the top of the regulatory processes controlling the trunk to tail transition. This project was funded by Fundacao para a Ciencia e a Tecnologia (FCT) and the research infrastructure Congento to the animal facility, co-financed by Lisboa 2020/FEDER and FCT (Portugal). AKH and YYK are supported by the NIH.

Program Abstract #327
Sniffing out olfactory neuroregeneration: neurogenesis in the context of Alzheimer's disease
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The loss of smell is an early potential biomarker for Alzheimer's disease (AD), which is perplexing given that the olfactory system is highly regenerative, with a population of basal stem cells responsible for the continuous renewal of olfactory sensory neurons (OSNs). Building on our previous findings of a Notch signaling-Insm1a feedback loop that drives developmental olfactory neurogenesis, we hypothesized that AD-associated Aβ42 peptide could disrupt this process. We first treated zebrafish embryos with Aβ42 peptide and found temporally-dynamic changes in the number of basal stem cells and OSNs. Next, we mosaically overexpressed Aβ42 *in vivo*, facilitating comparisons between individual Aβ42-expressing cells, and uncovered transcriptional changes and downstream effects pointing to a pro-neurogenic role for Aβ42. Our findings suggest that Aβ42 cell autonomously shifts the olfactory stem cell-neuron balance towards neuronal differentiation. Moving forward, we are assaying changes in basal stem cells and neuronal progenitors when Aβ42 is overexpressed, including how particular signaling pathways influence a cell's ability to self-renew or commit to a more differentiated cell fate. Thus, we hope to uncover new insights into how neurogenic mechanisms might be harnessed to improve outcomes for neurodegenerative diseases. This work was supported by the National Institute on Aging, National Institute of Child Health and Human Development, and the Alzheimer's Association.

Program Abstract #328
Investigating the role of critical developmental signaling pathways in organizing regeneration of the spinal cord
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In mammals, injury to the spinal cord is irreversible and characterized by an inability for neural stem cells to generate new, functional neurons. In contrast, simple vertebrates like zebrafish larvae undergo regeneration after spinal cord injury in as little as two days, in large part due to the successful re-initiation of neurogenesis. However, the mechanisms underlying neuronal re-patterning and differentiation in response to injury remains poorly understood. In development, ependymal radial glial (ERG) cells, the neural progenitor cells of the spinal cord, are patterned across the dorsal-ventral axis by signaling gradients, in particular, Sonic Hedgehog (Shh) and BMP/Wnt that emanate from the floor and roof plate respectively. The combination of morphogen gradients patterns the spinal cord into multiple progenitor

domains, which later specify into distinct neuronal cell types. During regeneration, ERGs again launch into a neurogenic program. How classic developmental signals continue to regulate dorsal and ventral neuronal populations, and how that affects function of the regenerating spinal cord is the subject of my investigation. In particular, our lab and others have found that Shh and Wnt inhibition during spinal cord regeneration results in the general reduction of neurons and leads to impairment of locomotion recovery. In contrast, we have recently determined that BMP appears dispensable for functional recovery after spinal injury. This suggests that key developmental signals critical for spinal cord patterning and specification during development may be re-configured during regeneration. To confirm this, I have generated a model to lineage trace neural progenitors upon spinal injury in zebrafish larvae, and propose to perform single cell analysis at a definitive regenerative endpoint in order to characterize the full spectrum of newly generated neurons after perturbations of critical developmental signaling pathways. NSFGRFP, NIHDP2, NIGMSR00, CZI.

Program Abstract #329

Breaking the barrier of retinal pigment epithelium neurocompetence

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The retinal pigment epithelium (RPE) is a monolayer of cells essential for retina health and physiology. RPE is a plastic tissue that can regenerate neural retina in embryonic amniotes via cell reprogramming. In chicken embryos, RPE reprograms into neural retina after retinectomy and FGF2 stimulation at embryonic day 4 (E4) but not at day 5 (E5), or later. We hypothesized that signaling pathways and intrinsic cell fate control during eye morphogenesis are coupled mechanisms that restrict RPE neurocompetence.
To identify transcription factors and signaling pathway candidates for functional perturbation that could promote RPE reprogramming in the late embryonic state, we used single-nucleus multimodal profiling to differentiate chicken RPE from E3-E7. This analysis pointed to 9 up-regulated and 9 down-regulated transcription factor-encoding genes and accompanying changes in motif accessibility that coincided with RPE neurocompetence restriction. Our data suggest that enhanced activity of the Hippo-YAP pathway and transcription factors such as NFIA and NFIB could restrict RPE neurocompetence. Inhibition of the Hippo-YAP pathway significantly increased cell proliferation in E4 and E5 RPE explants in the absence of FGF2, but did not induce retina formation, although it affected the expression of several genes, including EMT regulators and cell cycle-related genes, while suppressing RPE identity genes. In contrast, inhibition of NFIA increased the size of RPE explants, but only at E4. Altogether, our data suggest that the neurocompetence of embryonic RPE cells is jointly regulated by intrinsic and extrinsic cues, with differing effects on RPE cell behavior. Furthermore, these findings indicate that cell proliferation and gene regulatory networks may be responsible for controlling RPE reprogramming and restricting neurocompetence. This work was supported by F99 NS129167 to JAT, R01 EY026816 and R01 EY034980 to KDRT and the John W. Steube endowed Professorship to KDRT

Program Abstract #330

A TALE of heads and tails - towards understanding the regulatory dynamics of TALE factors in the regenerative patterning of the planarian anterior-posterior axis

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Planarians (main model species *Schmidtea mediterranea*) are an established model system to study whole-body regeneration and adult pluripotent stem cells (neoblasts), but the gene regulatory networks (GRNs) orchestrating regenerative polarity, patterning, and positional rescaling remain poorly understood. Planarian injury-induced regeneration can be subdivided into three phases: early generic wound response, axis re-establishment, and morphogenesis. During axis re-establishment, a signaling center forms at the tip of the blastema (anterior and posterior poles), which organize the regeneration of missing structures from the wound. The TALE factors prep and pbx have previously been shown to be required for the formation of these poles. Here, we present our ongoing and unpublished effort to understand the GRN of planarian regenerative patterning and re-establishment of the anterior-posterior axis. We have characterized the other TALE factors in the planarian genome and characterized the TALE

factor meis-2 and its role in posterior pole formation and posterior regeneration. To investigate the target genes and regulatory logic of prep, pbx and meis-2, we have integrated evidence from chromosome accessibility (ATAC-seq) and transcriptomics (RNA-seq) during the phase of axis re-establishment and pole formation. By silencing the TALE factors and performing RNA-seq and ATAC-seq, we have identified testable hypotheses of downstream target genes. In this analysis and following experiments, we have e.g. identified the Wnt signalling pathway (wnt1 ligand) as a putative direct target of pbx and meis-2. In addition, we have attempted to meta-analyze the available functional genomics data and literature to delineate an integrated understanding of the known regulatory interactions of position control genes (PCGs). We thank the Medical Research Council, the BBSRC, the Queen's College, the NaturalMotion Fund, the Osk Huttunen Fund, and the Saven Fund for support.

Program Abstract #331

ECM remodeling shapes *Hydra vulgaris* head regeneration

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The small freshwater cnidarian *Hydra vulgaris* is capable of whole-body regeneration. Its simple body plan consists of two epithelial monolayers separated by an extracellular matrix (ECM) containing the same core proteins as vertebrate ECM, making it a tractable model to study ECM dynamics during regeneration. We find that after head amputation, *Hydra*'s two epithelial layers quickly close the wound, then ECM proteins retract from the wound site, leaving a region of reduced ECM for approximately 48 hours. We show that several matrix metalloprotease (MMP) family members are expressed during head regeneration, and application of a broad-spectrum MMP inhibitor results in a reduction of the ECM gap, demonstrating that ECM is actively degraded during regeneration. Early in regeneration, ECM transcripts and MMP genes responsible for ECM breakdown are concurrently upregulated in the endoderm, indicating that the precise timing of ECM breakdown and new ECM synthesis may be coordinated at the post-translational level. We show through pharmacological inhibition that both MMP activity and collagen post-translational modifications are required for regenerating head morphogenesis. Finally, ECM dynamics may also play a role in the invasion of ectoderm-originating stem cells into the regenerating head endoderm. This stem cell population must migrate to the new head and invade endoderm to replace lost neurons and gland cells. We show that migration of these stem cells to the regenerating head requires new collagen synthesis, but that their invasion into the head endoderm occurs at regions of reduced ECM protein, including collagen, expression. Thus, complex tissue regeneration, which encompasses a suite of proliferative, migratory, and morphogenetic events, requires regulation of ECM levels that is precisely coordinated across space and time. Funding sources: SDB Emerging Research Organisms Grant, Hartwell Foundation Postdoctoral Fellowship

Program Abstract #332

Elevated Wnt signaling in non-regenerating tissue induces blastemas with ectopic posterior identity in the annelid *Capitella teleta*

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The canonical Wnt signaling pathway plays a crucial role in anterior-posterior patterning during development, but this pathway has been shown to be equally important in the context of regenerating tissues following injury. We sought to determine whether canonical Wnt signaling could explain differences in regeneration capabilities across the anterior-posterior axis in an annelid worm, *Capitella teleta*. *Capitella* can regenerate its tail but not its head, even though tail fragments can survive over a week following amputation. We characterized differences in gene expression, cell proliferation, and morphology between regenerating head fragments and non-regenerating tail fragments. We observed that regeneration-negative phenotypes were associated with an absence of Wnt signaling. We sought to increase the regenerative potential of tail fragments by pharmacologically manipulating canonical Wnt signaling. Tail fragments treated with a Wnt signaling inhibitor were not morphologically different than controls. However, tail fragments treated with two Wnt signaling activators, 1-azakenpaullone (AZA) and CHIR 98014, showed blastema formation at the cut site. Tails treated with AZA showed expression of stem-cell-marker genes and increased EdU incorporation at the cut site, as well as neurite extension into the wound. Furthermore, expression of several Wnt pathway components was upregulated at the wound

site of tails treated with AZA. These tail fragments also showed signs of terminal gut differentiation and gene expression indicative of ectopic posteriorization at the cut site. However, differentiation of regenerating tissue never reaches completion in tail fragments treated with either Wnt signaling activator, suggesting that Wnt signaling is not sufficient to fully rescue regeneration in this context.

Program Abstract #333

Unlocking the role of Histone methyltransferase MLL1/2 and its biochemical activity in planarian tissue regeneration

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Planarian flatworms are multicellular organisms with extraordinary regenerative abilities. Their regeneration relies on pluripotent and multipotent stem cells that differentiate to replace the missing or damaged tissues. However, these stem cells cannot organize into new, properly patterned tissue on their own. They rely on signals from other cells to induce their differentiation and direct their reorganization. Although many signaling pathways and molecules required for this process have been identified, significantly less is known about the mechanisms that translate these signals into the specific gene expression changes that drive regeneration. We hypothesize that histone H3 lysine trimethylation (H3K4me3) added to specific gene loci by MLL1/2 is an essential part of a chromatin regulatory mechanism that integrates signaling, tissue patterning, and cell fate choices during regeneration. Although knockdown of the planarian (Schmidtea mediterranea) MLL1/2 homolog, Smed-MLL1/2, does not cause patterning defects after transverse amputations, we wondered if we would see patterning defects in Smed-MLL1/2 knockdown animals after parasagittal amputations, as these fragments must regenerate a new midline and other positional information. Indeed, my preliminary data show that Smed-MLL1/2 does in fact play an essential role in tissue repatterning during the regeneration of parasagittal fragments in which the tissue needs to reestablish a new midline. As MLL1/2 has also been shown to regulate genes required for cilia formation and function, we are addressing whether this regeneration phenotype is operating through cilia genes or other targets. We are also functionally dissecting the planarian MLL1/2 protein complex to understand how its divergent DPY-30 subunits affect its biochemical activity and role in injury-induced gene expression. Funding for this work was provided by NIH grant 5R35GM142679.

Program Abstract #334

Elucidating drivers of angiogenesis during zebrafish caudal regeneration using a novel "AngioTag" transgenic line

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Due to a remarkable conservation of genetic and molecular pathways involved in regeneration between humans and zebrafish, the zebrafish caudal fin has emerged as a popular model for regenerative studies. However, there remains a significant gap in our understanding of how various cell types interact and coordinate responses during fin regeneration, particularly in the case of angiogenesis. Our lab has recently developed a unique "AngioTag" transgenic line that allows us to profile the active endothelial translatome. Briefly, this is achieved by using the endothelial-specific kdrl promoter to drive the expression of an HA-tagged ribosomal protein that can be pulled down using affinity purification, which enables us to isolate actively translating mRNAs from endothelial cells. This is a significant feat, especially considering the challenges posed by their low representation in global cell populations when using other sequencing techniques. Here, we employ the AngioTag line to isolate endothelial genes that initiate angiogenesis during early fin regeneration. Our approach involves examining two distinct age groups: young (~3 months old) and old (~2 years old). We aim to elucidate the expression dynamics of endothelial cells throughout regeneration and identify potential targets associated with angiogenesis defects in aged populations. In conjunction with data from a multi-omics fin regeneration project, we also plan to isolate potential epigenetic regulators driving angiogenesis for future study. Together, these data will enable us to identify novel endothelial genes involved in angiogenesis during fin regeneration and establish the foundation for future comprehensive studies across multiple -omics levels, including

genomics, transcriptomics, and epigenomics. This work is supported by the NICHD intramural program (to BMW).

Program Abstract #335
The Effects of Estrogen on Tail Fin Regeneration in Embryonic Zebrafish (*Danio rerio*)
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17-beta-estradiol (E_2) is a common steroid hormone that plays a role in sexual development, metabolism, and regeneration. The effects of estrogenic compounds on the regeneration process in zebrafish has not been clearly described. To elucidate the effect of estrogen on regeneration, the caudal fin of zebrafish embryos will be amputated 3 days post fertilization and treated with three concentrations of E_2. Exposure to E_2 in concentrations as small as 100 nM resulted in significantly decreased regenerated tail length and area. Treatment with 1000 nM (1 µM) resulted in incomplete tail regeneration, with a significant decrease in the proportion of embryos possessing normal bi-lobed morphology. The inhibitory effect observed in this paper is of concern with large amounts of estrogen being displaced into the environment on a global scale. The expression of selected genes associated with regeneration signaling networks will be monitored using RT-qPCR. Future work will implement treatments of ICI, an estrogen receptor antagonist that disrupts the function of all estrogen in an organism. Funding provided by Roanoke College Biology Department, Summer Scholars, and Research Fellows programs.

Program Abstract #336
Dissecting the impact of mTOR signaling on fish fin endochondral regeneration
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Lungfishes and the basal actinopterygian *Polypterus senegalus* are able to completely regenerate their fins after amputation at the endochondral level and represent promising fish models for appendage regeneration studies. Our group have previously shown that *Polypterus* regenerating fins and axolotl regenerating limbs share a core transcriptional program. Pathway enrichment analysis showed mTORC1 signaling as one of the enriched pathways in the regenerating fin transcriptome. The contribution of mTOR in appendage regeneration has only recently been uncovered in a study showing that axolotls use an evolutionary divergent hyperactivated mTOR protein to rapidly activate protein translation during limb regeneration. In the present study, we investigated aspects of the mTOR signaling during fish fin endochondral regeneration in *Polypterus senegalus* and in *Protopterus annectens*. Spatial transcriptomics analysis and immunostaining of P-RPS6Ser240/244(used as a read-out of mTOR activation) in both *P. senegalus* and *P. annectens* indicated a strong activation of mTOR signaling in the blastema cells and in the wound epithelium basal layer during fin regeneration. Although *Polypterus* mTOR protein does not have the modifications seen in the axolotl mTOR, we also observed a rapid increase in protein synthesis after fin amputation. Pharmacological inhibition of mTOR signaling with rapamycin resulted in a complete blockage of fin regeneration. Furthermore, analysis of the transcriptional profiles of control and rapamycin-treated regenerating fins indicated that mTOR inhibition increases the expression of fibrotic markers and decreases muscle tissue remodeling during fin regeneration. Our observations combined with published mTOR related data in axolotl indicate mTOR as a shared component of a rapid regenerative response in successful appendage regeneration models. Funding: LSUAM Bio Sci Startup (I. Schneider).

Program Abstract #337
Identifying drivers of mature ligamentocyte de-differentiation to a developmental state for scar-free ligament regeneration in zebrafish
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Acute ligament injuries are common and lead to long-term risks of recurrent ligament tears and osteoarthritis resulting from instability of the repaired joint. In mammals, poor healing results from a failure

in differentiation; cells rebuilding the ligament form a fibrotic scar rather than differentiating into ligamentocytes, as they do not express ligamentocyte marker Scleraxis (*Scx*). In contrast, we recently described how adult zebrafish ligaments heal with *scxa+* cells following a transection injury. Additionally, we find injury-driven cell cycle re-entry in adult zebrafish ligamentocytes where mammalian ligamentocytes remain quiescent after injury. Repeated live imaging of two ligamentocyte transgenic reporter zebrafish, *scxa:mCherry* and *thbs4a_p1:eGFP*, shows initial repression and later re-activation of ligament fate during the regenerative process. Lineage tracing of mature ligamentocytes driven by a new *thbs4a_p1:CreER* allele shows that pre-existing ligamentocytes downregulate key ligament fate genes, migrate into the wound site, then persist to reform the new ligament. scRNAseq of joints across the full one-month healing period highlight a transient population of injury-specific cells that reactivate developmental ligamentocyte genes. snATACseq analysis of joints during early regeneration points to mechanosensitive Yap-driven re-opening of developmental enhancers near ligamentocyte genes as a driver of plasticity after injury. Together, these results establish zebrafish as the first model of adult scar-free ligament regeneration, whose ligamentocytes have the unique potential to de-differentiate and divide as a progenitor pool in adult ligament healing. By further characterizing biomechanical and developmental cues repurposed for dedifferentiation and ligament fate restoration in regeneration, we aim to identify targets for therapies to improve mammalian ligament healing. We thank the NIDCR F31 and R00 awards and AR3T pilot grant for funding this research.

Program Abstract #338

Heavy metal-laden particulate dust alters transcriptional regulation in human colonoids

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Chronic heavy metals exposure in the southwestern United States co-occurs with greater incidences of metabolic and digestive diseases, particularly in Indigenous and Hispanic populations. There is a need to understand how this exposure drives a dysbiotic state and propose a molecular explanation as a result of these exposures. The objective of this study is to elucidate the mechanism behind the transcriptional and cellular changes present in the intestinal epithelia upon acute exposure to non-fissile uranium particulate dust. We hypothesize that non-fissile uranium exposure biases the differentiation of progenitor cells in the crypt to secretory roles through miRNA regulation. Human colonoids were exposed (18 hours) to non-fissile uranium dust obtained from the Jackpile uranium mine, one of the largest open pit uranium mines, located on the Laguna Pueblo in New Mexico. Control and dust-exposed colonoids (n=3 unique donors) were digested into single cells and processed for droplet-based single cell sequencing (scRNAseq). Upon exposure, enteroendocrine cells (EEC) expanded >4 fold, along with increases in serotonin and PYY, as well as goblet cells (>3 fold) which indicates changes to the secretory lineage. Downstream pathway analysis found microRNAs miR-625-3p, miR-320a, miR-29b-3p to be differentially expressed. These results suggest that acute uranium dust exposure induces changes in differentiation pathways which are modulated by regulatory miRNAs. We would like to expand these findings by performing miRNA sequencing and comparing miRNA expression profiles between UBD exposed and control samples. Our results also provide an improved understanding of uranium dust's impact on the gastrointestinal system, which may inform other physiological studies on the health of individuals exposed to heavy metals. This study was funded by K01DK106323, R56ES034400, P42ES025589, P20GM121176, P20GM130422, and AGA SURF.

Program Abstract #339

Apoptotic cell signaling in epithelial progenitor cell fate determination

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The regeneration of adult tissues after injury, or the replacement of cells in tissues with high turnover rates, is well understood in tissues with distinct stem cell populations. However, in the adult prostate, despite its ability to regenerate through cycles of castration-induced regression and regrowth, no identifiable stem cell population has been observed. It has been shown that differentiated epithelial cells behave as facultative progenitors to contribute to repopulation during regeneration. *We believe that apoptotic cells present during castration-mediated regression contribute to their acquired progenitor fate* Through histological analysis, we identify non-professional phagocytosis of apoptotic cells to be a predominant

route of cell clearance in the regressing prostate. By quantifying changes tissue morphology as well as cell type heterogeneity throughout the process I plan to characterize the process of prostate regression more in depth. To then determine the role of cell clearance in acquired tissue regeneration potential, we will block phosphatidyl serine (PtdSer) during prostate regression. I generated a mouse model in which I can induce expression of a dominant negative form of the potent PtdSer binding protein MFGE8, MFGE8-D89E in K8-expressing luminal cells (K8Cre^{ERT2}; MFGE8-D89E) to block engulfment *in vivo*. Using this system, I will assess the influence on regeneration potential of luminal cells through sphere-forming assays, surgical regeneration, and immunofluorescent analysis of progenitor markers. Through this study, we hope to gain a better understanding of the role of non-professional phagocytosis apart from cell clearance and to determine the contributions of apoptotic cell signaling to epithelial cell plasticity. This research is funded by the Canadian Institute of Health Resarch.

Program Abstract #340

Studying the regenerative capacity of the respiratory system in Xenopus tropicalis

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The respiratory system takes a central role in animal biology by allowing vertebrates to breathe air. While our understanding of the embryonic development and regeneration of the respiratory system has increased substantially, these studies have been limited by their focus on mammalian systems. Here, we hypothesized that some vertebrates, such as amphibians, can regenerate their respiratory system and asked what are the underlying molecular mechanisms that regulate it. To this end, we have established a protocol for lung injury in Xenopus tropicalis (X. tropicalis) and followed the healing process. Using chemically induced injury of the lungs with bleomycin, known to damage the lung through oxidative stress, we aimed to identify the cell populations that contribute to organ recovery post-injury. Combining various imaging techniques, such as histology, *in situ* hybridization, and immunostaining, we characterized and compared the healing process of lungs post-injury between froglets and juveniles X. tropicalis. Preliminary results show an elevation in collagen deposition and alveolar enlargement in X. tropicalis lungs post-injury, indicating bleomycin's effect, to cause lung injury and fibrosis in Xenopus lungs. We then analyzed the dynamics of sftp genes mRNA expression post-injury, Sftpc and Sftpb, showing Sftpb expression is decreased at the alveoli during healing. These results show for the first time the physiological similarities between the amphibian and the mammalian lung following organ injury, suggesting X. tropicalis as a potential model to study lung disease and regeneration processes, and therefore may have implications for both the biomedicine and evolutionary biology fields. This work is funded by the Chicago Fellows Program postdoctoral fellowship, The University of Chicago, and was supported in part by the Zuckerman STEM Leadership Program.

Program Abstract #341

FOXO and β-catenin Influence Naïve Pluripotency Downstream of AMPK During Hyperglycemia Induced Oxidative Stress

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Preimplantation exposure to high glucose levels can negatively affect early embryo development. Uncontrolled glucose levels in the mother can lead to decreased of blastocyst size, spontaneous abortion, and congenital malformations. Problematically, studying the effects of hyperglycemia on the development of the blastocyst and the embryo are difficult to do *in vivo*. Here, embryonic stem cells (ESCs) remain a powerful model in studying these effects due to their pluripotency and capacity to mimic embryonic development. Intriguingly, ESCs are typically cultured with diabetic glucose levels at 4.5g/L, but whether this affects pluripotency is questionable. Previous work in our lab has demonstrated that hyperglycemia can lead to increased levels of ROS and antioxidant enzyme activity coupled with a decrease in cell proliferation. Investigating the molecular mechanisms, we focused on the transcription factor forkhead box protein O (FOXO), a well-known protector from oxidative stress. Hyperglycemia targeted FOXO and β-catenin to the nucleus to upregulate genes encoding for antioxidant enzymes and cell cycle regulators. Though effectively removing ROS, FOXO nuclear inclusion has undesired side effects: a downregulation of pluripotent markers and premature differentiation. Therefore, understanding

the upstream regulators surrounding FOXO nuclear localization is crucial. One potential regulator of FOXO, that acts as an energy sensor, is AMP-activated protein kinase (AMPK). AMPK was inhibited in high glucose (25mM) conditions partnered with increased FOXO activity. Treatment with an AMPK activator, 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), led to decreased FOXO nuclear levels and rescued pluripotency and proliferation in ESCs. Together, these findings provide insights regarding how hyperglycemia may alter the embryonic environment, which a step toward delineating how hyperglycemia disrupts early embryogenesis. Funded by CIRM: EDUC4-12752

Program Abstract #342
Investigation of Cutaneous Wound Healing in Zebrafish
Leah Greenspan[1], Keith Ameyaw[2], Daniel Castranova[1], Van Pham[1], Gennady Margolin[1], Caleb Mertus[1], Brant Weinstein[1]
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Two percent of the US population is plagued by open chronic wounds, with a delay in vascular reperfusion being a major contributor to the defects seen in wound closure. This delay often occurs in aged or diabetic adults, yet why remains unclear. Mammalian models of wound healing have revealed many important cell types and signals that drive the different phases of wound healing, but these models do not permit high-resolution imaging of the healing process in real time, making it difficult to study transient cellular and molecular events. The zebrafish is an ideal model for visualization and experimental dissection of cutaneous wound healing in the context of a living animal, with numerous transgenic lines available that mark relevant cell populations and powerful methods for high-resolution imaging of these lines. We have established an innovative and reproducible cutaneous wound model in zebrafish using a rotary tool combined with cellular-level long-term confocal imaging of wounds in living adult fish. We find that skin re-epithelialization and neutrophil recruitment initiate within hours after injury and peak at one day, while macrophage activity and vessel regrowth increase between 1-4 days post injury, and vessel re-patterning takes many additional months. We have also devised novel tools for direct *in vivo* profiling of the endothelial translatome using TRAP-RNAseq of "AngioTag" transgenic fish and have uncovered common endothelial signatures between adult organs and unique endothelial genes within the vasculature of each organ such as *col17a1b* in the skin vessels. We are now using these tools to explore the cellular and molecular changes in angiogenesis during the wound healing process. Together these studies will uncover the cellular behaviors and molecular mechanisms that restore vascular networks after cutaneous injury, providing potential new targets for therapeutic approaches. Funding is from the NICHD intramural research program.

Program Abstract #343
LUTHOR – unparalleled sensitivity in single cell sequencing
Kristy Ramsey, Mantas Survila, Pamela Moll, Cynthia Wollner, Musashi Tsujita, Kathrina Radl, Mira Schaupper, Michael Moldaschl, Torsten Reda
Lexogen , United States
A key quality metric in single-cell RNA-seq is the ability to capture ideally all RNA molecules present in each cell. Here we introduce the novel LUTHOR 3' mRNA-Seq protocol based on THOR amplification that holds the power to capture mRNA transcripts close to the estimated maximum. THOR technology can be used to generate individual and pooled 3' mRNA-seq, or full-length individual libraries from ultra-low input RNA or single cells. The technology is template-switch-, and ligation-free, and employs a unique THOR reaction. The THOR reaction is initiated at oligo(dT) primed poly(A) tails introducing a T7 promoter sequence to all 3' ends of transcripts. The resulting structure allows swift **amplification of antisense RNA directly from mRNA templates**. With the help of LUTHOR we accessed the sequencing depth detection limits for single cell experiments and investigated R^2 calculations which define correlations of detected but also non-detected genes in cell replicates taking the statistical constraints through read depth limitations into consideration. Such metrices are needed to distinguish experimental noise from expression data in single cell experiments. To demonstrate the power of LUTHOR, the quantitative performance was also compared in a high-throughput compatible setup. LUTHOR libraries were generated from HEK293T cells captured in 1 nL size droplets using Onyx instrument (DropletGenomics) and compared to 10X Single Cell v3.1 data with a limited number of reads. The gene and UMI detection rates were minimum 2-fold

higher when compared to 10X at all initial read depths. Funded by Lexogen GmbH Research and Development.

Program Abstract #344
Transcriptional analysis of axial level differences within the premigratory vagal neural crest
Aria Fasse, Marianne Bronner
California Institute of Technology, USA
The neural crest is an embryonic stem cell population that gives rise to diverse cell types including the craniofacial skeleton, melanocytes, and most of the peripheral nervous system. Different subpopulations of the neural crest form at different axial levels, each of which have a characteristic developmental potential. One of these subpopulations, the vagal neural crest, arises within the hindbrain and undergoes extensive migration to populate portions of the heart as well as the enteric nervous system. Cells originating from other neural crest subpopulations (e.g. cranial and trunk) cannot substitute for the vagal neural crest, demonstrating critical differences in neural crest developmental potential along the body axis. Within the vagal neural crest, the more rostral "cardiac" neural crest cells migrate primarily to the heart whereas the more caudal "enteric" neural crest cells migrate primarily to the gut. Importantly, cells from the "cardiac" and "enteric" subpopulations of the vagal neural crest also fail to substitute for each other, suggesting that key differences exist even between these directly adjacent subpopulations. Here, we explore the differences between cardiac and enteric neural crest cells by profiling premigratory neural crest cells from either axial level at high resolution using Smart-seq3 single-cell RNA sequencing. To this end, we dissected the dorsal neural tube adjacent to somite 1-2 to obtain cardiac crest cells and adjacent to somites 4-7 to obtain enteric neural crest cells. The cells were then dissociated into single cell suspension and isolated in well plates via FACS sorting to enable the profiling of hundreds of cells per population. Our results reveal differences in key transcription factors and signaling molecules that may contribute to the unique developmental potential of cardiac and enteric neural crest cells. Funding acknowledgement: R01HL169287 to M.E.B.

Program Abstract #345
Developmental trajectories of condensing cranial neural crest cells in the mandibular arch
Sierra Marable, Marianne Bronner
California Institute of Technology, USA
The neural crest is a multipotent progenitor cell population arising in vertebrate embryos during neurulation and subsequently giving rise to various cell types, including elements of the craniofacial skeleton and the peripheral nervous system. While there are various neural crest subpopulations along the body axis, only the cranial neural crest has the unique ability to form craniofacial cartilage and bone. While many studies have investigated the specification and migration of cranial neural crest cells, much less is known about the molecular mechanisms that drive cell fate decisions underlying formation of facial cartilage and bone. Here, we tackle this question by examining the transcriptional network regulating early steps of cartilage condensation within the first pharyngeal arch. To this end, neural crest cells were FACS-isolated from the mandibular arch of E11.5 mouse embryos and transcriptionally profiled using single cell RNA sequencing. Bioinformatic analysis revealed heterogeneity within the cranial neural crest population, identifying several clusters reflecting distinct cellular states and expressing markers characteristic of chondroprogenitors and osteoprogenitors. Molecular signatures of each cell state were used to construct developmental trajectories of each lineage. To identify potential drivers of cell differentiation, we examined transcription factors expressed in the chondroprogenitor cluster and noted high expression of *Barx1*, *Sox5*, and *Sox6*. In situ hybridization analysis further validated expression of these genes in the mandibular arch. Perturbation studies will be used to evaluate the relationships between these key transcription factors and chondrogenesis. Taken together, our results provide insight into the gene regulatory networks coordinating neural crest differentiation into craniofacial cartilage and bone. This work is supported by the NIH/NIDCR 5R01DE024157-09 to M.B. S.M. is supported by the Helen Hay Whitney Foundation.

Program Abstract #346
Candidate metagenes define transcriptional heterogeneity in intrahepatic biliary epithelium
Kendall Kanakanui, Fransky Hantelys, Hannah R Hrncir, Sergei Bombin, Adam D Gracz

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Epithelial heterogeneity within tissues supports both routine tissue functions and response to environmental challenges or damage. In the liver, hepatocytes and biliary epithelial cells (BECs) function in (1) metabolism, protein synthesis, detoxification and (2) bile modification and transport, respectively. Single-cell and spatial transcriptomic assays have begun defining molecular heterogeneity in hepatocytes, expanding the understanding of cellular subtypes in the liver. While morphological and functional heterogeneity in BECs is well-studied, a definitive understanding of BEC transcriptomic heterogeneity remains elusive. Our lab has recently characterized BEC heterogeneity using a $Sox9^{EGFP}$ allele expressed at distinct levels in all BECs and a subpopulation of peribiliary hybrid hepatocytes (HybHeps). Here, we performed scRNA-seq on $Sox9^{EGFP}$+ liver epithelial cells isolated by FACS in both homeostasis and cholestatic injury induced by bile duct ligation (BDL). We identified 4 transcriptomically-distinct BEC subpopulations but failed to identify single-gene biomarkers. Alternatively, we identified candidate multi-gene biomarkers (metagenes) using NSForest 2.0 that are uniquely enriched in distinct BEC subpopulations. Application of candidate metagenes to BDL samples reinforced BEC subpopulations identified in homeostasis. Following BDL, we identify a *de novo* BEC subpopulation marked by *Mki67* where all metagene-defined subpopulations are represented, suggesting all BEC subpopulations have a proliferative capacity in response to injury. Lastly, we identified IL-6 activation in our injured HybHep subcluster and validated *in vitro* that IL-6 signaling upregulates genes represented in the injured HybHep subcluster in our data. Together, our candidate metagene approach defines novel transcriptional heterogeneity in the liver epithelia. Funded by the NIH R01 DK132653-01 (ADG), T32 GM14028391-02 (KGK), and the GRFP NSF 12-599 (KGK).

Program Abstract #347

Sex-dimorphism in liver cellular transcriptomes of the naturally short-lived Nothobranchius furzeri

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University of Southern California, USA
The African turquoise killifish (Nothobranchius furzeri) is an emerging naturally short-lived vertebrate model organism to study aging. Thus, a comprehensive catalog of turquoise killifish cell types is still lacking. To address this gap in knowledge, we generated a single-cell transcriptomic map using blood, kidney, liver, and spleen from male and female turquoise killifish. The liver was the most sex dimorphic tissue assayed, with female livers enriched for terms associated with "response to lipid". To determine the significance of these gene expression differences, we used Oil-Red-O staining, and showed that male livers from 2 strains of killifish were fattier than age-matched females. We were also able to demonstrate the female-biased expression of a universal female-predictive ncRNA transcript, ncFem1, using quantitative fluorescent in situ hybridization in female and male turquoise killifish livers. Funding is provided by the Dornsife Summer Undergraduate Research Fund from the University of Southern California.

Program Abstract #348

Harnessing developmental biology to understand sex differences in non-reproductive organs

Alexandra Wiscovitch, Brooke Armfield, Martin Cohn
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Biological differences between males and females, known as *sexual dimorphisms*, have long been recognized in reproductive organs and in secondary sex characters. Some sexually dimorphic traits are found in only one sex, whereas others occur in both sexes but with differences in anatomical form and function. Less is known about sexual dimorphisms in non-reproductive organs, particularly where gross morphology and function are similar between sexes. Numerous diseases of non-reproductive organ systems – including urinary, respiratory, nervous, digestive, musculoskeletal, cardiovascular, and immune – show sex differences in their prevalence, progression, responses to treatment, and outcomes. Whereas adult diseases of reproductive organs often involve mis-regulation of the same signals that regulate development and sexual differentiation in the embryo (*e.g.*, sex hormones), the causes of sex-biased diseases of non-reproductive organs are poorly understood. We asked whether the approaches that we have used to study sexually dimorphic development of external genitalia can be applied to non-reproductive organs to identify novel sex differences and the underlying causes. We investigated development of 2 non-reproductive organs – bladder and spleen – that are structurally similar in males and females. We find that both organs are sexually dimorphic at the single-cell level. Genetic

manipulations show that sexual differentiation of the bladder and spleen is regulated, at least in part, by the same mechanisms that act on the external genitalia. Moreover, as we have shown for the external genitalia, sexually dimorphic development of the bladder and spleen is not qualitative or binary (*i.e.*, presence/absence), but is granular and quantitative at cellular and molecular levels. The results show that developmental biology is uniquely positioned to advance our understanding of sex differences throughout the body. Funding:NIDDK & Koger Endowment

Program Abstract #349

In toto recording of cranial neural crest lineage relationships in the zebrafish head

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Cranial neural crest-derived cells (CNCCs) contribute to diverse cell types that support the vertebrate head and face. To understand the diversity of CNCC cell types in the head, I have constructed a single-cell atlas of RNA expression and chromatin accessibility of CNCC lineage cells throughout the zebrafish lifetime. This atlas uncovered unique CNCC cell types in the gills and skin, and uncovered enhancers and transcription factors linked to CNCC lineage decisions. However, prior studies relied on cell dissociation, making it unclear how these CNCC cell types are spatially distributed and lineage related in the head. Recently, I have adapted a multiplexed in situ hybridization technique (seqFISH) to detect 220 transcripts simultaneously at multiple stages of zebrafish head development. This has allowed me to identify the spatial distribution of CNCC-derived cell types. To integrate true lineage information with spatial transcriptomics, I have developed zebrafish transgenic for an intMEMOIR integrase barcoding system. I will report on using these intMEMOIR zebrafish together with seqFISH to define the types of CNCC lineage relationships across the zebrafish head. In addition, I am using intMEMOIR zebrafish to assess how CNCC lineage relationships change in craniofacial patterning mutants and during regeneration. This project is funded by R35 DE027550.

Program Abstract #350

Generating transgenic zebrafish for optogenetic control of FGF, BMP, & Nodal signaling

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A fascinating biological phenomenon is the development of a single cell into a mature organism with a diverse range of tissues and cell types. Signaling pathway activity is crucial to this development. To better understand how signaling generates diverse cell types, we use optogenetic "bOpto" tools to activate the FGF, Nodal, and BMP pathways in zebrafish with blue light. We use mRNA microinjection to introduce these tools into one-cell embryos, a time-consuming technique that results in transient and sometimes mosaic expression. We are now generating transgenic zebrafish lines that express these tools from ubiquitous promoters, which should greatly facilitate further experimental pathway manipulation by eliminating the need for microinjection and by providing continuous, ubiquitous expression. F0s injected with Tol2 transposase and plasmids containing ubiquitous promoters driving bOpto-FGF expression appear normal when reared in the dark, but have defects consistent with ectopic FGF activity when exposed to blue light. F1 embryos with transgene germline transmission also appear to experience ectopic FGF activity with blue light exposure but are healthy in the dark. We are currently raising F1s with confirmed germline transmission and will test the blue light sensitivity of the F2 generation. We also plan to use the site-specific pIGLET transgenesis system to generate bOpto lines with well-characterized transgene insertion positions. Some challenges we are working to overcome include fine-tuning expression levels, housing potentially light-sensitive adult zebrafish, and generating bOpto-BMP and -Nodal transgenics, which both require the separate expression of multiple proteins for optimal functioning. We believe these transgenics will improve the ease and precision of optogenetic signaling manipulation and facilitate longer term experiments than those currently allowed by mRNA microinjection. Funding: NIH Intramural ZIAHD009002-01 to KWR.

Program Abstract #351

Using endogenously tagged pioneer TFs to investigate the organization and behavior of transcription during the zebrafish maternal-to-zygotic transition.

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Yale University, USA

Pioneer transcription factors facilitate chromatin remodeling and accessibility to initiate zygotic gene transcription. Recently, it has been shown that the zebrafish pioneer transcription factor Nanog coordinates the organization of large transcription bodies during the maternal-to-zygotic transition. The clustering of Nanog protein into distinct subnuclear foci is essential for its role during this critical developmental transition. While live imaging of exogenous, fluorescently tagged Nanog has revealed important molecular behaviors, the physiological relevance of these experiments is unknown. Here, we report the behavior of endogenous Nanog protein during the first wave of zygotic genome activation in zebrafish. Using CRIPSR/Cas9 genome editing, we inserted a short N-terminal epitope tag into nanog. When bound by its cognate nanobody binder, Nanog is visualized by fluorescence and used for live and fixed imaging. When investigating Nanog localization and dynamics by live imaging, we find fewer Nanog clusters that are notably smaller in comparison to exogenously introduced Nanog, further underscoring the physiological relevance of probing endogenous targets and revealing principles of pioneer transcription factor function during genome activation.

This research is generously funded by an EMBO and HFSP Postdoctoral Fellowship.

Program Abstract #352

Photobleaching-Based Spatial Proteomics in Zebrafish

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Spatial proteomics can provide novel mechanistic insights into developmental biology, but challenges include isolating sufficient protein while preserving spatial information efficiently and with high throughput. These challenges are particularly profound for complex tissues such as the developing brain. We are interested in understanding the molecular basis of neural differentiation and central nervous system disease progression. To do so, we aim to create an atlas of protein expression across all developing neurons in the brain. Current methods, including laser capture microdissection or three-dimensional imaging of solvent-cleared organs coupled with mass spectrometry-based proteomics, allow for conserved spatial information but are limited by complex tissue preparation techniques, pipelines, and/or specialized equipment. Here, we offer an approach that is cost-effective, high-throughput, and accessible, labeling larval or adult zebrafish tissue sections with fluorescent dyes or transgenically-expressed fluorescent proteins. These samples undergo fluorophore-specific photobleaching at single-cell resolution, followed by dissociation and cell sorting. This process yields 'fluorescence-barcoded' cells for downstream liquid chromatography and tandem mass spectrometry (LC/MS-MS) proteomics. Our approach to spatial proteomics using larval and adult zebrafish tissue will reveal insights into both brain development and disease. Funding sources: NIGMS, NICHD, UAB Heersink School of Medicine Start-Up Funds.

Program Abstract #353

VitelloTag: a tool for high throughput cargo delivery into oocytes

D. Nathaniel Clarke[1], Akshay Kane[2], Margherita Perillo[2], Christopher J. Lowe[3], S. Zachary Swartz[2]
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Developmental and reproductive biology research broadly relies upon the delivery of molecular and genetic tools into oocytes. In diverse model organisms, including mouse, zebrafish, xenopus, and echinoderms, this is achieved through microinjection. Microinjection, while highly effective, has challenges in terms of cost of setup, the technical skill required, and the limited numbers of injected oocytes obtainable. It also proves challenging in organisms with delicate oocytes or restricted spawning

seasons. To overcome these limitations, we have developed VitelloTag, a simple, cost effective, and high throughput method of delivery into oocytes, comparable in terms of technical difficulty to transfection. Here, we present a delivery system that employs conserved regions of vitellogenin, a yolk protein precursor, recombinantly fused with the protein of interest for delivery via receptor-mediated endocytosis and endosome escape. We demonstrate this tool's utility and cross-taxa applicability by delivering GFP and Cas9/sgRNA complexes, with successful gene knockout phenotypes, in two distantly related species.
Financial support for this work was provided by the NICHD, grant number 5R00HD099315-04.

Program Abstract #354
Parallel 10-Plex Imaging of RNA and Protein Targets with HCR™ RNA-FISH
Chanpreet Singh, Cameron Earl, Mike Liu, Aneesh Acharya
Molecular Instruments, USA
HCR™ RNA-FISH serves as a fundamental tool for developmental biologists, empowering researchers with high performance, multiplex, quantitative imaging of RNA expression in any sample type, including highly autofluorescent whole-mount embryos and FFPE tissue sections. The ability to map spatial organization of RNA and protein molecules within the same sample is critical for advancing our understanding of key developmental changes like organogenesis, thus providing deeper insights into developmental disorders. Molecular Instruments® (MI) supports a vast community of developmental researchers worldwide, engaged in studying over 500 diverse species. MI is excited to announce the immediate availability of ready-to-use up to 10-plex assays that can be seamlessly adaptable to any sample type of interest to the scientist. Additionally, we also present an affordable, scalable, and automated platform capable of performing HCR™ assays to streamline high-throughput workflows.
Funding source: Molecular Instruments®

Program Abstract #355
Next-Generation HCR™ RNA-FISH Enables Protease-Free, Scalable Co-Detection of RNA and Protein in Any Sample
Adam Maddox, Joseph Melko, Cameron Earl, Aneesh Acharya
Molecular Instruments, Inc., USA
HCR™ RNA-FISH has become a cornerstone tool for visualizing RNA expression within intact sample types, ranging from cells in suspension, to tissue on slides, to whole-mount vertebrate embryos. Today, these kits utilize the HCR v3.0 architecture first developed in 2018, which itself enabled robust, turnkey RNA-FISH due to its innovative automatic background suppression implemented at a platform level, regardless of target or sample type. Molecular Instruments® (MI) supports thousands of users within the global evolutionary and developmental research community, working with over 500 unique species. Here, we demonstrate our latest innovation: the next-generation HCR™ v4.0 platform. Representing nearly ten years of engineering efforts, HCR™ v4.0 will drive cutting-edge biology research for the next decade by maintaining an enzyme-free approach, ensuring compatibility with any sample type, and significantly enhancing the performance of HCR™ assays by an order of magnitude

Program Abstract #356
Genetically encoded affinity reagents (GEARs): A toolkit for visualizing and manipulating endogenous protein function *in vivo*.
Curtis Boswell[1], Caroline Hoppe[1], Alice Sherrard[1], Liyun Miao[1], Mina Kojima[1], Srikar Krishna[1], Damir Musaev[1], Ning Zhao[2], Timothy Stasevich[3]
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Probing endogenous protein localization and function *in vivo* remains challenging due to limitations with gene targeting in certain model organisms and, once established, the inflexibility of precision targeted alleles. Affinity binding reagents such as antibodies can be used to detect endogenous proteins but are amenable to fixed samples, and their use is limited by reagent availability, feasibility of epitope binding and cost. Recently, developments in affinity-based reagents that can be genetically encoded, such as nanobodies and single chain variable fragments (scFvs), have enabled precision probing of protein targets *in vivo*. Here, we optimize these ***g****enetically* ***e****ncoded* ***a****ffinity* ***r****eagents (GEARs*) and show their effectiveness at rapidly probing and perturbing *in vivo* protein function in zebrafish. Using short epitope-

based tags and their cognate GEAR binder, we demonstrate that these tools can bind to a variety of protein targets that exist in various subcellular compartments. Notably, these tags can be used for targeted protein degradation in fish, mice and human cells. To improve the utility of GEARs, we developed a rapid CRISPR/Cas9 based protein tagging pipeline to generate endogenous knock-ins in zebrafish and demonstrate the installation of GEAR tags at several zebrafish loci. We further demonstrate that these tagged alleles can be used with GEAR reagents to visualize endogenous, native behaviour of tagged proteins, recapitulate loss-of-function mutant phenotypes with degron GEARs and interrogate protein interaction networks using proximity-based identification methods. Together, this system provides a rapid and powerful means to interrogate protein function while circumventing many challenges associated with conventional means of gene targeting. This work is supported by the Canadian Institutes of Health Research (CIHR).

Program Abstract #357

The Shh-Brachyury axis in the postnatal nucleus pulposus cells regulates intervertebral disc health.

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Degeneration of intervertebral disc (IVD) degeneration is a significant cause of chronic back pain, a top neurological and debilitating condition affecting ~70% of people, but with no cure as little is known about the cellular and molecular basis of IVD development and health, and changes associated with its degeneration. Notochord descendant Nucleus pulposus (NP) form the center of each IVD. Previously, we showed that mouse NP cells continue to express SHH and Brachyury and that SHH maintains growth and differentiation of the entire IVD. However, the expression of these crucial developmental regulators was lost with age and painful IVD pathology. Conditional targeting of SHH revealed that Brachyury is its downstream target. Hence, to test the role of Brachyury in postnatal IVD, we generated a conditional allele and targeted it at 1) early development using haploinsufficient allele, 2) node/notochord stage (E8), and 3) postnatal stage following tamoxifen induction. The effects were analyzed on cell growth, survival, and extracellular matrix expression. Additionally, we utilized spatial transcriptomics to observe transcriptional changes in specific disc compartments between Brachyury mutant and control littermates. Our results show that a normal expression level of Brachyury is critical for NP and IVD formation, growth, and survival, and its loss accelerated IVD degeneration. Spatial transcriptomics shows a loss of distinct regional transcriptomic profiles in the Brachyury mutant disc compared to controls, including deregulation of basement membrane and extracellular matrix genes and an indication of mesenchymal to epithelial transition of the NP cells. We conclude that Brachyury expression is essential for postnatal IVD health. Further studies are required to identify the downstream targets of Brachyury that form a signaling network and regulate the surrounding components of the IVD. Funding support by NIAMS R01AR077145 and OD 1S10OD026763 of NIH.

Program Abstract #358

TwinNet: Quantitative analysis of developmental dynamics with artificial intelligence

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Animal embryos stereotypically produce body plans with species-specific features and appearance, but the developmental dynamics to achieve appropriate growth and form differ widely between species. Accurate assessment of morphogenesis and developmental tempo is therefore central for quantitative studies of embryonic development, but given the fluid nature of embryogenesis as well as considerable variation between individuals, manual assessment remains subjective and challenging. To overcome this challenge, we have developed TwinNet, a neural network that allows to quantitatively analyze developmental dynamics in diverse model organisms using artificial intelligence and similarity calculations. We first created a dataset of thousands of images of zebrafish development and applied TwinNet to automatically assess embryonic age without the use of pre-defined stages. In addition, TwinNet allowed us to calculate developmental tempo and variations between individual embryos. We then extended our method to medaka, three-spined stickleback, and C. elegans, demonstrating that

TwinNet can be robustly applied in different species even when only limited data is available. Furthermore, TwinNet was able to automatically generate atlases of the main developmental epochs in diverse species, which quantitatively showed that development is characterized by the alternation of periods, in which embryonic morphologies change, and phases, in which embryonic morphologies undergo little change. In summary, TwinNet can be used as standardized approach with various possibilities for objective, quantitative and multiparametric studies of embryonic development. The code of our software as well as terabytes of training and evaluation data are freely available from https://github.com/mueller-lab/TwinNet and https://doi.org/10.48606/50, and the method is open to further development by the community. Funding: ERC 863952, MPG, IZKF, UKON Blue Sky EvoDevoGPT, EMBO YIP, DFG EXC 2117 – 422037984.

Program Abstract #359

Evaluating molecular mechanism(s) by which the Foxi3 transcription factor governs pre-placodal specification during vertebrate craniofacial development

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Early in vertebrate embryogenesis, four multipotent cell lineages coalesce within a small region of the anterior embryo known as the neural plate border. These progenitor lineages— neural plate, neural crest, pre-placodal, and epidermal— collectively give rise to the diverse cell types of the head and neck. Of interest to our lab is the pre-placodal lineage, the origin of most cranial sensory organs and non-sensory tissues, such as the lens of the eye. Despite the value of pre-placodal cells in craniofacial development, researchers have a poor grasp of factors driving the specification and differentiation of this lineage. Our lab studies a transcription factor, Foxi3, that specifically labels the pre-placodal lineage from the time of border formation in mice. We previously showed that mice lacking Foxi3 lose the inner ear and posterior placode-derived ganglia, but anterior placodes, including the lens, form normally. We used Cre-LoxP lineage tracing to generate mice that were functionally null for Foxi3 but retained expression of a reporter gene in all cells where the Foxi3 promoter was active at the time of Cre activation. We discovered that Foxi3 mutant cells become neural tube, crest, and epidermal derivatives instead of placodes. Together, these findings suggest that Foxi3 is required for pre-placodal lineage specification but differentially regulates posterior placode development. We are now using single cell RNA-sequencing to determine the molecular basis for Foxi3 function at the neural plate border. We established that anterior and posterior placode progenitor cells can be transcriptionally distinguished just 24 hours after border formation. We will next assess whether Foxi3 mutant cells acquire the molecular signature of other border lineages and identify candidate gene regulons for posterior placodes that are altered in mutant cells. Funded through the NIDCR (1F31DE032898-01) and R01 DC013072.

Program Abstract #360

The role of Sonic hedgehog signaling in cranial neural crest cell development

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Most cells within the embryo are restricted to give rise to derivatives of their respective germ layers, but cranial neural crest cells (CNCCs), often called "the fourth germ layer", represent an interesting exception. Although CNCCs are derived from ectoderm, they can differentiate into both traditionally ectodermal (e.g., neurons/glia) and mesodermal derivatives (e.g., bone, cartilage). Despite a robust interest in CNCCs, the molecular signaling mechanisms that convey this pleistopotency are not well understood. Previous in vitro studies suggested that exposure to Sonic hedgehog (Shh) signaling conveyed pleistopotency upon CNCCs, as it increased the proportion of CNCCs capable of differentiating into both ectodermal and mesodermal derivatives. To investigate the role of Shh signaling in CNCC development, we performed RNAscope on early mouse embryos and showed that Shh is expressed in the paraxial mesoderm, suggesting a possible role for Shh signaling in CNCC development. In vivo data supported these findings by revealing that Gli transcription factors (Gli TFs) were expressed in the head folds during CNCC induction and specification, and computational analysis revealed that a significant number of Gli motifs were present at predicted enhancers of pluripotency genes (e.g., Oct4 and Nanog). To determine the role of Gli-mediated Shh signaling, we are generating conditional knockout lines that delete Gli TFs at different stages of CNCC development (induction, specification, and

migration). These results will not only provide insight into the molecular mechanisms necessary for establishing CNCCs pleistopotency but will also determine novel roles for Shh signaling during CNCC development. This work is funded by NIH F31DE033565 (SJYH) and NIH R35DE027557 (SAB).

Program Abstract #361

Phenylalanine Affects Cranial Neural Crest Cell Differentiation Into Smooth Muscle

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Maternal phenylketonuria [MPKU] is a syndrome associated with several congenital anomalies including cardiovascular malformations [CVMs], microcephaly, and craniofacial dysmorphism. The cellular and molecular mechanisms of Phe teratogenicity are currently unknown. Neural crest cells [NCCs] are known to migrate and differentiate to form vascular smooth muscle cells populating the cardiac outflow tract [OFT], aortic arch arteries, and vessels in the face and head. O9-1 mouse neural crest cells (O9-1) were plated on glass coverslips and grown in smooth muscle differentiation media in the presence or absence of Phosphate Buffer Solution (PBS) (vehicle control), Phenylalanine (Phe), and/or TGF-β. Immunohistochemical staining for ?-Smooth Muscle Actin (?-SMA) antibody and 5-ethynyl-2'-deoxyuridine (EdU) on D3, D4, and D5 to analyze differentiation and proliferation. RNA was isolated from treated cells, cDNA synthesized and RT-PCR conducted for gene expression of stem cell and smooth muscle markers. Preliminary data suggests Phenylalanine delays differentiation as shown by a decrease in expression of ?-SMA and an increased percent of cell proliferation. This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award P20GM103447. Additionally, this project is based on work supported by a grant from the Office of High-Impact Practices, and Office of Research and Sponsored Projects, University of Central Oklahoma

Program Abstract #362

Gene network regulating the early human neural crest specification from pluripotent stem cell state

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The neural crest is an embryonic multipotent stem cell population. Studies have elaborated on gene regulatory networks involved in induction of NC at the neural plate border and more recent research has focused on the earliest origins of NC specification from a pluripotent stem cell state. Recent studies have implicated the expression and role of OCT4 and NANOG in mouse, chick, and Xenopus. These studies have suggested a continued expression of these two stem genes in NC cells, and further suggesting that OCT4 might play a role in regulating the chromatin state in NC cells. While these studies suggest a role of these two stem genes in NC development, the mechanisms by which these genes regulate NC specification from a pluripotent stem cell state is not well understood. We have taken an unbiased approach to first identify a unique transcriptional signature of human NC, as well as explore the expression of genes that are shared with non-neural and neural ectodermal, mesodermal, and endodermal cell populations. Co-expression of many genes between different cell fates is expected during early stages of cell fate specification; however, differences in the level of expression of these genes between cell fates provides a better insight into the unique regulatory program that governs each cell fate. We then focus on the NC cell fate to identify key sets of genes involved in their early specification. Worked funded by NIH grant R01DE017914 and R21DE028112 to M.I.G-C.; F32DE027862 and K99DE029878 to R.M.C.; UCR Medical School Dean's Postdoc to Faculty fellowship to M.S.P.

Program Abstract #363

Mitochondrial metabolism in the developing retina: the role of TFAM in retinal progenitor cells and neurogenesis

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The retinal progenitor cells (RPCs) are self-renewable and multipotent cells that in a tightly regulated spatiotemporal sequence exit the cell cycle to give rise to six types of retinal neurons and the M?ller glial cells. Mitochondrial function and metabolism have emerged as core factors of cell pluripotency, proliferation, and differentiation. Mutations in genes of metabolic enzymes result in neurodegeneration and vision loss, however, the signaling pathways that link metabolism and retinal development are

unknown. Our hypothesis is that mitochondrial metabolism is directly link to the RPC renewability and therefore can regulate the competency of RPCs to generate new neurons and glia over the course of retinogenesis. Prior studies suggest that a functional electron transport chain (ETC) is required for RPC proliferation and retinal neurogenesis. A functional ETC is coupled to the production of cellular energy in the form of ATP via oxidative phosphorylation (OXPHOS). Transcription of OXPHOS subunits is regulated by several transcription factors including the mitochondrial transcription factor A (TFAM). Loss of *Tfam* in neural stem cells *in vitro* reduced the transcriptional expression of OXPHOS subunits mtND4, mtND6, mtCO1. In the present study we generated a conditional knock out (CKO) of *Tfam* in the mouse developing retina using the *Chx10*-Cre::GFP mice. We assessed the activity of the ETC and determined the spatiotemporal consequences of loss of *Tfam* in neurogenesis. In addition, we conducted *in vivo* EdU (5-ethynyl-2'-dioxyuridine)-pulse studies to assess RPC proliferation. Our preliminary data indicates that loss of *Tfam* in the RPCs results in deregulation of late-born cells and a delay of the RPCs cell cycle exit. This work is partially funded by grants from the *Knight Templar Eye Foundation* & a UHD internal grant.

Program Abstract #364

***Vexin*, *Meis2*, and *Meis3* Control Multiple Fate Specification Events During Mouse Retinal Development**

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Optimal vision relies on the precise generation of seven major retinal cell types. These cells emerge in an overlapping fashion during retinal development, granting retinal progenitor cells diverse fate options at any given time. Our investigation focused on deciphering the mechanisms that control retinal fate specification, specifically how the balance between cones and rods is established. Both photoreceptor types originate from a common pool of precursors expressing the transcription factor *Otx2*. However, the molecular cues directing these precursors toward a rod or cone identity remain unidentified. To elucidate this process, we conducted a comprehensive transcriptomic screen analysis in mice, identifying genes preferentially expressed in Otx2+ precursors. Among these genes were the transcription factors *Meis2* and *Meis3*, as well as a poorly characterized gene called *Vexin*. All three genes also exhibit expression in subsets of mature interneurons and ganglion cells. Based on their expression patterns, we hypothesized that these genes play pivotal roles in determining rod versus cone fate. To test our hypothesis, we manipulated these genes using gain- and loss-of-function approaches in mouse embryonic retinal explants via electroporation of cDNA misexpression and CRISPR interference plasmids. Intriguingly, our results revealed that each of these genes suppressed cone fate choice via discrete mechanisms. Remarkably, sustained misexpression of Vexin and the Meis genes resulted in the conversion of Otx2+ photoreceptors into interneurons or ganglion cells. Together, our findings propose that *Vexin* and the *Meis* genes function as regulatory switches, shaping photoreceptor diversity and impacting other cell fate decisions during retinal development. This work has been supported in part by funding from the NIH to JB (R01-EY024272).

Program Abstract #365

9-cis-Retinal Supplementation Accelerates Photoreceptor Development and Maturation in Human Stem Cell-Derived Retinal Organoids

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Retinal organoids (ROs) derived from human-induced pluripotent stem cells (hiPSCs) closely resemble the native structure and composition of the human retina. Consequently, they serve as potent in-vitro models for studying human retinal diseases and testing therapeutic interventions. However, the prolonged culture periods necessary for photoreceptor development limit the practicality of using ROs in these applications. We grew ROs from three distinct hiPSC lines and assessed five different supplementation conditions for their ability to promote the development of mature photoreceptors before day 180. The supplements tested were 1uM retinoic acid (RA), 10uM RA, 1uM 9-cis-retinal, 10uM 9-cis-retinal, and no treatment. These supplements were introduced at day 63 of differentiation and continued until the end of the culture period (180 days) to evaluate their impact on photoreceptor development and maturation. Outcomes were assessed by immunofluorescence, confocal microscopy followed by quantification using FIJI software, and RT-qPCR. Live ROs were also longitudinally evaluated throughout the culture time by

measuring photoreceptor outer segment (OS) length. Our experimental results indicate that 1uM 9-cis-retinal promotes the acceleration of photoreceptor OS elongation and increases overall length by day 150, which may indicate increased maturation. Through qPCR, we discovered that *OPN1LW* and *OPN1MW* gene expression were significantly higher by day 120 in 1uM 9-cis-retinal treated ROs, suggesting accelerated red and green cone photoreceptor development. Our improved protocol may expand the applications of retinal organoids in basic and translational research. This work was supported by the CellSight Development Fund, a Challenge Grant to the Department of Ophthalmology at the University of Colorado from Research to Prevent Blindness, and by the Linda Crnic Institute for Down Syndrome.

Program Abstract #366

The Transcription Factor *Myt1*, an Atoh1 target, is Necessary for Cochlear Patterning

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The mammalian auditory organ, the cochlea, contains an elongated sensory epithelium comprised of a mosaic of mechanosensory hair cells and non-sensory supporting cells. Precise patterning of this mosaic into rows of inner and outer hair cells and at least six types of supporting cells is necessary for auditory function. To achieve this cellular diversity, sensory progenitor cells undergo a series of cell fate decisions governed by transcription factors including *Atoh1*. *Atoh1* is known to be necessary and sufficient for hair cell differentiation, and deletion of *Atoh1* results in a complete loss of hair cells. To identify novel genes involved in hair cell development, we performed RNA-seq on cochlear epithelia from E15 *Atoh1*$^{+/+}$ and *Atoh1*$^{-/-}$ mouse embryos. We identified several genes significantly downregulated in *Atoh1*$^{-/-}$ cochleae whose function is unreported in the inner ear. We determined the expression patterns of genes of interest found in hair cells and/or supporting cells and confirmed their downregulation in *Atoh1*$^{-/-}$ cochleae. Of particular interest was the transcription factor *Myt1*, which is known to be important for neural fate selection in other systems. In the cochlea, *Myt1* is specifically expressed in hair cells during differentiation, from E15 to at least P5. *Myt1* conditional mutants have an increased number of hair cells and a disrupted cellular mosaic, suggesting that expression of *Myt1* is important for cochlear patterning. *Myt1* has been demonstrated to suppress Notch downstream targets and to upregulate proneural genes in other tissues. We hypothesize that loss of *Myt1* disrupts Notch-mediated lateral inhibition, leading to the conversion of supporting cells to hair cells, and we are investigating the mechanisms through which this occurs. These studies should enhance our understanding of the factors needed for proper hair cell differentiation and patterning of the cochlea. Supported by funds from the NIDCD Division of Intramural Research (DC000059).

Program Abstract #367

The transcription factor Prox1 regulates cellular patterning and differentiation in the mouse organ of Corti

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The organ of Corti (OC) of the mouse cochlea is an extraordinary model for studying mechanisms of tissue patterning and cellular differentiation. This auditory sensory organ is made up of hair cells and supporting cells (SCs) that are arranged in a precise lattice array. OC patterning divides the SCs between inner and outer phalangeal cells that surround inner hair cells and outer hair cells respectively. These inner and outer cell populations are divided from each other by a single row of inner pillar cells. While the cellular anatomy has been well characterized, the genes regulating SC patterning and development are poorly understood. PROX1 is a great candidate because it is a transcription factor restricted to outer phalangeal cells, and its expression starts just prior to SC differentiation. Since the *Prox1* ortholog *Prospero* is required for tissue patterning in *Drosophila*, we asked whether *Prox1* was similarly required for patterning the cochlear duct. To answer this question, OC-restricted *Prox1* conditional knockouts were generated and evaluated by immunofluorescent labeling of micro-dissected cochleae using antibodies that label hair cells and the different populations of SCs. All images were captured by structured illumination microscopy using the Zeiss Apotome system. In the absence of *Prox1*, we see an increase in SC number, but not hair cells, that disrupts SC patterning. The increase in SCs is not associated with increased proliferation or decreased apoptosis and therefore is likely due to a cell fate transition. This is

accompanied by a decrease in markers and morphological characteristics of outer phalangeal cells with a corresponding increase in the number of cells expressing inner phalangeal markers that are located beyond the inner pillar cell boundary. Overall these results indicate that Prox1 controls tissue patterning and differentiation in the OC by regulating inner versus outer phalangeal cell fate. This work was supported by NIH R01DC018040.

Program Abstract #368

Understanding the gene regulation dynamics in embryonic heart development

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Recent advancements in multiomics analysis of *Drosophila* embryo development have shed light on the intricate regulation of zygotic activation during cellularization. Pioneer factors such as Odd-paired (Opa) have emerged as crucial regulators, alongside transcription factors like Twist (Twi), Runt (Run), and Suppressor of Hairless (Su(H)). Notably, Opa and Twi have been observed to coexist during cellularization, potentially orchestrating the expression of cardiac genes, including early and late enhancers in sloppy paired (slp), paired (prd), Dorosocross (Doc), and tailup (tup) genes. While Opa's involvement in heart development is well-documented, its specific role in the early embryo remains elusive. Mutations in Opa have been linked to heart defects across species, from humans to flies, underscoring its significance. We analyzed occupancy data to examine the regulatory mechanisms of Opa, Twi, Su(H), and Run for both dorsal-ventral (DV) and anterior-posterior (AP) cardiac genes. Our findings indicate distinct regulatory mechanisms for AP and DV genes. Specifically, Su(H) regulates heart-specific AP genes but not DV genes. Leveraging techniques such as live-imaging microscopy, and single cell multi-omic analyses, our study aims to elucidate the regulatory mechanisms through which cellularization-expressed transcription factors govern heart cell specification during early embryogenesis. Funding: STARs program UTA

Program Abstract #369

A CRISPR-mediated genetic screen to identify genes necessary for the maintenance of endocardial cell identity

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The primitive vertebrate heart tube consists of two tissue layers: the myocardium and the endocardium. The endocardium forms the heart's inner lining, connecting it to the vasculature in the rest of the body. *tal1*, a bHLH transcription factor, is required for the specification and preservation of endocardial identity. In *tal1* mutant zebrafish and mice embryos, endocardial cells gradually express genes characteristic of myocardial differentiation, indicating *tal1* is involved in repressing myocardial gene expression in endocardial cells. To identify other genes important for maintaining endocardial identity, we have been developing a CRISPR-mediated genetic screen for use in zebrafish embryos. This screen involves injecting one-cell stage embryos with a vector containing the flk-1 promoter driving Cas12a expression. Also on the plasmid is a U6 promoter followed by a CRISPR guide array targeting multiple exons in a single gene, such as *tal1*. A library of vectors against different genes is then created. We are utilizing two transgenes, Tg(fllk1:mcherry) and Tg(myl7:egfp), to assay ectopic myocardial differentiation in endocardial cells. *tal1* mutations result in egfp and mcherry double positive cells, as well as other known phenotypes, such as the aggregation of endocardial cells in the ventricle. Our future studies will seek to leverage this system to identify genes important for the development of a variety of cardiovascular cell types. Funding provided by the NIH and AHA

Program Abstract #370

TET1 safeguards lineage allocation in intestinal stem cells.

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Intestinal stem cells (ISCs) sustain intestinal homeostasis by producing all mature intestinal epithelial cell (IEC) lineages. IEC differentiation is tightly regulated by cell autonomous and non-autonomous signaling. The chromatin-modifying enzyme Tet1 fine-tunes genomic accessibility and gene expression by catalyzing DNA demethylation and co-recruiting other chromatin modifying enzymes in a catalytically independent manner. We hypothesized that Tet1 facilitates ISC identity transitions through activation of

cell type specific gene expression. Here, we define the role of Tet1 in IEC differentiation and chromatin regulatory mechanisms along the crypt-villus axis. We generated a conditional, inducible Tet1 knock-out model (Tet1iKO; Tet1fl/fl:villin-CreER). The impact of Tet1 on IEC differentiation was evaluated at the cellular and transcriptomic level by immunofluorescence and scRNA-seq. We functionally challenged IEC differentiation in vitro by treating Tet1 knock-out organoids with lineage-specific growth factors. Tet1iKO mice exhibited elevated numbers of enterocytes and goblet cells as well as a reduction in ISCs, progenitors, and enteroendocrine cells in vivo. Pseudotime analysis of scRNA-seq demonstrated premature loss of stem cell and progenitor transcriptional signatures and early bias towards absorptive transcriptional signatures in the absence of Tet1. Paradoxically, we observed a reduction of 5hmC over upregulated absorptive genes in Tet1iKO intestines. In vitro, Tet1iKO organoids exhibited no differentiation phenotype in basal culture condition, but exacerbated IEC differentiation towards secretory lineages upon growth factor treatment. Together, our results support a non-catalytic role for Tet1 in regulating adult ISC differentiation by inhibiting transcriptional priming and fine-tuning cell fate in response to environmental cues. This project is funded by NIH 5R35GM142503.

Program Abstract #371

Development of intestinal smooth muscle cells and fibroblasts in zebrafish

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The development and homeostasis of the intestine is dependent on mechanical and signaling stimuli from its surrounding environment. Cells providing these stimuli include intestinal smooth muscle cells (iSMCs) and other fibroblast populations that provide mechanical forces driving villi formation and extracellular signals such as WNTs and BMPs that regulate epithelial proliferation and differentiation. iSMCs are identified by their orientation, as one layer aligns along the longitudinal axis of the gut while another layer wraps circumferentially around the gut. However, until recently few transcriptional differences between iSMC layers had been identified. scRNA-seq data from our lab on whole zebrafish embryos throughout development identified previously undescribed complexity among the mesenchyme surrounding the intestine, including transcriptional differences between the circular and longitudinal iSMCs and additional uncharacterized fibroblasts. Fluorescent in situ hybridization of markers for these uncharacterized populations identified the localization of these cells around the gut. One such cell type is marked by foxl1 and is transcriptionally similar to FOXL1+ telocytes that are important signaling centers in mice and humans but have not been described in zebrafish. Each iSMC and fibroblast cell type expresses different signals and signal response genes, suggesting differing roles for each cell type in driving intestinal cell fates, as well as differences in how they are specified. To investigate how each iSMC type and fibroblast cell type is specified and the specific roles they play in intestinal development, I am performing scRNA/ATACseq on developing intestines and surrounding cells to identify gene regulatory networks and signaling responses active within each developing cell type. This study will increase our understanding of the crosstalk between the intestinal epithelium and its surrounding cells. Funding: NICHD Intramural Program (ZIAHD008997) to JAF.

Program Abstract #372

Secrets of joint cartilage development: Discovering new molecular players and decoding WNT-BMP signaling interplay

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Limb development starts from a single contiguous cartilage template, segmenting into distinct skeletal elements. The cartilage, often called as the transient cartilage (TC), eventually gets replaced by bone while permanent or articular cartilage (AC) at the joint site remains, ensuring smooth joint movement. Osteoarthritis, one of the most prevalent degenerative disorders affecting the elderly population, is characterized by pain in the joint and the formation of osteophytes within the AC, akin to what happens during embryonic endochondral ossification. Thus, a comprehensive understanding of embryonic AC development may provide insights into its homeostasis in adult life. Our previous findings suggest that a common pool of progenitor cells expressing collagen II when exposed to BMP signaling forms bone and when exposed to WNT signaling differentiates into AC. It is of notable significance that the interzone (the presumptive joint site) is the source of WNT ligands, whereas the cells that experience active WNT

signaling that ultimately differentiate into AC, are situated just below the interzone. We have developed a novel in vitro model system mimicking embryonic AC differentiation using this fundamental knowledge. Utilizing this model, we conducted transcriptomic studies to identify novel WNT-responsive genes that may promote AC differentiation. We have successfully characterized two regulators, KLF4 and RGS2, functionally and found them critical for AC maintenance. Our findings also shed light on the interplay between BMP and WNT signaling in AC maintenance. AC cells are non-proliferative, but our previous studies show that during OA, there is a proliferation surge in AC but due to altered tissue microenvironment, these cells differentiate into TC. The genes discovered from this study will have a role in maintaining AC integrity and will serve as molecular markers and potential therapeutic targets for managing OA.
Funding: SERB, IIT Kanpur

Program Abstract #374

A Conserved Transcription Factor Regulatory Program Promotes Tendon Fate

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Tendons, which transmit force from muscles to bones, are highly prone to injury. Understanding the mechanisms driving tendon fate would impact efforts to improve tendon healing, yet this knowledge is limited. To find direct regulators of tendon progenitor emergence, we performed a zebrafish high-throughput chemical screen. We established Forskolin as a tenogenic inducer across vertebrates, functioning through Creb1a, which is required and sufficient for tendon fate. Putative enhancers containing cAMP response elements (CRE) in human, mouse and fish, drove specific expression in zebrafish cranial and fin tendons. Analysis of these genomic regions identified motifs for Ebf/EBF transcription factors. Mutation of CRE or Ebf/EBF motifs significantly disrupted enhancer activity and specificity in tendons. Zebrafish *ebf1a/ebf3a* mutants displayed defects in tendon formation. Notably, Creb1a/CREB1 and Ebf1a/Ebf3a/EBF1 overexpression facilitated tenogenic induction in zebrafish and human pluripotent stem cells. Together, our work reveals functional conservation of two novel transcription factors in promoting tendon fate. We are thankful for support from the HSCI, the Arthritis National Research Foundation, and NIH/NIAMS for J.L.G. (AR074541 and AR079495) and A.M.C. (AR073821).

Program Abstract #375

The regulatory mechanism of long bone development through mechanical stress anisotropy promoted by chondrocyte hypertrophy

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The length of long bones is regulated through column formation by proliferative chondrocytes and subsequent chondrocyte hypertrophy in the growth plate throughout bone development. Although mechanical loading is crucial in long bone formation, the mechanical conditions of the cells within the growth plate, such as mechanical stress field, remain unclear because of the difficulty in investigating spatiotemporal changes during multicellular dynamics within growing tissues. This study investigated the regulatory mechanisms of long bone development through mechanical analysis within the growth plate using continuum-based particle models (CbPMs; Yokoyama et al. *J Mech Behav Biomed Mater* 2023). To simply describe essential aspects of a biological signaling cascade regulating cell activities within the growth plate, a one-factor model of cell differentiation was developed and incorporated into CbPM

(Yokoyama et al. *Bone* 2024). The study identified the anisotropic mechanical stress field, consisting of in-plane tension in the transverse plane and compression in the longitudinal direction, within the proliferative zone of the growth plate through the simulation of long bone development. This mechanical stress field affected bone elongation through chondrocyte column formation. Furthermore, the anisotropy in the mechanical stress field within the proliferative zone was promoted by chondrocyte hypertrophy in the growth plate. These findings provide new insights into the mechanical regulation of multicellular dynamics during bone development. Thus, this study will contribute to the fields of developmental biology and biomedical engineering by regulating bone shapes *in vivo* or *in vitro*. This work was supported by Grant-in-Aid for JSPS Fellows (JP23KJ1234) and Scientific Research (A) (JP20H00659) and (C) (JP22K03827) from JSPS; JST-CREST (JPMJCR22L5); and JST FOREST Program (JPMJFR222D).

Program Abstract #376

Temporal transcriptomics reveals a molecular signature of developmental delay upon minor spliceosome inhibition.

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Development is marked by tissue-specific milestones such as shape, size, and differentiation state. Developmental delays occur when these milestones are not met within the expected timeframe. Extensive research has characterized the milestones necessary for embryonic and postnatal limb development, including progenitor cell expansion, patterning, hand plate formation, chondrogenic condensation, interdigital apoptosis, and constant longitudinal growth. Previous studies relied on RNA labeling of systemic markers to establish developmental delay of the limb; however, a comprehensive molecular understanding of developmental delay remains elusive. To address this, we leveraged RNA sequencing data from *Rnu11*$^{Flx/Flx}$*::Prrx1-Cre*$^{+}$ (mutant) forelimbs, where U11 ablation inhibits the minor spliceosome. This leads to mis-splicing of minor intron-containing genes (MIGs) at E10.5 that regulate cell cycle, resulting in developmental delay by E11.5. Additionally, E11.5 mutant limbs showed increased gene expression. These upregulated genes showed expression patterns like the wildtype E10.5 limb, highlighting their potential role in facilitating developmental delay. Temporal RNA sequencing from E10.5 to E13.5 revealed elevated expression of genes regulating chondrogenesis at E12.5, suggesting that the developmental delay observed at E10.5 and E11.5 is followed by rapid differentiation. We then performed single-cell RNA sequencing which confirmed delayed differentiation of osteochondroprogenitor cells at E11.5 and altered abundance of neuronal and muscle progenitor cells. 3D reconstruction of migrating muscles and axons revealed disrupted differentiation of systems development. Finally, histological analysis of the U11-null growth plate demonstrated a increase in proliferative columnar chondrocytes, suggesting their differentiation into hypertrophic chondrocytes was disrupted. Taken together, these findings establish how developmental delay precipitates upon U11-loss.
Funding: NS102538

Program Abstract #377

Hox11 transcription factors regulate osteochondral differentiation during limb development

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Hox genes are essential for the proper development of the axial and appendicular skeleton during embryogenesis. Posterior *Hox* genes *(9-13)* are critical for patterning the skeletal elements of the limb along the proximodistal axis. *Hox11* genes pattern the zeugopod (radius/ulna and tibia/fibula). Recent studies have demonstrated that *Hox11* genes are expressed in a skeletal stem/progenitor cell population that self-renews and gives rise to osteoblasts, chondrocytes, and adipocytes of the zeugopod. Despite well understood genetic contributions to limb development, the mechanisms by which *Hox11* regulates differentiation remain unknown. Loss of *Hox11* function in stem/progenitor cells impairs their capacity to differentiate into osteoblasts. During differentiation, *Hox11* controls and mutants turn off Hoxa11eGFP reporter expression as the cells initiate differentiation, turning on early differentiation markers. However, *Hox11* mutant cells fail to complete differentiation. These findings are consistent with a model in which *Hox11* is required to establish differentiation potential. Taking advantage of our newly generated epitope-tagged mouse *Hox11* alleles, we aim to define the DNA binding sites utilized by Hox11 during

differentiation. Using CUT&RUN, we have identified hundreds of binding sites. We are also intersecting active and repressive histone modification profiles at Hox11-bound loci to predict how these sites regulate differentiation. These findings will be correlated with RNA-seq analyses from *Hox11* control and mutant cells during *in vitro* osteogenic differentiation. These analyses have revealed that *Hox11* mutants maintain a chondrogenic transcriptomic signature in response to osteogenic differentiation signals, with expression deficits in focal adhesion and extracellular matrix-receptor interaction pathways. Our findings will provide significant insights into the gene regulatory networks regulated by Hox11 to mediate proper differentiation. Funding:R37AR06140211

Program Abstract #378

Rab35 regulates liver cell fate and loss leads to cholangiocyte hyperplasia and hepatocyte transdifferentiation

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Rab35, a regulator of endosomal vesicular trafficking also regulates primary cilia length and is required for embryonic development, and kidney and ureter tissue homeostasis in the mouse. Using a conditional Rab35$^{fl/fl}$;CaggCre^{ERT} mutant mouse model, we also show that Rab35 is required for liver homeostasis in juvenile and adult mice. Loss of Rab35 led to cholangiocyte hyperplasia, a phenotype previously associated with loss of primary cilia structure or function. We confirmed that disruption of Rab35 on cholangiocytes also results in shortened cilia similar to what we observed in the kidney. Additionally, the loss of Rab35 led to the formation of atypical cholangiocytes, which were characterized by cells forming bile ducts that lacked normal cholangiocyte expressed markers. Unlike ciliopathic cholangiocyte hyperplasia, our data suggests this is not a proliferation driven phenotype. Instead, we found evidence of bile ducts and cholangiocyte progenitors emerging outside of established bile duct areas, a phenomenon often observed in intrahepatic cholangiocarcinoma or hepatocyte-to-cholangiocyte transdifferentiation. Changes in hepatocytes indicated bile canaliculi remodeling linked to changes within E-cadherin and EGFR expression. Finally, we found that loss of Rab35 led to activation of de-differentiation genes that we predict drive changes in liver cell fate and hepatocyte transdifferentiation. This was associated with hepatocytes expressing both hepatocyte and cholangiocyte transcription factors HNF4 and Sox9. Altogether, we demonstrate that the liver phenotypes in Rab35 mutants are associated with increased bile duct formation, altered bile canaliculi maintenance, and hepatocyte to cholangiocyte transdifferentiation and are likely driven by Rab35's regulation of epithelial cell homeostasis. Funding sources include 5R01HD089918-05to JFR and BKY, 2R01DK115751 to BKY, 5T32GM008111-34 and 5T32DK116672-05 to KRC.

Program Abstract #379

The role of the Pdx1 IDPR during pancreatic endocrine development and β-cell proliferation

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Diabetes is characterized by a reduction in insulin-producing β cells. Establishment of postnatal β-cell mass occurs during gestation and is variable across the human population. Individuals born with fewer β cells may have increased risk of developing Type 2 Diabetes (T2D). It is therefore critical to investigate the molecular mechanisms controlling both β-cell differentiation and proliferation during development. Global loss of the transcription factor pancreatic and duodenal homeobox 1 (Pdx1) results in pancreas agenesis. Pdx1 also plays important roles in β-cell differentiation and proliferation. β cells exhibit altered Pdx1 subcellular localization and changes in Pdx1 protein levels during cell cycle progression. Dynamic regulation of Pdx1 levels is thus required for β-cell proliferation and maintenance of functional β-cell mass, however, little is known about how changes in Pdx1 levels and localization drive the switch between β-cell proliferation and differentiation. Many transcription factors, including Pdx1, contain domains that mediate physical interactions with other transcriptional regulators, to affect target gene selection, protein stability, or function. While the homeobox and transactivation domains of Pdx1 have been characterized, the functional role of the Pdx1 C-terminus is less well-defined. An intrinsically disordered protein region (IDPR) exists within the Pdx1 C-terminus. IDPRs lack a fixed secondary structure, making them amenable to flexible conformations, which can facilitate key protein-protein interactions that lead to the formation of

large transcriptional complexes critical for development. Mutations within the Pdx1 C-terminus, including the IDPR, have been linked to T2D. We are using unique *in vivo* mouse models to determine if the Pdx1 IDPR is an essential domain required for development of the endocrine pancreas and β-cell proliferation. 5T32DK007563-35, R01DK127270-01A1

Program Abstract #380
Understanding HOX Transcription Factor Diversification
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Sequence-specific transcription factors (TFs) drive an astonishing number of cellular states during development. Paralogous TFs drive distinct cell fates despite similar DNA-binding preferences. However, the mechanism by which TFs of the same family diverge in overall binding patterns is not entirely understood. HOX TFs are a prime example of TF family diversification. Posterior HOX TFs (HOX9-13) arose from one common ancestor and bind very similar DNA binding motifs, yet, they induce different spinal cord fates along the main body axis. Specifically, HOXC9 and HOXC13 can bind to sites in inaccessible chromatin, whereas HOXC10 cannot. However, which HOX TF domain and specific residues within the TF allow HOX to bind inaccessible motifs is not known. To address this knowledge gap, I am generating HOX chimeras, swapping each protein domain/specific amino acids, and analyzing their binding and patterning phenotypes in the posterior HOX-relevant neuromesodermal progenitor cell type. In addition, TF DNA binding frequency and binding duration to chromatin contribute to pioneering. However, which of these two features contributes to HOX TF diversification is obscure. I will explore what biophysical properties contribute to pioneering. I will use single molecule tracking to analyze and compare the residence time (1/k_off), and chromatin-bound versus freely diffusing fractions (k_on) of posterior HOX TFs and their chimeras. This work aims to understand how an ultra-conserved TF family diversifies their activity to ensure proper development. By determining the differences in binding dynamics and the determinants of posterior Hox binding, this research will shed light on the molecular underpinning of cell fate determinations, which will have implications for understanding an important aspect of developmental biology and disease mechanisms. This work is supported by the NIH R01GM138876 grant.

Program Abstract #381
Identifying INSM1-associated protein networks that drive neuroblastoma differentiation
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Neuroblastoma (NB), the most common cancer in children under the age of one, is sometimes seen to resolve by differentiating into neurons. Chemotherapeutic treatments can aid in differentiation but demonstrate varied efficacy and significant side effects. Using a zebrafish xenotransplantation system, we identified several neurogenesis-promoting factors that are necessary, but not sufficient, for inducing NB differentiation *in vivo*. We separately found that the transcription factor Insm1a plays a role in the differentiation of zebrafish olfactory progenitor cells into sensory neurons, leading us to query its effects in NB cells. Human INSM1 has been previously shown to promote NB cell survival/proliferation, but its effect on NB differentiation has remained unclear. Intrinsically disordered transcription factors such as INSM1 regulate multiple cellular functions by acting as hubs for transient protein interactions, and thus we hypothesize that INSM1 drives survival versus differentiation via differential binding partners. To delineate the roles of INSM1-associated interactors, we have generated a 3xFLAG-tagged INSM1 construct to pull down protein complexes for MS/MS-based identification and compare INSM1's protein interactome in progenitor-like NB cells to that in differentiating NB cells. Candidate interactor proteins will be perturbed prior NB injection into distinct embryonic microenvironments to evaluate a shift in the balance between NB survival and differentiation. We hope to identify binding interfaces of INSM1-associated interactors that offer new targets for therapeutic drug development. This project is funded by Alex's Lemonade Stand Foundation grant 22-26834 and UAB Heersink School of Medicine Start-Up Funds.

Program Abstract #382
Cytomics Reactome Forges an Integrative Systems Biology Resource of Cell Lineage Paths and a Toolset

for the Analysis of Single-Cell Data

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Reactome is a comprehensive open-source biological pathway database, containing manually curated and peer-reviewed pathway diagrams, detailed mechanistic biochemical reactions, and literature summations. The Cytomics Reactome project extends the data structure used for molecular-level annotation of cell biology to the annotation of cell lineage paths. Cell lineage paths are organized by organ systems, cross-referenced to Gene Ontology (GO) biological processes, and organized into causally connected cell development steps. Trillions of cells in the adult human body, specialized to fulfill diverse roles in tissues, organs, and organ systems, originate from a single cell. Reaction-like cell development steps detail transitions between cell states during development or differentiation, characterized by positive and negative regulators and required input components. Curated cell states correspond to a unique combination of cell type (Cell Ontology), anatomical location (UBERON), and biomarkers (manually annotated and cross-referenced to open source data: CellMarker and PanglaoDB). Each differentiation step is regulated by a distinct combination of regulatory molecules present in the microenvironments of the differentiating cells. These pathways detail differentiation steps regulated by distinct combinations of regulatory molecules present in the differentiating cell microenvironment. Reactome provides an open source, comprehensive, continuously maintained reference repository of cell lineage paths framed on the pre-existing knowledge of tissue morphogenesis, as well as a cell type-specific pathway resource, which serves as a framework for interpretation and integration of experimental findings. Reactome offers a toolset for the analysis of single-cell data using existing web applications and pathway visualization tools, enabling display, navigation, and search of Cytomics Reactome data. Funding: NIH (U24 HG012198, U24 HG011851 and U01 CA239069), EMBL, and Open Targets

Program Abstract #383

Longitudinal transcriptomic analysis of zebrafish embryonic explants reveals a cell type-independent network of timekeeping gene modules.

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Gene expression changes across embryonic development reflect both differentiation of an increasingly diverse array of cell types and genes whose expression varies strictly with developmental time, independent of cell type. Multiple embryonic timing systems set the onset and pace of developmental events, and blocking transcription arrests many of these events, suggesting their temporal coordination involves expression of timing genes. A core set of embryonic time-keeping genes has not been identified, so we employed zebrafish embryonic explants to define genes whose expression varies strictly with developmental time. These cell aggregates exhibit timely germ layer specification and gastrulation morphogenesis upon Nodal signaling activation, but very limited differentiation in its absence. Longitudinal transcriptional profiling of Nodal-activated and naive explants from blastula to neurula stages revealed that the temporal expression dynamics of most genes is shared between conditions. Using consensus clustering on the 5000 most variable genes, we derived 24 gene modules, 20 of which exhibit essentially identical temporal trajectories between naïve and differentiated explants. These "chrono-constitutive modules" (CCMs) were also found in intact zebrafish embryos, with temporal trajectories matching those of explants. We used the CCM genes to train a random forest machine learning model and found that it accurately staged both explants and embryos (R^2=0.99), demonstrating that CCMs provide sufficient timing information to infer stage. Enrichment analysis of microRNA targets and transcription factor regulons within the CCMs further revealed distinct putative regulators of several modules. These results support the existence of transcriptional time keeping during early development, and suggest that their activity could be partially modulated via microRNAs and transcription factors. This work is supported by R01 HD104784 to MKW and MH134392 to CC

Program Abstract #384

Decoding differential gene expression responses through optogenetic activation of zebrafish developmental signaling

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Signaling pathways coordinate cell fate determination and developmental patterning to form a multicellular organism from a single cell during early embryogenesis. The process of cell fate determination involves multiple signaling molecules including BMP, FGF, and Nodal, which generate spatial signaling gradients in developing zebrafish embryos. These signaling gradients activate sets of signaling pathway specific target genes—"developmental programs", thereby specifying cell fates. Different target genes can have distinct spatiotemporal expression in response to the same incoming signaling gradients, suggesting that genes respond differently to signaling levels or dynamics. An ideal experiment to test this would require the ability to manipulate signaling levels and dynamics independently. Molecular optogenetic signaling activators are appropriate tools to address this question because of their precise and selective control of signaling levels and dynamics. We will use these tools to profile differential gene expression responses to BMP, FGF, and Nodal signaling levels and durations during zebrafish embryogenesis. First, we are establishing a pipeline for RNA sequencing to read out gene expression. Next, we will optogenetically introduce signaling pulses of different levels and durations to examine how genes respond to these features—for example, which genes require high levels or long signaling durations for activation? These results will help identify pathway-specific gene expression profiles and what signaling features are critical for cells to decode signal into fate decisions.**Funding: NIH Intramural ZIAHD009002-01 to KWR.**

Program Abstract #385

An upstream enhancer drives *sox32* activity at the onset of endoderm induction

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The zebrafish-specific *sox32* gene encodes a transcription factor critical for Nodal-responsive endoderm emergence. In addition to lacking all endoderm, the *sox32* mutant *casanova* features disrupted organization of neighboring mesoderm-derived tissues that depend on the endoderm for migration. However, the gene-regulatory elements controlling *sox32* induction and expression during endoderm initiation remain unknown. Further, if *sox32*-expressing cells are definitively fated to endoderm or retain dual mesendoderm potential is also unknown. We have developed a pipeline for discovering early ventro-lateral *cis*-regulatory elements using ChIP-seq tracks for the Nodal/BMP-responding transcription factors EomesA, FoxH1, and MixL1 (E/F/M). Using this pipeline, we identified a putative E/F/M-responsive enhancer upstream of the *sox32* locus. Transgenic reporter lines based on this enhancer recapitulate endogenous *sox32* expression in earliest endoderm progenitors and their descendants. Functional dissection of *sox32* enhancer reporters with serial deletions of the E/F/M consensus binding sites narrowed down necessary regulatory modules. Endogenous deletion of the *sox32* enhancer causes cardia bifida as well as a global reduction of the definitive endoderm marker *sox17*, all features of the classic *casanova* mutant. Together, these results show that our identified E/F/M-controlled enhancer is necessary and sufficient for *sox32* expression in zebrafish. Using *sox32:creERT2*-based lineage tracing with tamoxifen-inducible Cre/lox labeling, we establish that *sox32*-positive cells have selective endoderm potential, with minimal to no mesoderm contribution. We are now investigating the source of the limited mesodermal lineage traces, and if the upstream regulation of endoderm-specific sox32 induction is conserved across chordates. This work is supported by CU School of Medicine, Dept. of Pediatrics and by the Children's Hospital Colorado Foundation.

Program Abstract #386

Temporal requirement for Hedgehog signaling in asymmetric gene expression

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Bilaterally symmetric vertebrates have a complex internal body plan that displays left-right (LR) asymmetries in organ structure and position. LR patterning is the process by which LR asymmetry is

established in these organs, including the heart. When LR patterning goes wrong, it can result in congenital heart disease (CHD), the most common structural birth defect, affecting 1% of all live births. The left-right organizer (LRO) plays a critical role in LR asymmetry, which is essential for proper LR axis specification. Cilia-mediated fluid flow within the LRO is believed to break symmetry and biases the expression of Nodal, a TGF-beta morphogen, towards the left side. Previous research has suggested that Hedgehog (HH) signaling influences the asymmetric expression of Nodal, but its exact role in LR patterning is still unknown. Modulating HH signaling can affect LR patterning in various organisms, but its specific role remains unclear because the loss of HH signaling affects multiple stages of development. For instance, HH signaling is required in mice and chicks to develop the notochord and floor plate, which are necessary to establish a midline barrier that divides the LR axis. To investigate the effect of HH on LR patterning, we used BMS-833923, a Smo inhibitor, to block the HH pathway during zebrafish development. Our research revealed a crucial two-hour window during embryogenesis when Smo inhibition affects the expression of LR patterning genes. This finding led us to hypothesize that cilia-mediated fluid flow biases HH responsiveness, and an asymmetric HH response signaling is essential for the induction and maintenance of LR patterning. Our work has significant implications for CHD and human health, as mutations in HH pathway genes are often found in patients with CHD and Holoprosencephaly. A better understanding of LR patterning will provide insight to the molecular and genetic etiology of human disease. This research is funded by T32GM00738.

Program Abstract #387

Non-ciliary roles for the cilia-motility associated gene *Cfap298*(*kurly*) in zebrafish and mouse

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Cfap298/kurly is important for regulating cilia motility and is mutated in ciliopathy-related disorders in both humans and zebrafish. Cfap298 is thought to act as a protein folding chaperone in a complex with RuvBL1/2 that is important for assembly of dynein arm complexes required for cilia motility. However, *Cfap298* is expressed in tissues that do not make motile cilia in zebrafish and mice. To investigate the role of *Cfap298* in cells without motile cilia, we generated two mutant alleles in mice. *Cfap298*^{delta3aa} affects cilia motility and left-right patterning as expected. Surprisingly, our *Cfap298*^{null} mutant is early embryonic lethal between E4.5-E5.5 peri-implantation stages due to a failure to maintain epiblast cells. *cfap298* mutants are not embryonic lethal in zebrafish. However, we observe that maternal *cfap298* mutants have abnormal cell divisions and centrosome organization at early blastula stages yet recover by MZT. To further investigate the role of CFAP298 in tissues without motile cilia, we developed a *Cfap298* floxed allele for tissue-specific knockouts in mouse. Conditional removal of *Cfap298* in the developing mouse epidermis severely impacted skin formation, leading to post-natal death by dehydration. From E15.5 - E17.5 stages, we observe reduced stratification and skin barrier function along with an increased in cell death. We are investigating whether effects on spindle orientation and function, as well as protein misfolding may underlie the non-motile cilia associated phenotypes we observe in our different systems. Overall, our work uncovers novel functions for *Cfap298* in vertebrate development that extend beyond cilia motility. Funding from the National Institutes of Health (HD105009, AR066070, AR071486) and the Genetics and Molecular Biology Training Grant of the Molecular Biology Department at Princeton University (T32 GM007388).

Program Abstract #388

Role of a novel zinc finger protein in morphogenesis of the zebrafish left-right organizer.

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In humans, mice, and fish, patterning of internal organs along the Left-Right (LR) body axis is mediated by motile cilia in a transient structure known as the Left-Right Organizer (LRO). Defects in LR patterning in humans can result in heterotaxy syndrome characterized by a spectrum of birth defects. In zebrafish, the LRO—called Kupffer's vesicle (KV)—moves posteriorly through the tailbud of the embryo as it develops. Ciliated KV cells undergo morphological changes along the anteroposterior (AP) axis during this process—referred to as KV remodeling—to create an AP asymmetric density gradient of motile cilia that generates a leftward fluid flow inside KV. Yet, the mechanisms that drive KV remodeling are not fully

understood. Here we report the identification of a novel gene, *xicof6*, that is predominantly expressed in the posterior region of the KV. *xicof6* is predicted to encode an uncharacterized zinc finger protein. Previously, copy number variant screens have identified alterations in zinc finger proteins in heterotaxy patients. In zebrafish, *xicof6* mRNA is not detected in KV before remodeling but is then detected predominately in posterior KV cells after remodeling is complete. Our functional analyses indicate knockdown or overexpression of *xicof6* results in heart laterality defects, suggesting that *xicof6* expression is important for the establishment of the LR axis. Overexpression of a fluorescent Xicof6-mScarlet fusion protein localizes to the nucleus of KV cells and disrupts KV cell shape changes during remodeling. We hypothesize that Xicof6 regulates the transcription of genes that are involved in KV remodeling. To this end, we are using RNA sequencing approaches to determine the effects of the misexpression of *xicof6* on the transcriptome in the KV. The expected outcomes of this project include uncovering new mechanisms of KV remodeling. This work is supported by NIH grant R01HD099031.

Program Abstract #389

Charting New Connections: Unveiling CPLANE's Associations with Rab23 in Ciliary Functions

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Cilia, highly conserved microtubule-based organelles on cell surfaces, are vital for organism development and homeostasis. Ciliogenesis, the formation of cilia, involves intricate processes including vesicular trafficking and protein recruitment. A key protein complex for ciliogenesis is CPLANE (ciliogenesis and planar polarity effector), consisting of INTU, FUZ, WDPCP which bind to JBTS17 and RSG1. Although the contributions of INTU, FUZ, WDPCP, and JBTS17 to ciliopathies are well-documented, RSG1's role is less clear. To address this, our laboratory, in collaboration with the Peter Jackson lab, used proteomics to identify novel RSG1 interactors, leading to the discovery of its interaction with Rab23, a Ras-like GTPase essential for embryonic development. Notably, mutations in Rab23 have been associated with Carpenter Syndrome, a disorder linked to ciliopathies. Despite evidence of a direct interaction between Rab23 and CPLANE components and their involvement in ciliopathies, the specific mechanisms uniting them in ciliary formation and function remain poorly understood. This study focused on clarifying Rab23's role in ciliogenesis by knocking down gene expression of CPLANE components and Rab23, analyzing the impact on ciliary function and formation, including protein localization, basal body docking, and ciliary axoneme length. Our preliminary results offer insights into the CPLANE-Rab23 interplay in ciliogenesis. Future work will investigate Rab23 mutations found in Carpenter syndrome patients to further understand the CPLANE-Rab23 dynamics. This effort aims to illuminate the mechanisms linking these proteins, thereby advancing our knowledge of ciliogenesis and its relevance to ciliopathy-related disorders. This work is funded by : 5R01HD085901-05.

Program Abstract #390

MYRF Represses Nodal Signaling via Furin during Left-Right Patterning

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Variants of *myrf* are associated with congenital heart disease, diaphragmatic hernia, lung hypoplasia and urinogenital syndromes in humans. However, a molecular mechanism that connects *MYRF*, myelin regulatory factor, a gene previously known for the myelination of neurons in the central nervous system, to these congenital phenotypes is unknown. Here, we show that *myrf* is essential for global LR patterning in *Xenopus*and unexpectedly acts via the Nodal pathway. Depletion of *myrf* in *Xenopus tropicalis* leads to left right patterning defects including cardiac looping and the global marker *pitx2c*. However, *dand5*, an LR markers at the Left Right Organizer (LRO) was found to be normal indicating that LRO signaling was intact. Nodal signaling communicates the break in LR symmetry from the LRO to the lateral plate mesoderm, and in embryos depleted of myrf, nodal expression was expanded. Normally, nodal is expressed only on the left, but in myrf depleted embryos nodal mRNA expression was more on the left and expanded into the right side of the embryo. Ectopic expression of the wildtype *myrf* rescued the abnormal *pitx2c* in *myrf* depleted embryos but previously reported patient variants failed to do so. Moreover, the depletion of *nodal* in *myrf* crispants also rescued expression of *pitx2c*. We then identified *furin*, a nodal proprotein convertase, which was highly expressed in the *myrf* crispants and depletion of *furin* rescued the abnormal *pitx2c* in *X. tropicalis* embryos. This study demonstrates

that *myrf* plays a role in defining left and right sides of the body at the early stages of the *X. tropicalis* development through the regulation of *nodal* by modulating the expression of its proprotein convertase *furin*. Funding for this study was provided by the grant NIH R01HD102186.

Program Abstract #391

TNRC18 May Contribute to Congenital Heart Disease Through Disruption of Cilia Function

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Congenital heart disease (CHD) affects 1:100 children born, and it is the leading cause of birth-defect related mortality. While genetics are predicted to contribute to 90% of CHD, the underlying mechanisms remain poorly understood. Programs such as the Pediatric Genomics Discovery Program aim to identify candidate genes from patients with CHD, which can then be studied in model organisms. One such candidate gene is *TNRC18*. The function of the encoded TNRC18 protein is not well established, but it may be involved in chromatin binding to control gene expression. Preliminary studies using CRISPR/Cas9 sgRNA directed to *tnrc18* in *Xenopus tropicalis* show embryos with decreased cilia flow. Because proper cilia flow is critical in establishing the left-right organizer allowing for normal heart development, disruption of cilia via *TNRC18* may contribute to CHD. Funding: Yale Medical Scientist Training Program T32 Grant through NIH/NIGMS

Program Abstract #392

***IL2RB*: bridging the gap between cell mediated immune responses and congenital heart defects.**

Stephen Kamuli, Emily Mis, Mustafa Khokha
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Congenital heart defects (CHDs) comprise functional and structural cardiac abnormalities that occur in the developing fetus during pregnancy, and represent the leading cause of infant mortality and hospitalization cases. Technological advancements have accelerated the discovery of CHD disease-causing genes using next generation sequencing. So far, over 5000 genes have been shown to be associated with this disorder. There still exists some challenges in the molecular diagnosis of CHD with up to 60% of cases still lacking a genetic underpinning, due to the heterogeneity of the disorder compounded by the genotypic diversity of the global population. Among the genes that have been purported to cause CHD, based on clinical phenotyping and genotypic analysis is the interleukin-2 receptor subunit beta (*IL2RB*) which has widely been reported as a major regulator of cellular mediated immunity but never reported to have a causative role in CHD pathogenesis. The diploid *Xenopus tropicalis* has enabled us to perform functional studies on *il2rb* using CRISPR-CAS9 to knockout *il2rb* and morpholino to knockdown protein expression, both of which show robust cardiac looping phenotypes in *Xenopus* stage 45 embryos. Preliminary data indicates that *IL2RB*, largely known for its role in immunity, is also a significant player in embryonic cardiac morphogenesis and further work needs to be done to determine where *IL2RB* acts to ensure proper cardiac development. This will be a giant step in tailoring bench to bedside treatments for patients with *IL2RB* variants and providing further insights into understanding how immune related factors affect embryonic development. This work is supported by funding from NIH R01HD102186.

Program Abstract #393

Time window of caffeine-induced embryotoxicity during *Xenopus* development

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Caffeine is an alkaloid that occurs in leaves, seeds, or fruits multiple plant species. Caffeine is rapidly and completely absorbed in human digestive track after ingestion. Because it is structurally similar to several endogenous metabolites, absorbed caffeine is distributed in body fluids across the blood-brain barrier and placenta. Some reported physiological effects of caffeine include increased heart rate, stimulant properties, and enhanced muscular activity. Although some studies have suggested that high coffee intake by mothers may increase the risk of miscarriages and low birth weight, animal studies show that caffeine is only teratogenic at concentrations that are not achievable in the human body through ingestion of caffein-containing food products. To contribute to the understanding of the potential effects

of caffeine during embryonic development, our study has examined the morphological outcomes of caffeine exposure on the developing amphibian *Xenopus laevis*, , with a focus on determining the time-window during which the embryos are the most vulnerable. Embryos were exposed to various concentrations of caffeine (1000, 500, 250, and 125µg/ml) starting from different developmental stages (32-cell, 9, 12, and 20) until the tadpole stage (43-44). Our results show that exposure during cleavage stage leads to massive embryonic death at high concentrations, whereas later exposure leads to various morphological defects, including stunted development, short body axis, curved body, wavy fins, and microcephaly, which is accompanied by holoprosencephaly and cyclopia at high concentrations. These findings contribute the be better understanding of the potential adverse effects of caffeine on human health and wildlife. Our current effort is aimed at elucidating the molecular mechanism of the observed phenotypes. This work was supported by the NIH-RISE grant # 5R25GM106995-08.

Program Abstract #394
Investigating GLI Localization during Mouse Neural Tube Development
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Ventral neural tube patterning during embryonic development requires Hedgehog (HH) signaling, which acts through the GLI family of transcription factors (GLI1-3 in mammals). Previous studies have defined essential roles for GLI2 and GLI3 during mouse neural tube patterning. However, the precise localization of GLI proteins within neural progenitors remains largely unexplored, particularly with regard to different ventral and dorsal cell fates, as well as over developmental time and at different axial levels (e.g., forelimb vs. hindlimb). This study seeks to define the subcellular localization of the GLI transcription factors during HH-dependent neural tube patterning. To achieve this goal, we employed a novel mouse model generated in the lab, carrying endogenous epitope-tagged Gli alleles (Gli1FLAG/FLAG;Gli2HA/HA;Gli3V5/V5), referred to as GliFHV mice. Using these animals, we are able to simultaneous investigate the subcellular localization of all three GLI proteins across different dorsal-ventral regions of the neural tube at various axial levels (i.e., forelimb and hindlimb) during different embryonic stages (E8.5-E11.5). Our preliminary data indicate that we can successfully detect epitope-tagged GLI1, GLI2 and GLI3 in the neural tube, including GLI protein localization to both primary cilia and within nuclei in neural progenitors. We are currently investigating how GLI subcellular distributions change in distinct HH-responsive populations over developmental time and at different axial levels. Correlating GLI protein distribution with changes in HH-dependent gene expression and HH-dependent cell fate decisions will shed light on the role that GLI proteins play in ventral neural tube patterning during mouse embryogenesis. Future studies will investigate potential alterations in GLI localization following the modulation of HH pathway activity. Funding Sources: R25 GM086262, R01 CA275182

Program Abstract #395
Autocrine Signaling at the Vertebrate Neural Plate Border
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Vertebrate ectoderm during gastrulation shows variable molecular characteristics along the medial pre-neural and lateral non-neural domains. The overlap of these domains, called the neural plate border (NPB), may comprise of progenitors for all four ectodermal derivatives—CNS, neural crest, sensory placodes, and epidermis. The mechanism of this differentiation event remains to be fully resolved. Studies from our laboratory and others have reported paracrine signaling pathways such as FGF, BMP and WNT, that are important for the precise patterning of the NPB. However, much less is known about autocrine signaling across the neural and non-neural domains. In this study, we dissected the anterior-lateral ectoderm of mid-gastrulation mouse embryos to study the NPB patterning. We performed high-depth single cell RNA sequencing, via MATQ-seq, and identified approximately 9000 unique transcripts per cell. This allowed us to cluster the cells of the anterolateral ectoderm and discover previously unknown signaling pathways and cell-cell interactions across the clusters using CellChat, a computational tool to infer cell-cell communication. One signaling pathway significantly enriched at the NPB was the Midkine(Mdk)-Nucleolin(Ncl)-Pleiotrophin(Ptn) pathway. Mdk and Ncl were ubiquitously expressed, however asymmetrically higher expression of Ptn was observed in the neural/neural-crest clusters. Mdk and Ptn are known to be closely related pleiotropic factors that bind to receptors like syndecans, with

roles in cell migration and mitosis. Mdk-Ptn genes are also evolutionarily conserved with expression reported in zebrafish and chick neural plates. We hypothesize that this signaling pathway is an early indicator of migratory properties of neural crest progenitors at the NPB. Our future experiments will investigate expression of Mdk signaling components between gastrulation and neurulation, followed by gain and loss of function experiments. Funding- NIH RO1 DCO13072 to AKG.

Program Abstract #396

Early FGF signaling is Necessary for the Specification of the Organizer of Crepidula atrasolea

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The formation of the dorsal ventral axis in Crepidula atrasolea, a gastropod mollusc, is caused by the presence of an organizer cell. This cell is being studied in a broader group of organisms to better understand its role in the formation of body plans and axis formation. It was previously shown that FGF signaling is necessary for the specification of this organizer cell in Owenia fusiformis (Seudre et al. 2022). Crepidula atrasolea is an ideal model organism for this research, as it has a short generation time and is able to grow easily under lab conditions. Previously, two FGF and FGFR orthologs were found in Crepidula atrasolea. Their expression patterns were characterized using HCR, showing expression through early development stages. The involvement of FGF in the development of the organizer was tested with the inhibitor SU5402 before and after the development of the organizer cell. Of the embryos treated at early stages before organizer development, we found that approximately 75% of the SU5402 treated snails were radialized, lacking a dorsal ventral axis. However, of the embryos treated after organizer development, all developed normally and did not display any signs of a radialized embryo. Overall, the inhibition of FGF/FGFR appears to affect the formation of a dorsal ventral axis when embryos are treated before the organizer is developed rather than after its development. Phenotypes were characterized by the absence of the eyes and shell of treated embryos, structures known to be induced by the organizer in other spiralian species. This work was funded by NIH MIRA grant awarded to Deirdre C. Lyons

Program Abstract #397

Grid formation and segmentation in Parhyale hawaiensis

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Over the past several decades much has been learned about the mechanisms that generate segments in *Drosophila melanogaster*. At the same time, we have come to realize that these mechanisms have diverged extensively during arthropod evolution and show significant differences even between relatively closely related species. In the present study, we use *Parhyale hawaiensis*, a crustacean that adds segments sequentially, which is thought to be a more ancestral system of arthropod segmentation and more like the pattern of vertebrate and annelid segmentation. *Parhyale*, however, is unique in that each parasegment arises from a single precursor row during the process of segmentation. This results in the formation of a highly ordered grid composed of precisely oriented rows and columns of cells. We have examined the expression of several Parhyale orthologs of genes involved in segmentation in other species, as well as some general signaling systems, and suggest that Parhyale segmentation may be driven by a pattern of asymmetric cell division. We are testing this model by using CRISPR-Cas9 to mutate several of these genes, as well as searching for novel genes that may be involved in the process.

Program Abstract #398

Atypical dorsal-ventral body segmentation pattern in the tadpole shrimp, *Triops longicaudatus*.

Jessica Wilkins, Heather Bruce, Nipam Patel

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A striking characteristic of Arthropods is their segmented body plan. Each segment is typically a circumferential unit spanning the entire dorsal-ventral axis. An exception is seen in millipedes, where two ventral segments, each with a pair of legs, is connected to what appears to be a single dorsal body segment. An even more striking example of uncoupling of dorsal and ventral segmentation is found in the freshwater crustacean, *Triops*. In *Triops longicaudatus*, the anterior 10-12 segments show dorsal-ventral alignment, with each ventral segment in line with a dorsal segment bearing a pair of limbs. Subsequent segments, however, show a gradual uncoupling between the dorsal and ventral sides. Ultimately, the

most posterior dorsal segment may bear more than 7-8 pairs of ventral limbs. Previous studies have attributed this peculiar anatomy to segmental fusion or mechanical annulation ventrally. We show here that instead this pattern represents independent segmentation on the ventral versus dorsal side. Differential division versus cell growth may cause the ventral segments to form from smaller cells, thus producing more tightly packed segments in comparison to the dorsal side where the cells remain a consistent size throughout development.

Program Abstract #399

Investigating changes in gene networks through early stages of multipotency during early fetal mouse mammary development.

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University of Maryland, USA

Reciprocal signaling between embryonic mammary epithelia and mesenchyme drives the specification of early mammary tissue around E14.5. Subsequent growth and developmental potential, however, occur independently of mesenchymal signaling. Sexual dimorphism also occurs around E14.5 as androgen signaling via fetal testosterone causes mammary rudiment degeneration. Together, the establishment of autonomous multipotency and new responsiveness to androgen signaling at E14.5 indicates a major stage of fetal mouse mammary development. To date, no in-depth spatiotemporal transcriptomic analyses have been performed before luminal/basal lineage segregation by E16. Therefore, we aim to understand the genetic variation through this transitional period using multiplex quantitative RNA fluorescent *in situ* hybridization. Using the bipotent HC11 mouse mammary cell line, we first validated key genes by qRT-PCR (*Notch1, Elf5, Pthlh, Sox9, Tbx3, Snail2, Gata3, and Lmo4*) and then validated hybridization probes. CDH1 and CK14 antibodies were also validated for cell segmentation analysis. We are currently performing multiplexed quantitative *in situ* hybridizations on mouse mammary rudiments from E13.5 to E15.5 using validated specification, luminal, and basal lineage regulator probes to evaluate transcriptomic signatures through the E14.5 transition. These results will be coupled with single-cell RNA-Seq data at the same stages. Together, this work will contribute to uncovering the gene regulatory networks directing multipotency before E16 and will contribute to understanding how dysregulation of fetal mammary gene networks can lead to decreased milk production, breast hypoplasia, mastitis susceptibility, and even breast cancer. (This research is funded by a UMD Grand Challenges Grant).

Program Abstract #400

Maternal and embryonic metabolism instruct cellular programming during gastrulation

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Specification of the principal lineages of the future body occurs at the pivotal and evolutionarily conserved gastrulation stage. Despite the high prevalence of pregnancy loss during the early post-implantation period, when gastrulation occurs, a detailed understanding of cell and molecular workings at this stage of development remains elusive. Early embryonic cells are known to be sensitive to the changes in the metabolite availability in their immediate surroundings, but very little is known about the metabolic environment of gastrulation and how it shapes embryo viability. Using an inducible mutation that elevates the epimetabolite 2-hydroxyglutarate in the maternal bloodstream, we demonstrate that maternal metabolic dysfunction can severely disrupt gastrulation. We additionally investigate the mechanistic role of nutrient utilization in instructing cellular programs to shape developing embryos. Our findings show that glucose utilization undergoes two spatially-resolved waves of activity in a cell-type- and stage-specific manner. By blocking key enzymes of different branches of glucose metabolism, we identify that the first finely-tuned spatiotemporal wave of glucose metabolism occurs through the hexosamine biosynthetic pathway to drive fate acquisition in the epiblast, while the second wave uses glycolysis to support mesoderm migration and lateral expansion. Glucose influences these phenotypes via cell signaling, with glucose metabolism increasing ERK activity via distinct mechanisms in each wave. Our findings show that cellular metabolism helps guide cell fate and specialized functions in development in communication with genetic mechanisms and morphogenic gradients. Overturning the notions that cellular metabolism is generic and housekeeping, this work revolutionizes our knowledge of

metabolism in the context of development. Funding provided by NIH Director's New Innovator Award, DP2-HD112040.

Program Abstract #401

Exploring Extra-embryonic Endoderm Specification in Early Human Development Model

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Distinguishing between extra-embryonic endoderm and embryonic definitive endoderm in stem cell culture poses a challenge due to their similar transcriptional profiles. While models simulating post-implantation human development include extra-embryonic lineages, not many investigations have been done to explore these differences in a 3D context. Here, we utilize a multi-lineage differentiation approach with human pluripotent stem cells (hPSCs) that self-organize into structures mimicking early embryonic development. This approach reveals spontaneous differentiation into extra-embryonic hypoblast-like lineages and patterning within the embryonic epiblast-like lineages in the absence of a trophoblast lineage. To confirm the extra-embryonic endoderm identity of our system, we isolated hypoblast-like cells from our structures and compared them to embryonic definitive endoderm cells derived from conventional differentiation protocols from primed hPSCs. Chimeric competency assays in mouse embryos demonstrated successful integration of hypoblast-like cells into the primitive endoderm lineage of mouse blastocysts, while differentiated-definitive endoderm cells were excluded. Our functional assays using small molecule inhibitors highlight the necessity of TGFβ/NODAL and FGF signaling for the formation of hypoblast-like cells. Our single-cell transcriptomics comparison with the published datasets also correlates with hypoblast-like cells. Taken together, these findings underscore the robustness of our strategy in generating cells resembling extra-embryonic-like identity and provide insight into the key signaling pathways involved. Overall, our system captures critical aspects of early human embryonic development, providing a scalable platform that will offer new opportunities for high-throughput dissection of developmental disorders. **Acknowledgments:** Funded by the Richard and Susan Smith Family Foundation. The Yale Stem Cell Center Chen Innovation Award and Reprograms

Program Abstract #402

Remodeling of the basement membrane guides anterior-posterior body axis specification

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Establishing the body axis is a key event for metazoan development, which lays out the blueprint for patterning the embryos for subsequent development. In humans and mice, the symmetry-breaking cue for the specification of the anterior-posterior (AP) axis first emerges in the hypoblast in humans or visceral endoderm (VE) in mice as the embryo implants into the uterus. In mice, a subset of these VE cells, the distal visceral endoderm (DVE), migrates to the proximal end of the embryo before gastrulation to facilitate the formation of the anterior visceral endoderm (AVE). During gastrulation, the AVE restricts the formation of the primitive streak at one end of the embryo, thus establishing the AP axis. How this unidirectional and collective migration of the DVE is regulated is not well understood. Here we show that the remodeling of the basement membrane guides this symmetry-breaking process. Through single-cell RNA sequencing of pre- to post-implantation mouse embryos and mapping the basement membrane between the epiblast and the VE with 3D super-resolution imaging, we find that spatially biased expression of matrix metalloproteinases leads to uneven perforations in the basement membrane before DVE migration. Through *in toto* morphometric analyses and physical modeling coupled with perturbations

of cultured embryos, we find that the heterogeneity in the basement membrane instructs DVE migratory trajectories by creating biased tissue fluidity in the VE. Furthermore, observations of cultured preimplantation human embryos and a stem cell-based human embryo model indicate the role of the basement membrane between the hypoblast and the epiblast in informing the AP axis specification in human embryogenesis. Overall, our results point to a mechanical asymmetry that guides AP axis specification in mammals. Funding: NICHD K99HD111676, Open Philanthropy, The Weston Havens Foundation, and The Shurl and Kay Curci Foundation.

Program Abstract #403

Development of the human limb progenitor cells from pluripotent stem cells

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Research using model animals contributes to our understanding of developmental mechanisms. In contrast, the study of human development is limited due to ethical limitations and the availability of samples for research. The use of human pluripotent stem cells (PSCs) offers a unique opportunity to study human development. We are interested in understanding the mechanisms of cell fate specification and gene regulatory networks that regulate the development of human limb buds. Recent studies have demonstrated a guided differentiation scheme of human iPSCs into limb bud mesenchyme cells. This differentiation is achieved by guiding iPSCs by manipulating the activities of various signaling pathways, including the Activin, BMP, WNT, FGF, and Hedgehog pathways. Pluripotent human iPSCs are first directed to differentiate into the primitive streak status, then into the hindlimb progenitor-like cells in the lateral plate mesoderm. The cells are further guided to become the hindlimb bud mesenchyme-like status. In order to test gene regulatory systems for human limb bud development in this in vitro system, we generated knockout iPSC lines that lack *ISL1*, *SALL1*, *SALL4*, *IRX3*, or *IRX5*. In our previous study, conditional knockout of *Isl1* in mouse embryos using *TCre* in mesoderm progenitors caused failure to initiate hindlimb bud development. The *TCre; Isl1* conditional knockout mouse embryos exhibited downregulation of a hindlimb marker *Tbx4*, while expression of another hindlimb marker *Pitx1* was maintained in hindlimb progenitor cells. In order to test the *ISL1* requirement in human hindlimb progenitor cells, we directed *ISL1* knockout human iPSCs into the hindlimb progenitor status. *ISL1* knockout cells exhibited downregulation of both *TBX4* and *PITX1*. These results suggest common and distinct regulatory systems in human and mouse hindlimb progenitor cells. Supported by a grant from the NIAMS/NIH. Grant number: R01AR064195.

Program Abstract #404

Mechanosensing of Cytoskeletal Stress by the Planar Cell Polarity Protein Prickle2

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Planar cell polarity (PCP) complexes are highly conserved membrane-bound macromolecular assemblies that confer directional polarity to cells along a specific axis. During vertebrate development, PCP coordinates proper elongation of tissues along the anteroposterior axis. It achieves this by enabling changes in cell shape and motility required for the collective cell behavior termed convergent extension (CE). Accordingly, understanding how PCP regulates and is regulated by the actin cytoskeleton is critical for understanding vertebrate tissue morphogenesis. Recent work has implicated the PCP protein Prickle2 (Pk2) as a mediator of PCP – actin crosstalk, as Pk2 and actomyosin activity are highly correlated during CE. Pk2's LIM domains, which are predicted to bind actin filaments under mechanical stress, may account for the close yet poorly understood association between PCP and actomyosin. To determine the role of Pk2's LIM domains in localizing PCP molecules in cells undergoing CE, we expressed fluorescently tagged Pk2 LIM-Containing-Region (Pk2-LCR) in X. laevis embryos alongside markers of the actin cytoskeleton and Zyxin-LCR, a well-characterized LIM construct that binds stressed actin. Results show Pk2-LCR binds Zyxin-LCR-positive actin filaments, while expression of Pk2-LCR-F/Y(1-3)A, a mutant predicted to abolish mechanosensing, did not exhibit similar recruitment. Inhibition of actomyosin activity also perturbed LIM recruitment. Together, these results suggest that Pk2-LIM domains bind stressed actin via a conserved mechanism. Interestingly, full-length Pk2 does not exhibit similar stress-sensitivity, hinting at inhibitory mechanisms regulating LIM activity that we will explore in the future. These findings provide evidence for a direct molecular coupling between PCP and actomyosin and help clarify the poorly

understood relationship between PCP and mechanical stress. This work was funded by NICHD (R01HD099191-08S1) and NSF (DGE 2137420)

Program Abstract #405
Investigating the role of myocardial protrusions during the early stages of heart development in zebrafish
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University of Mississippi, USA
A pivotal event in heart development is cardiac fusion, wherein bilateral myocardial populations collectively migrate towards the midline, initiating the formation of a heart tube—an essential precursor for a fully functional heart, across vertebrates. Previous studies have revealed the importance of extrinsic influences such as the adjacent endoderm in cardiac fusion, however, recently we have uncovered an intrinsic mechanism involving the platelet-derived growth factor receptor alpha (Pdgfra) - phosphoinositide 3-kinase (PI3K) signaling pathways that is also required (Shrestha et al., 2023). In our current study, we are investigating how these intrinsic influences steer the myocardium towards the midline. Through high-resolution time-lapse studies, we have observed myocardial protrusions oriented medially in the direction of movement. These protrusions display different morphologies – thin and transient, or wider and longer-lasting, reminiscent of filopodia and pseudopodia, respectively. Furthermore, in embryos in which the PI3K pathway is inhibited, these protrusions lose their polarisation, with a corresponding decrease in directionality and speed. Our ongoing investigations are focused on determining whether protrusions are required for cardiomyocyte movement at the collective and/or individual cell level? and if so, what is the role of the different protrusion morphologies? This work is supported by funding from the NIH.

Program Abstract #406
Investigating the biomechanical properties of the myocardium during heart tube formation
Harini Saravanan, Tess McCann, Joshua Bloomekatz
University of Mississippi, USA
Collective cell migration is crucial for organ formation, as seen in heart development where bilateral cardiomyocyte populations migrate to the midline, a process known as cardiac fusion, prior to forming the heart tube. We are investigating the biomechanical properties that underlie the collective movement of cardiomyocytes during cardiac fusion in zebrafish. Our studies of cardiomyocyte cell morphology during cardiac fusion reveals an intriguing change in cell size and elongation. By quantitating whole-mount immunofluorescence staining of ZO-1 we have found that during the early stages of cardiac fusion, cardiomyocytes display a mostly isometric uniform morphology. As cardiac fusion proceeds, cardiomyocyte morphology becomes heterogeneous with medial cells displaying smaller surface areas and more compact morphologies compared to larger, elongated lateral cells. Changes in cell shape (cell deformation) can result from changes in tension or viscoelasticity. To measure viscoelasticity, we have taken a microrheology approach involving the injection and tracking of inert beads. Our preliminary findings indicate that myocardial cells exhibit a mostly viscous behavior. Furthermore, these cells have a lower elastic modulus during the early stages of cardiac fusion compared to later stages. Our current efforts are directed at investigating whether viscoelasticity, like myocardial morphology, differs between regions of the myocardium. And we are investigating the molecular underpinnings of cardiac morphology and viscoelasticity dynamics. Ultimately, we expect our results help reveal the fundamental biomechanical principles underlying normal and pathogenic collective cell migration. Funding provided by AHA and NIH.

Program Abstract #407
Camsap2 functions in the extraembryonic yolk cell to regulate zebrafish epiboly
Ashley Bruce, Haoyu Wan
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Gastrulation positions the three germ layers which is essential for formation of the adult body plan. In zebrafish, epiboly is a dominant gastrulation movement and describes the thinning and spreading of the blastoderm to internalize the underlying extraembryonic yolk cell. The yolk cell provides the major motive force for epiboly via accumulation of actomyosin in the yolk syncytial layer (YSL) to form a contractile ring. In addition, macropinocytosis in the YSL removes excess yolk membrane ahead of the spreading

blastoderm. The molecular control of the yolk cytoskeleton during epiboly is not well understood. We identified Camsap2a as a novel regulator of epiboly. *camsap2a* transcript is confined to the YSL during epiboly and mutant embryos exhibit epiboly delays. Actomyosin accumulation is severely delayed and reduced in mutant embryos. PIV analysis showed that retrograde flow of actin to make the contractile ring is misoriented while FRAP analysis showed that actin turnover is unchanged in mutant embryos. Consistent with reduced actomyosin accumulation, tension in the contractile ring is reduced in mutant embryos as measured by laser cutting. In addition, macropinocytosis and yolk membrane ruffling are also reduced in mutants. We identified a pool of actin beneath the contractile ring that is likely to be involved in macropinocytosis and which is abnormal in mutant embryos. The small GTPase Rab5ab is required for yolk cell macropinocytosis and morphants also have reduced actomyosin accumulation. We postulate that the main role of Camsap2a might be position or activate Rab5ab for yolk macropinocytosis. Consistent with this hypothesis is the finding that the *C. elegans* Camsap homolog co-localizes with Rab5. We are currently testing this hypothesis by attempting to rescue *camsap2a* mutant embryos with constitutively active Rab5ab. This work provides new insights in the control of morphogenesis by an extraembryonic tissue. Funding: NSERC.

Program Abstract #408

Intestinal fibroblasts are key contributors to postnatal crypt morphogenesis

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Stromal fibroblasts are key components of the small intestinal stem cell niche, supplying morphogens essential to maintain the active stem cell population. In the adult mammalian small intestine, intestinal stem cells (ISCs) reside at the base of cup-like invaginations called crypts and differentiate as they migrate upwards to the finger-like protrusions called villi. The structural compartmentalization of the intestine exposes epithelial cells to different niche factors secreted by heterogeneous fibroblasts for precise regulation of stem cell behaviors during homeostasis. In mice, villi form embryonically while crypts develop in the first two weeks after birth by epithelial invagination of intervillar regions into the underlying stroma. While we have some understanding of the mechanisms by which mesenchymal signaling and contractility regulate villus formation, whether epithelial-mesenchymal interactions are required for crypt morphogenesis is unknown. Here, we genetically ablated fibroblasts at the early postnatal stage and found that they are required to establish crypts by facilitating epithelial invagination. Our results show that as crypts invaginate, a contractile fibroblast subtype expressing high levels of PDGFRa (telocytes), is recruited and aligns beneath the intervillar region. Single-cell RNA sequencing analysis of intestinal fibroblasts and epithelial cells during postnatal development demonstrates robust maturation programs in both cell populations throughout crypt morphogenesis. These transcriptional analyses decipher the dynamics of epithelial-fibroblast crosstalk and will inform our mechanistic studies of how fibroblasts regulate epithelial crypt formation. Our findings imply the potential regulatory roles of telocytes in crypt morphogenesis and will provide new insights into the significance of the stem cell niche in shaping tissue architectures. Funded by NIH R35GM150645, the Yale Stem Cell Center Chen Innovation Award and NIH 5T32HD007149.

Program Abstract #409

Investigating the role of protoporphyrinogen oxidase (*Ppox*) during murine mesoderm lineage differentiation.

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Protoporphyrinogen oxidase (Ppox) is the penultimate enzyme in the heme biosynthesis pathway. Autosomal dominant loss-of-function *Ppox* mutations cause a rare metabolic disorder called variegate porphyria, an episodic disease of the liver most prevalent in women. The cellular and systematic biology of porphyrias are poorly understood and have rarely been studied in a developmental context. To address knowledge gaps in the roles of Ppox and heme during mammalian development, the Knockout Mouse Project (KOMP)-obtained *Ppox* heterozygous line was examined. *Ppox* null embryos are developmentally delayed beyond embryonic day 7.5 (E7.5) and fail to complete chorioallantoic fusion and placentation by E9.0, resulting in lethality by E10.5. Wild-type embryos display high levels of *Ppox* expression in the fetal blood cells of the yolk sac and placenta, and low to undetectable expression

elsewhere in the conceptus. *In vitro* adhesion assays suggest that the mutant allantoids are capable of adhesion and display abnormal vascular branching. Whole embryo immunofluorescence demonstrates that this defect can be observed throughout the embryo. Furthermore, *Ppox* mutants display abnormal somite segmentation, suggesting that mesoderm differentiation is impaired. Intriguingly we note red blood cell abnormalities in pregnant heterozygous dams. Further experiments are aimed at identifying the role of Ppox in the mesoderm and the possibility of non-apoptotic cell death through loss of heme. Future research on the impact of porphyrin accumulation during embryonic development could greatly aid in the understanding and treatment of variegate porphyria. This research is funded by NIH R01HD096073 to KDT.

Program Abstract #410

Control of Muscle and Spine Morphology through Urotensin Signaling

Johnathan O'Hara-Smith, Elizabeth Bearce, Samuel Bertrand, Rachael Giersch
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As animals mature, the body grows rapidly and must properly scale its tissues and organs to maintain the right shape. Failure to properly grow and develop the musculoskeletal system can result in scoliosis, that is the breakdown of the morphology of the spine. Idiopathic scoliosis (IS), defined by acquisition of spinal curves during adolescence in the absence of explicative neuromuscular or vertebral hard tissue defects, is highly prevalent, affecting about 3% of humans. Despite the commonality of IS, treatments for IS and to address scoliosis broadly are invasive and limited, owed to poor understanding of scoliosis etiology. Using zebrafish genetic models of IS, we have identified 2 cyclic neuropeptides, the Urotensin II-related peptides 1 and 2 (Urp1 and Urp2), which are secreted from within the spinal cord and control spinal morphology as well as muscle development. When Urp1 and Urp2 are deleted, spine morphology breaks down severely during juvenile growth, producing scoliosis-like phenotypes. To understand the molecular developmental mechanisms leading to this spinal dysmorphology, we performed bulk RNA-sequencing of Urp1/Urp2-deficient embryos, identifying multiple dysregulated transcripts encoding proteins critical for the basic function of muscle. Additionally, spinal dysmorphology occurred when we mutated the Urp1/Urp2 receptor, Uts2r3, which is typically expressed in muscle tissue. In mutants, histological approaches and birefringence assays revealed structural defects in muscle tissues. Overall, this work aims to advance understanding of how and why the factors involved in developing and maintaining a normally shaped spine (in this case, the components of Urotensin signaling) are required for development. These studies were supported by: National Institutes of Health (NIH) Pathway to independence award to (R00AR70905 to D.T.G.), and a Ruth L. Kirschstein Postdoctoral Individual National Research Service Award, (F32AR078002 to E.A.B.).

Program Abstract #411

The Reissner Fiber assembles downstream of local motile cilia activity to control body morphology

Elizabeth Bearce, Sophie Fisher, Sam Bertrand, Adamend Freya, Dan Grimes
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The linear body axis is a key feature of vertebrate life. It forms during embryogenesis based around the notochord and later the spine, a rigid but flexible column of vertebrae. We study the mechanisms that give rise to proper spine morphology. In zebrafish, generation and maintenance of the body and spine requires the function of motile cilia. Zebrafish embryos lacking cilia motility fail to undergo axial straightening and instead exhibit a curved early body axis and, later, scoliosis-like spinal curves. Remarkably, we found that returning cilia motility to mutants allows the self-correction of body and spine morphology, suggesting a mechanism in which abnormalities are sensed and corrected. Motile cilia are required to assemble the Reissner Fiber (RF), an extracellular thread in the central canal composed of the protein Scospondin which we hypothesize to be important for the "sense-and-correct" mechanism. However, while Scospondin mutants do demonstrate morphological defects, we currently do not understand how RF forms downstream of motile cilia or how RF functions to promote a linear spine. To address this, we are performing live-imaging of cilia motility and RF in a variety of mutant backgrounds. This revealed that dynamic, rostral-caudal RF formation is dependent on local cilia motility in the central canal. When cilia are immobilized, knot-like RF structures form and eventually, the RF breaks down into diffuse monomers, something which correlates with the onset of spine dysmorphology. Our imaging has also revealed that central canal neurons uptake RF material in mutants that develop spinal curves. We

propose that neuron-RF interactions, followed by endocytosis of RF material, function as a sensory system that allows the large-scale shape of the body and spine to be sensed, facilitating a path to maintain and correct organ morphology during growth, aging, and disease. This work is funded by NIH NIAMS F32 AR078002 (Bearce) and NIH NIGMS R35 GM142949 (Grimes).

Program Abstract #412

Investigating the cell and tissue-scale movements that drive avian foregut morphogenesis

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Respiration and digestion are carried out by organs that arise from an embryonic structure known as the gut tube. How the definitive endoderm is internalized and transformed from an epithelial sheet to a cylindrical tube remains remarkably understudied in amniotes. Formation of the anterior segment of the tube, the foregut, begins with posterior descensus of the anterior intestinal portal (AIP), a crescent shaped invagination that forms in tight coordination with the head-fold. Because the AIP is externally accessible and is a key organizing center for heart tube fusion, much of the efforts on understanding foregut tube morphogenesis have focused on descensus of the AIP, without directly examining links between AIP movement and elongation of the nascent foregut tube (Bellairs 1953, Seidl & Steding 1978, Hosseini 2017). Beginning with a morphometric analysis of tube formation, we find that AIP movement is poorly correlated with length and surface area of the elongating foregut tube. Further, through live imaging of cell movements along the AIP, we find relatively little in-rolling of cells over the midline of the AIP, despite elongation of the foregut floor by as much as 200%. We have also observed anterior ward collective cell movements of presumptive foregut roof cells through the AIP, suggesting a previously unappreciated source of cells driving growth of the foregut tube. Ongoing studies using tissue-specific disruption of acto-myosin activity in the endoderm are aimed at understanding the causal role of endoderm-generated forces in driving the tissue deformations that lead to foregut formation. Ultimately, we aim to understand how long range coordination of gut tube formation is achieved across the foregut, midgut, and hindgut, and to gain insights into why amniote embryos evolved to construct a simple continuous tube through three distinct mechanisms at disparate times and locations. Funding: Blavatnik Family Foundation (OP), NIH R35GM142995 (NN)

Program Abstract #413

Identifying mechanical signatures in convergent extension by simulating passive and active cell behaviors

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What can we learn about the mechanical processes that shape tissues by simply watching? A variety of schemes suggest that static cell morphology or junctional connectivity alone can reveal whether a tissue acts in a fluid- or solid-like fashion, where chains of cells transmit force, or where force asymmetries drive cellular rearrangements. Here we hypothesize that dynamic cell shape changes from time lapse sequences can yield "signatures" that can be used to distinguish mechanisms of passive response and active force production during convergent extension (CE). CE describes tissue scale deformation wherein a planar tissue narrows in one direction and lengthens in the other. It is tempting to assume that forces driving CE reside within cells of the deforming tissue; however, CE may reflect a variety of active processes or can even reflect passive responses to forces generated by adjacent tissues. In this work, we first construct a simple model of epithelial cells capable of passive CE in response to external forces. We leverage the same model to simulate CE from active anisotropic processes such as crawling and contraction modes. Next, we develop an image analysis pipeline for analysis of both live-cell and simulated-cell morphogenetic changes using a panel of mechanical and statistical approaches. This pipeline can identify mechanistic signatures of passive and active modalities in simulations and apply the same analyses to live cell data collected from Xenopus neural plate CE. Model data reveals signatures that distinguish active force generation from passive motions, as well as identifying active modes contributing to CE. Thus far, live cell data suggests signatures of both active forces and passive motion across the neural plate. Our modeling framework allows us to gain insight from tissue timelapse images and assess the relative contribution of specific cellular mechanisms to morphogenesis. Supported by the National Institutes of Health (R37 HD044750).

Program Abstract #414

Central role of centrioles: Tissue-specific requirements for coordinating craniofacial development

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Centrioles make up the core of centrosomes which function as microtubule-organizing centers of the cell. Centrioles perform two distinct cellular functions: (i) they form core components required to build the centrosome and (ii) they form the basal body that templates formation of the cilium, a microtubule-based specialized signaling organelle. CENPJ is a central component of centrioles required for centriole duplication. Mutations in *CENPJ* cause Seckel syndrome and primary microcephaly, leading to craniofacial defects including hypoplasia of the lower jaw (micrognathia), facial midline abnormalities, premature closure of cranial sutures (craniosynostosis), and tooth abnormalities. Understanding how centriole loss affects craniofacial development is critical for developing novel therapeutic interventions. Our goal is to uncover the molecular basis for craniofacial dysmorphology upon centriole loss and dysfunction. To investigate the role of centrioles in craniofacial development, we conditionally deleted *CenpJ* in craniofacial tissues of mouse embryos using the *Sox9-cre* driver. The resulting mice displayed underdeveloped jaws, midline facial defects, and skull abnormalities similar to Seckel syndrome patients. Defects appeared at embryonic day 10.5 (E10.5), starting with a smaller jaw and widened midface. By E11.5-12.5, midline facial clefting occurred. Increased cell death in the mandibular arch and p53 activation were observed. Remarkably, deleting *p53* fully rescued the craniofacial phenotypes in *CenpJ*-deficient mice, emphasizing p53's role in craniofacial dysmorphology due to centriole loss. This discovery sheds light on the pathways involved in craniofacial development and highlights the p53 pathway as a potential target for therapeutic interventions. Funding: This research was supported by the Intramural Research Program of the NIH, NIDCR, NIH ZIA DE000748 (to LK) and the Independent Research Scholars Program (to SA).

Program Abstract #415

Using Natural Diversity To Understand The Regulation Of Developmental Timing

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Genetic variation in wild populations is a powerful tool for discovering novel aspects of developmental regulation. Model systems such as C. elegans have been a powerful system driving discoveries in developmental processes. However, the mechanisms behind developmental variability in early embryogenesis caused by intraspecific genetic variation remains poorly understood. C. elegans serves as an attractive model to explore this question due to its invariant body plan and cell lineage, allowing straightforward, meaningful comparisons on a cell-by-cell basis between individuals. Our lab recently developed a machine learning pipeline to allow for automated cell lineage tracing in nematode embryos using label-free microscopy. We have used this pipeline to characterize developmental features of 6 genetically diverse wild isolates of C. elegans. Two isolates exhibit drastic variation in the timing of organogenesis events, morphogenesis, and hatching. Initial characterization of recombinant intercross lines suggests that increased time to hatch may be a recessive phenotype. These phenotypes represent an exciting potential model for the study of temporal regulation in organ development and mechanisms of cross-organ coordination. We are now pursuing QTL mapping and genetic experiments to dissect the regulation of these processes. This work was supported by the NIH grant R35GM151199 awarded to PKS.

Program Abstract #416

Microfluidic intubation of the embryonic eye alters intraocular pressure (IOP) and disrupts corneal morphogenesis

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During development, the embryonic eye is fluid-filled and undergoes dramatic changes in size and shape. Growth of the developing cornea is correlated temporally with the expansion of the eye, and changes in intraocular pressure (IOP) are thought to be important for both eye and corneal development. In the avian embryo, the eye and cornea undergo a rapid phase of growth between embryonic days (E) 4 and 10. This expansion is associated with an accumulation of vitreous fluid, and previous experiments have shown that altered IOP can disrupt normal eye development. Even so, it is not

clear how changes in IOP influence the 3D geometry of the developing cornea nor how patterns of proliferation might be modulated by differences in fluid pressure. Here, we used pulled glass micropipettes to intubate the vitreous cavity of the developing eye in windowed avian embryos at E4. The embryos were then incubated until either E5 or E8 and fixed. (Glass microneedles, which lack a luminal space, were inserted into the eyes of other embryos and used as controls.) Microfluidic intubation at E4 caused the embryonic eye to collapse, and by E5 and E8, optical coherence tomography (OCT) reconstructions of control and intubated eyes revealed macroscopic changes in the 3D geometry of the developing cornea. Morphometric measurements indicated that decreased IOP causes an increase in corneal thickness, a decrease in corneal diameter, a reduction in the volume of both the vitreous cavity and anterior chamber, as well as a decrease in corneal curvature (with respect to the surrounding sclera). In addition, quantification of phosphohistone H3 immunofluorescence revealed decreased proliferation within the cornea following microfluidic intubation. Taken together, these data highlight the importance of mechanical forces during eye development and suggest that embryonic IOP can influence the cell behaviors that shape the cornea. This work is supported by the NIH grants R01HL145147 and R01EY030190.

Program Abstract #417

The role of zic genes in Convergent Extension

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Convergent Extension (CE) is the morphogenetic process where tissue narrows (convergence) along one axis and lengthens (extension) in a perpendicular axis. It occurs during gastrulation in the dorsal mesoderm and during neurulation in the neural ectoderm in Xenopus embryos. The planar cell polarity pathway regulates convergent extension, which is mediated by core proteins that are asymmetrically localized. These include frizzled, Vangl, flamingo and the cytoplasmic effectors prickle and disheveled. Mediolateral polarization, elongation, and intercalation are lost when these localized core PCP components are disrupted, leading to a failure in CE. During gastrulation, the zic1-3 genes are expressed in the involuting mesoderm during convergent extension. During neurulation, the zic1-3 genes are expressed in the neural plate border region. Zic proteins are transcription factors that are involved in neural development, left-right axis patterning, somite development and in cerebellum formation. We have found that inhibiting zic2 or zic3 expression causes a defect in convergence, but not in extension in Keller explants. Most genes that are required for CE affect both convergence and extension. To our knowledge, zic2 and zic3 are the only known genes that affect convergence only. Thus, we will test the mechanism by which inhibiting the zic2 and/or zic3 genes causes a defect in convergence, but not in extension. The Prickle and Vangl core PCP proteins are localized to the anterior cell membrane in dorsal mesoderm cells undergoing CE. Our goal is to determine if inhibition of zic2 or zic3 expression, which leads to convergence defects, will disrupt the anterior localization of Prickle and Vangl proteins. In order to test this, we will use zic2MOs or zic3MOs and prickle-GFP injected Keller explants. Vangl can be tested directly by immunofluorescence staining for detection using confocal microscopy. This work is funded by the SDB Choose Development! Program and by the NSF.

Program Abstract #418

Supracellular mechanics and counter-rotational polonaise flow during morphogenesis of the Xenopus tailbud.

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After gastrulation, the tailbud emerges as a dynamic region independent of anterior structures, where neuromesodermal progenitors (NMPs) provide cells for posterior axial tissues. In mammals and avians, tailbud morphogenesis begins at the posterior-neuropore (PNP) which generates germ layers through somitogenesis and secondary neurulation. Here, we describe posterior morphogenesis during the transition from late neural to early tailbud stages in *Xenopus laevis*. We have identified large-scale counter-rotational tissue flows, aka "polonaise flow," lateral to the blastopore. Image analysis of tissue movements suggests long range mechanical interactions between the ventral and dorsal tissues; complex strain patterns that suggest supracellular transmission for forces in the tailbud suggesting distinct sites of compression and tension shape tissue movements in and around the tailbud. Timelapses of nuclei-

and membrane-labeled ectodermal cells reveal rapid cell rearrangement in regions of high compression and polonaise-flow. Inspections of the deep tissues in fixed embryos reveals extensive fibronectin and laminin fibrils emanating ventrally like spokes from the blastopore between ectoderm and mesoderm. These observations lead us to hypothesize that tailbud morphogenesis is driven by long-range supracellular forces that integrate dorsal extension with ventral morphogenesis. We have begun mechanical testing of this hypothesis via tissue cutting and find large and rapid recoil, indicating the posterior end of the dorsal axis is under ventral generated tension. This suggests that NMPs exist in a highly anisotropic mechanical environment, where the dorsal posterior axis is "towed". This work is revealing a complex interplay of multiple force-generating processes drive posterior morphogenesis, and lays the groundwork for future analysis of the role of mechanobiology on the NMP niche. Supported by the National Institutes of Health (R37 HD044750 and R21 HD106629).

Program Abstract #419

Environmental stress regulates stomatal development via direct inhibition of the stomatal bHLH transcription factor module

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How does an ever-changing environment affect the optimization of stomatal formation? Stomata are pores required for the uptake of gases necessary for photosynthesis, but open stomata also increase the risk of water evaporation from the plant. To balance gas exchange and water evaporation, plants have developed sophisticated mechanisms that regulate stomatal development to adjust the optimal number of guard cells. It is well-studied that the number of stomata is reduced under osmotic stress; however, the mechanism by which key transcription factors (TFs) are regulated under osmotic stress has not been identified. This study proposes a new mechanism involving basic Helix-Loop-Helix (bHLH) TFs in modulating guard cell numbers under osmotic stress conditions. During stomatal development, bHLH TFs play central roles in specifying cell fate transitions. SCREAM (SCRM) and SCRM2 are expressed throughout stomatal development, while SPEECHLESS (SPCH), MUTE, and FAMA are expressed sequentially. SCRM respectively interacts with SPCH, MUTE, or FAMA by forming bHLH-bHLH heterodimers to specify cellular identity. Unlike bHLH TFs, (b)HLH proteins lack a typical basic region required for DNA binding, leading to the inhibition of DNA binding for transcription. We hypothesize that the uncharacterized (b)HLH TFs, CREAM, acts as a negative regulator by forming heterodimers with bHLH TFs during stomatal development. Interestingly, CREAM expression is notably induced by osmotic stress, particularly in meristemoid cells governed by the MUTE-SCRM module. To elucidate CREAM's regulatory mechanism on stomatal bHLH TFs, we employ biochemical and biophysical approaches, revealing CREAM's direct binding to MUTE and displacement of its partner, SCRM. A comprehensive understanding of the function of CREAM will provide new insights into the antagonistic interaction of (b)HLH-bHLH proteins regulated by environmental factors in stomatal development. This work was supported by the HHMI and UT-Austin.

Program Abstract #420

Using Transcriptomics to Detect Developmental Mechanisms in Larval and Adult Flies Adapting to Extreme Environments

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Introduction. Adaptation of an individual organism to new environments is achieved by gene expression changes. Stress from adaptation initiates a repertoire of transcription factors, as well as epigenetic changes to the genome, such as DNA methylation, which consequently reactivate transposable elements (TEs) residing in genomes. Expression of TEs in most cells is normally suppressed to avoid uncontrolled transposition another important contribution of TEs is regulation of neighboring genes in *cis*, particularly in early embryos and stem cells. Little is known about TEs as potential developmental mechanisms in adaptation, especially adaptation to extreme environments, such as space. **Objective & Methods:** We created a novel bioinformatics pipeline, Transcript & TE Signature Analysis to test our hypothesis that stress from adaptation to space environment, alters expression of TEs and gene networks for developmental systems in larval and adult flies (*D. melanogaster*). **Results:** Flies exposed to either microgravity or simulated hypergravity implicate adaptation affects multiple developmental systems,

including growth and fertility while stimulating genes regulating stress and immune responses. Some TEs, were substantially altered during adaptation to microgravity (8-100X), especially Long Terminal Repeat (LTR) retrotransposons, such as Gypsy, Copia, Diver LTRs, have strong effects on fruit flies, particularly fertility, and can act as promotors, encode long noncoding RNA, and create new gene products with new biological functions. **Conclusion**: Our results corroborate the novel hypothesis TEs may be a molecular mechanism reshaping developmental gene networks during adaptation to new gravitational environments. These experiments facilitate understanding the role of TEs in development by inducing robust transcription of TEs normally not observed on earth and serves as translational model for human adaption to altered gravitational environments (FL Space Agency).

Program Abstract #421
Developmental and neurologic effect of arsenic in *Drosophila*
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The risk of developing neurologic diseases, such as Alzheimer's and related dementias, is heightened due to increased arsenic exposure. Despite known neurotoxic effects of arsenic, its mechanisms and impact on neurodevelopment remain unclear. Here, we investigate the developmental and neurotoxic impacts of arsenic on *Drosophila*. To model developmental exposure, first instar larvae were exposed to varying arsenic doses. We noted a significant dose-dependent delay in pupariation and eclosion caused by arsenic exposure. Neurological effects of arsenic exposure included an increase in brain volume and mitotic indices, indicating disrupted cell cycle progression. Consistent with this model, we identified a decrease in the frequency of neural stem cells within S-phase. Live imaging analyses indicated no change in cell cycle length, yet neural stem cells exposed to arsenic exhibited prolonged time in metaphase. This deregulation of mitotic progression led to elevated rates of chromosomal instability in neural stem cells of arsenic exposed larvae. In adults, arsenic exposure resulted in marked behavioral defects. The climbing assay showed sex-specific arsenic responses. Specifically, wild-type female flies were more tolerant to arsenic exposure, as compared to males. Interestingly, this sexually dimorphic response reversed within an established tauopathy model. Arsenic exposure reduced climbing ability in tauopathy females flies, but increased it in males. Sex-specific differences were also observed in circadian rhythms following arsenic exposure. Arsenic also affected circadian rhythms, altering sleep patterns differently in Tau mutants. Our findings demonstrate the multifaceted effects of arsenic on *Drosophila* neurodevelopment and behavior, underscoring its potential to disrupt neurological processes through interaction with Tau-related pathways. This study further highlights the need to expand analysis of gene x environment interactions in human disease mechanisms.

Program Abstract #422
High sugar consumption alters parental provisioning of offspring
Manaswini Sarangi, Laura Buttitta, Monica Dus
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While food is crucial for an organism's survival, certain nutrients in the wrong proportions deter its ability to protect bodies against chronic diseases. A major shift in the dietary environment during the past 5 decades shows a startling increase in calorie consumption among which diets rich in sugar (among other highly palatable foods) remains one of the characteristic components of Western food. Given that developing organisms are far more receptive to environmental changes, we subjected wild-type Drosophila melanogaster to a high sugar diet (SD) environment and collected their embryos at the end of the dietary regime. We find that SD parents suffer from loss of perception of sweet taste, overeating, increased levels of glucose and triglycerides in the body and their offspring experience developmental delay. High throughput metabolomics analysis of offspring embryos revealed depleted states of nucleotides, amino acids, carbohydrates, lipids, cofactors and vitamins in those from SD-fed parents. Genes regulating neurodevelopment, nucleotide and one carbon metabolism were also differentially expressed in such embryos exhibiting strikingly different profiles between parental diet types. Interestingly, supplementing the parental diet with folic acid rescued the developmental delay phenotype as well as reversed or reset several of the differentially expressed genes initially observed in SD conditions. Ongoing work on DNA damage in fly embryos and implications on cell cycle phases are showing differential impact of sugar diet consumption compared to their control counterparts. These experiments are helping

us characterize if and what reprogramming occurs in the inherited nutritional state of offspring that likely alters offspring development when fly parents reproduce consuming high sugar food. Funding: NIH DHH-US1-009449, NIH 1DP2DK-113750 and NSF CAREER Award to Monica Dus.

Program Abstract #423
Environmental affects on wing shape in the painted lady butterfly, *Vanessa cardui*
Madison Kline
High Point University, USA
The wings of Lepidoptera offer a particularly attractive system in which to investigate the mechanisms of developmental plasticity. Species of Lepidoptera differ greatly in the size and shape of their wings, but all have the same venation pattern, consisting of a standard set of homologous veins that make it possible to map homologous locations on the wing throughout development. This makes the wing a particularly suitable system in which to study the interplay between development and evolution in morphogenesis because it is possible to ascribe changes in shape during development to quantitative changes in the growth of specific regions across the entire wing surface. Using the painted lady butterfly, *Vanessa cardui*, we trace the development of forewing and hindwing shape during the last larval instar under different nutritive and temperature regimes. We will discuss aspects of differential growth and ongoing research directions regarding wing shape plasticity in Lepidoptera. We thank HPU's Natural Science Fellow grants for supporting this research.

Program Abstract #424
Larval color plasticity in the painted lady butterfly, *Vanessa cardui*
Chloe Buffalino
High Point University, USA
Although Conrad Waddington had shown almost a century ago the ability to destabilize a trait by exposing organisms to extreme environmental conditions, few studies have shown a plausible molecular mechanism for destabilization. Waddington classically used heat stress to unveil hitherto unseen phenotypes. The work of Rutherfod and Lindquist in the late 1990s hinted at the possibility that the molecular chaperone Heat Shock Protein 90 (HSP90) may be destabilized at elevated temperatures, allowing for the penetrance of some genetic variants to change. The authors demonstrated that selection for mutant phenotypes dependent on HSP90 inhibition, be that chemical inhibition or hypomorphic HSP90 alleles, could increase the prevalence of those phenotypes in subsequent generations. These traits could be driven to near fixation, presumably due to a selective sweep wherein a previously rare or obscure allele(s) increases rapidly in frequency due to selection. We have found that heat shock of the painted lady butterfly, *Vanessa cardui*, during the 4th larval instar changes the caterpillars normal 5th instar black pigmentation to white. We will discuss the variability of this plastic response and preliminary data on the molecular mechanism underlying the unveiling of this novel phenotype. We thank HPU's Natural Science Fellow grants for supporting this research.

Program Abstract #425
Environmental regulation of metamorphosis in a gregarious beetle
Allyssa Winegar
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Metamorphosis is a crucial biological transformation wherein an organism undergoes complete reconstruction to reach maturity. Significant adult structures such as wings and legs are developed during metamorphosis, initiated by the synthesis of steroid hormones during the pre-pupal stage. This process is tightly regulated, with distinct environmental influences observed across species. Our research concentrates on a specific species of darkling beetle, *Zophobas morio*, known for its ability to delay molting when in a crowded environment and commence molting when isolated. This unique behavior represents an interesting environmental cue for evolutionary control over the neuroendocrine axis. We will discuss the potential mechanisms underlying *Z. morio* pupal commitment. We thank HPU's Natural Science Fellows grants for supporting this research.

Program Abstract #426

Disruption to a Bmp-*nkx2.3* genetic pathway sensitizes embryos ethanol-induced defects to the endoderm pouches.

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Fetal alcohol syndrome disorders (FASD) describes a wide range of birth defects associated with prenatal alcohol exposure, including to the facial skeleton. The formation of the face involves complex interactions between the neural crest and the endoderm. Neural crest cells migrate and condense into the pharyngeal arches while lateral protrusions, called pouches, form from the endoderm. These pouches serve as signaling centers that guide neural crest cells to form various cartilage elements of the facial skeleton. An important signaling pathway necessary for pouch formation is the Bone Morphogenetic Protein (Bmp) signaling pathway. The Bmp pathway regulates pouch formation by regulating multiple genetic targets including the transcription factor, *nkx2.3*. Previous studies show that blocking Bmp signaling from 10-18 hpf reduces *nkx2.3* expression, disrupting pouch specification and subsequent morphogenesis. Here, we show that mutations in the Bmp pathway sensitize embryos to ethanol leading to defects in pouch morphogenesis and facial development. Using hybridization chain reaction, we identified Bmp receptors expressed in the pre-pouch endoderm at 16 hpf, during pouch morphogenesis. We also show that knockdown of *nkx2.3* using a translation blocking morpholino results in craniofacial defects similar to what is observed in other pouch mutants. Furthermore, ethanol exposure exacerbates these defects, resulting in phenotypes similar to what is observed in ethanol-treated Bmp mutants. Finally, we show that morpholino knock down of *nkx2.3* exacerbates ethanol sensitivity in *bmp4* mutants. Overall, this suggests that Bmp signaling, and its downstream target, *nkx2.3*, play a crucial role in ethanol-induced disruptions in pouch morphogenesis. We are currently validating an *nkx2.3* Crispr/Cas9 mutant line to characterize the ethanol-sensitive epistasis of Bmp signaling and *nkx2.3* in pouch morphogenesis and facial development. This research was supported by R00AA023560 to CBL.

Program Abstract #427

Investigating the Role of Gadd45ba in Adaptive Responses to Anoxia

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The zebrafish is one of several vertebrate organisms capable of surviving environmental changes, such as hypoxia (low oxygen) or anoxia (no oxygen). The anoxia-tolerance of zebrafish embryos is due to their ability to enter a reversible state of metabolic suppression. Despite this energy-conserving response and the general suppression of transcription, the expression of genes that promote anoxia survival are up-regulated. Using an RNA-Seq approach, the Brewster lab found that over 1000 genes were increased twofold or more in embryos exposed to anoxia compared to normoxic controls and stress response gene, Growth arrest and DNA-damage-inducible beta (*gadd45ba*) was among them. The goal of my project is to carry out a functional analysis of *gadd45ba*. Previously, I validated the upregulation of *gadd45ba* using qPCR and wholemount in situ hybridization to examine the spatial distribution of this gene. The tissues where *gadd45ba* is expressed are known to be hypoxia-responsive. Under normoxic conditions, I found that *gadd45ba* is expressed in the diencephalon and the hematopoietic stem cell niche. Interestingly, in response to prolonged anoxia, *gadd45ba* expression expands to the forebrain and circulating red blood cells. The forebrain, where the diencephalon is located, is a region of the brain that regulates respiration. In the hematopoietic stem cell niche, newly developed stem cells undergo proliferation to increase the number of RBCs. These findings suggest that gadd45 mediates these adaptive responses, possibly by functioning as a DNA demethylase. Future experiments will test this model using gadd45 knockdown embryos. This research was supported by Brewster lab (T34GM136497), Meyerhoff Scholars program, Howard Hughes Medical Institute (HHMI) undergraduate scholars' program (52008090), and U-RISE Program at UMBC (T34GM136497).

Program Abstract #428

Ndrg1b is a regulator of N-cadherin that modulates cell adhesion during early development and under anoxia

Prableen Chowdhary, Gabriel Otubu, Rachel Brewster

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Early embryonic development is dependent on the tight regulation and complex coordination of many genes and signaling molecules. One such family of molecules is cadherins, cell adhesion molecules that regulate processes such as cell migration, sorting, and tissue organization. With cellular junctions being so dynamic during early development, levels of cadherins are carefully controlled through either their endocytosis and degradation or their synthesis and recycling. As such, disruptions to the levels or subcellular localization of cell adhesion molecules can have detrimental effects during embryonic development. Recently, many researchers have studied the N-Myc Downstream Regulated Gene family (NDRGs), molecular adapters that are activated by environmental stressors and can function in vesicle trafficking pathways. In particular, NDRG1 is a target of the Hypoxia Inducible Factor (HIF) and is hence hypoxia responsive. Our lab has previously identified an adaptive role for zebrafish Ndrg1a in orchestrating the downregulation and degradation of the sodium-potassium ATPase pump (NKA) under anoxia in order to conserve energy. Here, we characterize and identify a novel function for zebrafish Ndrg1b in maintaining homeostasis and promoting proper embryo development by regulating N-cadherin. In contrast to *ndrg1a*, the expression of *ndrg1b* is much broader and suggests a function in many tissue types. We show that knockdown of *ndrg1b* results in severe developmental defects similar to loss of cell adhesion phenotypes seen in N-cadherin mutants, consistent with a previous report suggesting that NDRG1 regulates E-cadherin-based cell-cell adhesion via Rab4-mediated endosomal recycling in prostate cancer cells. This work was funded by NIH R21 (R21HD089476) and personal funding by the Graduate Research Training Initiative for Student Enhancement (G-RISE) program through NIH (T32GM144876).

Program Abstract #429

"Forever" impacts: How “chemicals” like PFAS disrupt gene regulatory programs during development.

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Our current environment exposes us to pollutants such as the "forever chemicals" like PFAS, which resist degradation and linger for a very long time in our system. PFAS, or per- and polyfluoroalkyl substances, encompass a group of chemicals commonly used and often found in substances with non-stick and/or waterproof properties. Their detection in human samples varies by geographical location and racial/ethnic backgrounds, with numerous studies highlighting their adverse effects on multiple organ systems and potential links to developmental delays in children. Exposure during pregnancy has been associated with childhood leukemia and developmental delays in toddlers. The precise mechanisms by which PFAS disrupts cellular processes, that could be impacting early development and have repercussions later in adult physiology, remain incompletely understood. To address this knowledge gap, we are using zebrafish as our model system to investigate how PFAS affects embryonic development. To decipher the level at which PFAS plays a role, our approach involved collecting embryos and treating them with a low and high dose of PFAS. Additionally, to explore potential epigenetic inheritance with PFAS exposure, we treated adults and then collected embryos. We processed all the collected embryos for RNA expression changes at two developmental stages critical for organogenesis. We observe that some key developmental genes for aligning anterior-posterior body axis or involved in organ development are differentially impacted with varying amounts of PFAS exposure. Given the dynamic nature of early development, we plan to further elucidate the extent of PFAS's differential impact on embryos through single cell sequencing experiments. This methodology will offer insights into the divergent organ-specific effects of PFAS exposure, enhancing our understanding of potential long-term ramifications, that may extend into adulthood, of early PFAS exposure.
Funding:UNC Collaboratory Chapel Hill

Program Abstract #430

Early exercise disrupts a pro-regenerative extracellular matrix program during zebrafish fin regeneration

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Adult zebrafish robustly regenerate amputated fins including their characteristic bony ray skeleton. In

contrast, human injury repair is modest but can often be enhanced by rehabilitative exercise. We investigated the impact of exercise on recovery in pro-regenerative zebrafish. Fin regeneration begins with wound epidermis formation, followed by a transition through blastema establishment to a progressive outgrowth phase. We used a swim tunnel and staggered swimming onset to find that exercise uniquely disrupted the early stages of regeneration. Daily imaging of fluorescently labeled fibroblasts demonstrated that swimming impaired blastema formation. Transcriptomic profiling implicated exercise-disrupted extracellular matrix deposition, including hyaluronic acid (HA). We used Biotinylated Hyaluronic Acid Binding Protein (bHABP) staining to confirm exercise decreased blastema-localized HA, and HA microinjection into the blastema prior to exercise improved regenerative outcomes. Further, exercise-independent disruption of HA synthesis and localized enzymatic HA degradation phenocopied exercise effects. We did not detect HA-ligand receptors in the blastemal mesenchyme. Thus, we considered if HA supports a pro-regenerative environment for mechanotransduction. bHABP and Yes-associated protein (Yap) antibody staining showed HA density across the blastema correlated with Yap nuclear localization. Finally, both exercise loading and HA synthesis inhibition decreased nuclear Yap and reduced proliferation. We conclude early exercise disrupts expression of a pro-mechanotransduction extracellular matrix program, including HA production, that supports fin blastema establishment. Zebrafish fins provide a new model to explore exercise and regeneration, and our work suggests HA-based therapies could moderate the detrimental effects of early exercise on skeletal appendage repair. Funded by NIH F32GM140712 (VML), R01GM149999, and the Wu Tsai Human Performance Alliance.

Program Abstract #431

Naproxen exposure during early development inhibits cranial chondrogenesis in axolotl embryos

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Naproxen (NPX) is a non-steroidal anti-inflammatory drug (NSAID) commonly used to alleviate pain and inflammation via inhibition of the cyclooxygenase (COX1/2) enzymes. Embryonic exposures to NSAIDs are linked to preterm birth, neural tube closure defects, abnormal enteric innervation, and craniofacial malformations. Each of these anomalies may be caused by abnormal neural crest cell (NCC) development. NCCs are stem-like cells that differentiate into diverse adult tissues including craniofacial cartilage and bone and neurons of the peripheral and enteric nervous systems. Our lab has identified that COX1 and COX2 transcripts and proteins are expressed during the early stages of vertebrate embryonic development, and that targeted knockdown of COX2 and its receptor, EP3, leads to aberrant neural crest cell maturation in vertebrate embryos. To investigate the phenotypic and molecular effects of NSAID exposure on the development of NCCs and their derivatives, we exposed axolotl embryos to various concentrations of NPX during NCCmigration and differentiation stages and then performed immunohistochemistry (IHC) for markers of NC-derived cells. We identified that NPX-exposed embryos exhibit molecular changes by tailbud stage and gross anatomic changes by tadpole stages. Specifically, NPX-exposed embryos have reduced migration of SOX9-positive NCCs, resulting in abnormal formation of discrete craniofacial cartilage structures, including Meckel's cartilage, in the developing jaw. NPX exposure also disrupts normal differentiation of sensory cells and organs in the developing face. Future work will focus on defining the specific COX signaling pathway effectors involved in normal NCC development and formation of craniofacial bone and cartilage. Funding was provided by the UC Davis School of Veterinary Medicine and the American Veterinary Medical Foundation.

Program Abstract #432

Investigating gene co-expression network modularity and robustness across mammalian limb development

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Gene networks are a common tool for representing and investigating developmental pathways, but the effects of their or topology on development remain understudied. Previous work shows that the network active in early limb development exhibits more expression variation than the later network. Though the network reorganizes through developmental time, it remains unclear to what extent that changing topology contributes to the measured variation. To investigate the role of network topology in the

variability of limb development, we use RNA-seq data from mouse, bat, pig, and opossum to generate gene co-expression networks and analyze their structure in the context of mammalian limb diversity. Each species was sampled at three stages of development for both the fore- and hindlimb. We generate gene co-expression networks centered around limb patterning genes, as well module networks in which highly co-expressed genes are grouped together. Thus, we assess network topology at two scales – gene-gene interactions, and interactions of modules of highly co-expressed genes. We measure modularity and robustness as proxies for developmental integration and resilience. Preliminary results show that the opossum has the most modular limb development, which likely is linked to the severe heterochrony between its fore- and hindlimbs. Robustness analyses show that later development is less resilient to perturbations, which supports previous work from the lab that showed increasing variability in expression throughout development. This study leverages the diversity of mammal limbs to understand the role of network topology in shaping the variation that development can produce. We assess patterns of modularity across species and of robustness through time. Preliminary results suggest modularity is increased for species with distinct fore- and hindlimb development, and that limb development is more robust early than later. This work is supported by NSF-GRFP DGE-1650604, DGE-2034835.

Program Abstract #433

Alternative splicing as a mechanism of gene dosage mediation during limb development and evolution

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Gene regulatory networks (GRNs) governing limb development are highly conserved throughout tetrapods; however, the resulting morphology is incredibly diverse. Differences in the amount and temporospatial dynamics of gene expression are thought to underlie this variation. Previous work has shown that differences in regulatory sequences and epigenetic modification contribute to evolutionary differences in gene expression. We hypothesize that alternative splicing (AS) is an additional mechanism contributing to limb GRN differences. AS produces multiple protein isoforms from a single precursor mRNA. However, the impact of these isoforms on downstream targets is less understood and may alter effective GRN levels. To test this hypothesis, we used RNAseq data from mouse and opossum limbs at the ridge, bud, and paddle stages to evaluate changes in splicing patterns between species and developmental stages. Our analysis revealed that splicing of key limb development GRNs including essential limb development gene *Fgf8,* and its receptors changes over developmental time and between species. Splicing of *Fgf8* is known to produce two key conserved protein isoforms, Fgf8a and Fgf8b, which have been shown to play different roles during the development of tissues such as the brain and have differing binding affinities to their receptor, yet their impact on downstream *Fgf8* targets is less well known. To further elucidate this impact, we will be incubating mouse fibroblasts with either Fgf8a or Fgf8b protein and quantifying changes in downstream signaling targets, target gene expression, and mitotic output. Funding from this project was received from NIH R15 DE026611-02

Program Abstract #434

Extending the base – the elaboration of proximal elements and models of fin to limb evolution

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Since descending from a common antecedent appendage, teleost fins and tetrapod limbs have evolved highly divergent structures and functions. The differences between these appendages are extensive and make homology associations between specific elements difficult from anatomy alone. However, recent functional genetic studies have revealed that distal fins and limbs share common developmental histories and specification, demonstrating that deep homology relationships can be identified between appendage regions. While normal teleost fins lack a region comparable to the limb zeugopod (radius/ulna 'middle'), we identified zebrafish mutants that form novel articulating skeletal fin elements which have regionality and molecular identity similar to that of the middle portion of the limb. These data define a retained potential in fins to pattern and elaborate the proximal-distal axis in a manner similar to limbs. Extending our analysis of latent higher order patterning mechanisms in the fin skeleton akin to limb developmental programs, we have identified new mutants that produce surprising articulations in the fin base, further extending the proximal fin skeleton and shoulder girdle. To understand how proximal patterning mechanisms are modified in these mutants, we generated knock-in reporter

lines to examine the expression and function of genes required for shoulder and proximal appendage development in limbs. Shockingly, we find that these markers are absent from the appendage proper and restricted to the shoulder mesenchyme in wild type zebrafish fins. Distal markers, however, were found throughout the fin. This suggests that a domain comparable to the limb stylopod (humerus) is not realized in wild-type fins but can be revealed via genetic perturbation. While previous models have emphasized terminal addition in the evolution of the limb, our data suggest the specification of a new proximal domain was a critical early step in the fin-to-limb transition.

Program Abstract #435

Altered PTHrP & Ihh expression associated with the loss of the mammalian metatarsal growth plate

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Unlike the major long bones of the limbs, those in the hands and feet of mammals [metatarsals (MT), metacarpals, and phalanges] form a growth plate at only one end. The opposite end undergoes direct ossification with the primary center of ossification invading the cartilaginous epiphysis. The MT is a natural model to understand gene expression specific to growth plate formation. PTHrP (the product of the *Pthlh* gene)/IHH feedback loop is a key regulator of growth plates. Resting chondrocytes express *Pthlh* which limits differentiation of proliferative cells. Just prior to undergoing hypertrophy, pre-hypertrophic chondrocytes express *Ihh* which signals back to resting cells to promote the expression of *Pthlh*. We quantify the expression of *Pthlh* and *Ihh* in neonatal (P0), 4 day old (P4), and P9 mouse MTs to test the hypothesis that the PTHrP/IHH feedback loop is specific to growth plate formation. *In situ* hybridization shows that *Pthlh* is located in the perichondrium and articular chondrocytes at both ends of the MT3, but a strong band of expression occurs only in resting chondrocytes in the distal growth plate. Strong *Ihh* expression is observed in pre-hypertrophic chondrocytes at both ends of the P0 and P4 MT3. However, the boundary of *Ihh* expression is irregular across the proliferative/hypertrophic transition in the non-growth plate forming end. At P9, *Ihh* is expressed in pre-hypertrophic chondrocytes of the distal growth plate and throughout the proximal epiphysis. RT-PCR identifies a quantifiable difference in *Pthlh* expression between the ends of the MT. The reduction of *Pthlh* expression in the non-growth plate forming end results from the loss of PTHrP+ cells in the resting zone. This population has been hypothesized to act as a progenitor pool of growth plate chondrocytes, and its loss underlies the differential growth of the mammalian metatarsal. Funding provided by NSF grants IOS-1656315 & BCS-1638812.

Program Abstract #436

Developmental basis for evolutionary loss of digits in horses

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A pentadactyl (5 digits) limb pattern was stabilized early in tetrapod evolution. Variation in the number of fingers and toes reflects evolutionary reduction and loss of digits, which has occurred numerous times during the diversification of tetrapods. Comparative developmental studies have shown that evolutionary reduction of digit number has been accomplished by different mechanisms in different lineages. Equidae, which includes horses, zebras, and donkeys, are a classic and extreme example of digit reduction, with each limb bearing only a single functional digit. The earliest equids were pentadactyl, and the fossil record shows that incremental reduction and loss of digits led to the monodactyl limbs of modern equids. To identify the mechanisms responsible for digit loss during horse evolution, we collected a developmental series of horse embryos and performed a transcriptomic analysis of their limb buds at different stages. Comparison of horse limb transcriptomes with those of stage-matched mice, which served as a pentadactyl model, showed that the limb development signaling network is conserved in early limb buds, but differences emerge prior to digit formation. The BMP antagonist GREMLIN1 is central to the genetic feedback loop that coordinates limb outgrowth and digit patterning. After the SHH/GREM1/AER-FGF circuit is established in horse limb buds, Grem1 then undergoes quantitative and spatial changes in expression, which functional studies show to be sufficient to account for the horse autopod phenotype. Our results suggest that the pattern of digit alteration that occurred during horse evolution, including elongation of the digit 3 and reduction of digits 2 and 4, can be explained by two modifications to Grem1 regulation.

Program Abstract #437

Fgf8 dosage regulates first pharyngeal morphogenesis and symmetry

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Fgf8 is expressed in the oral ectoderm of the first pharyngeal arch (PA1), lateral ectoderm of the pharyngeal clefts, the foregut endoderm of the pharyngeal pouches, and the anterior heart field mesoderm. PA1 is the precursor of jaws whereas the first pharyngeal pouch (pp1) and cleft (pc1) contribute to the middle ear. To further investigate the role of *Fgf8* in pharyngeal development, we utilized an allelic series of mutant mice, that generates embryos expressing different levels of *Fgf8* during development, including a mild and severe mutant. *Fgf8* mutant mice have directionally asymmetric jaws with the left side being more affected than the right. In mild mutants, unilateral fusion occurs in approximately one-third of individuals, only on the left side. In *Fgf8* mutants, pp1 fails to extend along the proximal-distal axis and the first and second arches fail to separate. This defect is associated with alterations to epithelial patterning. For example, pp1 normally exhibits regional identity in that the ventral side is Sox2 positive while the dorsal side is Sox2 negative. In *Fgf8* mutants, the ventral side of the pouch loses Sox2 identity. Here, we further investigate how *Fgf8* expression (mRNA) levels impact known downstream signaling protein targets such as pERK and pAKT in order to more directly determine how Fgf signaling affects pharyngeal epithelial patterning. We find that Sox2 and pERK activity occupy complementary domains in the pharyngeal endoderm. We have also investigated pp1 patterning and morphogenesis in different vertebrate taxa (mouse, chick, lamprey) and described taxa-specific shape and patterning. NIDCR R03 DE028984

Program Abstract #438

Investigating a surprising developmental relationship between vertebrate rods and short-wavelength cone photoreceptors

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Rod and cone photoreceptors are highly specialized cells that have typically been considered to have separate developmental pathways. Our studies however have found a consistent theme of developmental relatedness between rods, the cells responsible for vision in dim light, and the subpopulation of cones responsible for detecting low wavelength light (sws1, e.g. "blue cones" in humans). The transcription factor nrl is necessary and sufficient for rod cells in mice and zebrafish; when nrl is absent rods are lost and a concomitant increase in sws cones is observed. Transgenic expression of nrl in sws1-fated precursor cells is sufficient to enforce an apparent rod-fate switch. Our results provoke a surprising hypothesis that these two cell types may share an evolutionary and developmental history that is distinct from other cone subtypes. Ongoing work aims to further elucidate the specificity of this effect between rods and sws1 cones, as well as the evolutionary conservation of this phenomenon across the vertebrate lineage. we have produced various lines of transgenic zebrafish expressing homologs of nrl from mice, chicken, lamprey and hagfish, which have demonstrated that the ability of nrl to enforce the rod fate in otherwise sws1 fated cells is extremely well conserved across the vertebrate lineage. Additionally, we've examined the specificity of this rod-cone relation by ectopically expressing nrl behind various other cone promoters in order to determine whether this fate shifting effect is unique between rods and sws1 cones specifically, or whether other cone subtypes can be persuaded to take on a rod-like fate via nrl. The capacity to influence developing photoreceptor precursors between a rod and sws cone fate in particular suggests an exciting possibility that rods and sws cones share a previously unknown evolutionary relation, distinct from that of other cone subtypes. Funding provided by NSERC.

Program Abstract #439

Understanding how the Retinoblastoma pathway affects newt eye development

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Although humans are limited in their ability to mount regenerative responses following injury, newts are capable of regenerating injured tissues/organs such as limbs, heart, brain, lens, and retina. The molecular mechanism of regeneration shares similarities with cancer growth including the loss of cell identity, division of cells, and even the reduced expression of tumor suppressor genes. This prompts the assumption

that newts must be tumor-resistant to be able to downregulate genes that suppress tumors from forming and instead regenerate tissues. We have generated a double CRISPR/Cas9 knockout of the tumor suppressors *Retinoblastoma 1* (*Rb1*) and *Retinoblastoma-like 2* (*Rbl2*) in the newt *Pleurodeles waltl* to investigate the effects of this compound loss. *Rb1/Rbl2* crispants display eye defects, including what appears to be an overgrown retina that we are characterizing. In order to investigate this, we will first examine the process of eye development and characterize the cell types of the retina that develop throughout the process. We will be employing histology, HCR in situ hybridization, and immunohistochemistry for such characterization. We hope to understand whether the *Rb1/Rbl2* newt crispants are resistant to the development of retinoblastoma, an eye tumor, and eventually what the implications are of reduced tumor suppressor genes on the regenerative capacity of newts. This work was supported by the Society for Developmental Biology "Choose Development!" Program to SMR, the Department of Biology at Miami University, the Swedish Cancer Society (Cancerfonden), and the Knut and Alice Wallenberg Foundation.

Program Abstract #440

A Conserved Mechanism in Vision Development: Lens Nucleus Centralization in *Xenopus Laevis*

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Developing optics of the eye, determined by the lens and cornea, must coordinate with the axial length of a growing eye to focus light onto the retina to form an image. It was found that the zebrafish *(Danio rerio*) lens nucleus in is initially anteriorly localized in the optical axis in larvae and then centralizes at juvenile stages, resembling non-aquatic species. This adaptation increases lens power to enable a functional visual system in young zebrafish, where the eye axial length is extremely short. The clawed frog, *Xenopus laevis*, is another fully aquatic species that similarly relies on vision for survival at stages where the eye is small and lens-retina distance is short. We examined if the lens nucleus asymmetry found in young zebrafish also occurs in *Xenopus*. Dissected tadpole and adult frog lenses were imaged perpendicular to the optical axis (axial orientation) to quantify the relative distance of the center of the lens nucleus to the center of the lens. We found that young tadpoles had lens nuclei localized closer to the anterior pole while adult frog lens nuclei were located centrally in the optical axis. Lens nuclei were centrally localized when viewed through the optical axis. Initial studies also showed that the lens nucleus of a regenerated tadpole eye is also asymmetrically localized. These findings suggest that lens nucleus centralization is a conserved evolutionary mechanism across at least two fully aquatic species, the zebrafish and clawed frog, supporting the hypothesis that it is a requirement of a functional visual system. An understanding of the key mechanisms that regulate cross-talk between eye optics and eye axial length will aid in discoveries of therapies to prevent or delay formation of diseases resulting in a mismatch between these, as occurs in short-sightedness, when the eye grows too long for eye optics. This work is supported by SDB CD Fellowship (KG), NIH EY031587 (IV), and NIH GM146672 (KT).

Program Abstract #441

Xenbase: Improvements in *Xenopus* gene nomenclature annotations

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Xenbase, the Xenopus Model Organism Knowledgebase, is the official resource for gene, genome and biological research data supporting the international Xenopus frog research community. Xenbase plays a foundational role in supporting biomedical science, developmental, cell and cancer biology. Xenbase sources genome build data from the UCB Xenopus genome group, the NCBI and ENSEMBL, to produce curated gene page entities and integrated Xenbase general feature format (GFF) genome annotation files. The most recent genome builds for X. laevis and X. tropicalis had thousands of instances where

previously well characterized genes lost key identifying metadata such as gene symbols and gene names. This presents a major opportunity to improve the utility and potential impact of the latest X. laevis and X. tropicalis genome annotations, by addressing the underlying gene nomenclature metadata of individual genes and large gene families that lost this key information, in addition to those that have never had proper annotations. In this poster, we outline the various approaches and strategies we have undertaken at Xenbase to address gene symbol name assignments for specific groups of genes and for large gene families. In an effort to first carry over annotations from v9 to v10 genome builds, we were able to recover annotations for hundreds of genes based on sequence similarity and by assigning gene synteny scores. We also addressed specific gene families, naming over one thousand genes belonging to the Olfactory Receptors (OR), Heat Shock Protein Beta (HSPB) genes and the FC receptor gene families. We are in process of integrating data sharing pipelines with the Alliance of Genome Resources group which will facilitate future integration with the DIOPT pipeline for automated gene annotations by identification of cross-species gene orthologs from protein sequences. Xenbase is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH)

Program Abstract #442

Metabolism and the evolution of developmental rate in *Caenorhabditis* nematodes

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The evolution of developmental rates has long been thought to be a major driver of morphological change. The nematode *Caenorhabditis* inopinata is the closest known relative of the widely studied developmental genetic model system, *C. elegans*. The time of reproductive onset is nearly doubled in *C. inopinata* compared to *C. elegans*, revealing *C. inopinata* has a much slower developmental rate compared to its sister species. This comparative system then offers the opportunity to understand the genetic basis of heterochronic change. In *C. elegans*, the gene *clk-1* encodes a hydroxylase that is required for synthesizing a key component of the electron transport chain (ubiquinone). Mutations in *clk-1* reveal much slower and variable rates of development compared to wild-type animals. Moreover, mutant *clk-1* slow growth phenotypes can be pharmacologically rescued by supplying animals with a ubiquinone precursor analogue, 2,4-dihydroxybenzoate (DHB). Transcriptomics experiments revealed a one-fold decrease of clk-1 transcript abundance in *C. inopinata* compared to *C. elegans*. This then raised the possibility that *C. inopinata* grows slowly because of reduced ubiquinone biosynthesis. To test this hypothesis, we reared *C. inopinata* on DHB to see if restoring CLK-1 activity can increase the developmental rate of this species. We were able to observe a consistent, significant increase in developmental rate in *C. elegans clk-1* mutants grown on DHB. *C. inopinata* revealed a modest increase in developmental rate on DHB in one experimental trial (8%), whereas no changes in developmental rate were observed in two other trials. We then conclude that divergent CLK-1 activity may not be a major driver of the evolution of slow development in this species. We will also discuss ongoing work on the role of wild microbial food sources on the evolution of developmental rates in nematodes. This work was supported in part by an award from the NSF (#2238788).

Program Abstract #443

Investigations of Morphological Plasticity Among Migratory and Non-Migratory Monarch Butterfly Populations

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Each fall, millions of monarch butterflies (Danaus plexippus) in North America migrate thousands of kilometers to Mexico to overwinter. How monarchs achieve this feat is not well understood. In order to tackle this problem, we leverage the fact that monarchs exist as both migratory and resident populations across the globe. The goal of this project is to understand differences in brain development between monarch butterfly populations that may contribute to migration differences. Theory predicts that migratory monarchs should invest more in morphologies that lead to increased migration success, such as larger brains for increased navigational capacity or larger wings for greater flight capacity. Previous studies showed that fall migratory monarchs' brains show experience-independent plasticity (growth) post-eclosion (Henize et al. 2013). Fall migratory monarch brains grow significantly larger over time either when individuals live in natural fall conditions and gain migratory experience or in a fall-like environmental

chamber with no migratory experience. The mechanisms of this brain plasticity are unknown and of interest. We address two specific unresolved questions in our research: 1) is experience-independent brain growth season-specific and 2) is experience-independent brain growth specific to the North American migratory population. We use advanced imaging approaches and gene expression analysis to provide an initial profile of brain development in different monarch populations under different environmental conditions. Understanding how monarchs' brains develop differently sheds light on key developmental and environmental factors required for a migratory brain phenotype. We acknowledge Choose Development! for summer student funding and consistent support (to D.M.) and the UM EEB Block Grant (to M.S.) for research support.

Program Abstract #444

Co-odination of robust pattern and plastic size in *Drosophila melanogaster* wing

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The shape of an organism is determined by the spatial arrangement of its morphological traits, referred to as pattern. To preserve function, pattern must be maintained as body size varies with genotype and the environment. How pattern and size are developmentally coordinated is poorly understood. Canonically, pattern formation is orchestrated by diffusible signaling molecules called morphogens. These morphogens are secreted to form a gradient across developing tissue. Cells at different positions along the gradient are exposed to different concentrations of the morphogen, determining their fate. Previous data demonstrate that morphogen gradients scale proportionally to the size of the growing tissue, a phenomenon called dynamic scale invariance, which is hypothesized to account for the maintenance of pattern regardless of the tissue size. This hypothesis, however, has not been tested, particularly when final tissue size varies with environmental condition. In this study, we use *Drosophila melanogaster* wing disc as a model to test the effect of environmental variation on morphogen scaling, focusing on the Dpp signaling gradient, which controls the position of the longitudinal veins in the wing. We show that, consistent with previous studies, the Dpp signaling gradient (as assayed by phosphorylated mothers against Dpp, pMad) is dynamically scale invariant under standard laboratory conditions (25^{o}C,21%O2), throughout ontogeny. However, at low oxygen (10%O2,25^{o}C) and low temperature (25^{o}C,21%O2) Dpp signaling gradient underscales: That is, the gradient gets disproportionally narrower as the disc grows. Despite this underscaling, adult wing pattern does not vary within or between environmental conditions. These data suggest that dynamic scale invariance is not an explanation for the maintenance of pattern across a range of body sizes and indicates another uncharacterized mechanism must be involved. This work is supported by the national science foundation IOS-1901727.

Program Abstract #445

Resolving the function and evolution for two transcription factor genes that pattern a sexually dimorphic fruit fly pigmentation trait

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Animal morphological traits develop through the actions of and evolve through changes in genomic-encoded Gene Regulatory Networks or GRNs. Therefore, a robust understanding of the evolutionary developmental biology of traits requires knowledge about the architecture of GRNs and how such architecture evolves. Generally speaking, GRNs are hierarchical in structure, with their highest tier consisting of regulatory genes like transcription factors and the lowest tier consisting of the realizator genes whose encoded proteins end up making the trait. For several evo-devo model traits, some of the regulatory and realizator tier genes are known and their evolution have been chronicled. However, the architecture and evolution of an entire GRN has remained out of reach. This includes the GRN responsible for the sexually dimorphic pigmentation on the abdomens of *Drosophila* fruit flies. Here, new insights will be shared for two upper-tier transcription factors in the *Drosophila melanogaster* abdomen pigmentation GRN whose function and evolution have remained mostly un-studied. This work is supported by the NSF award IOS-2211833 to TMW.

Program Abstract #446

Combining bioinformatic and transgenic approaches to better understand the regulatory control of genes for a *Drosophila* pigmentation trait

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Spatially- and temporally-regulated patterns of gene expression are an essential feature of animal development. Moreover, changes in expression patterns are known to underlie cases of evolution and disease. These expression patterns are sculpted by the activity of sequences often referred to as *cis*-regulatory elements or CREs. The collective number of CREs in a typical metazoan genome greatly exceed the number of genes. However, even in the most thoroughly studied animals, like *Drosophila* (*D.*) *melanogaster*, most CREs remain to be found and/or characterized. We previously used a small set of known CREs that function in a gene regulatory network (GRN) for the sexually dimorphic pigmentation of *D. melanogaster* to predict additional CREs genome-wide. Using the transcription factor motif-blind SCRM-shaw bioinformatic tool, more than 500 potential pigmentation GRN CREs were predicted. Here, we share our findings that are part of a follow-up study that focused on CRE predictions residing in or near to regulatory genes, including those populated by multiple predicted CREs. This includes the eight CRE predictions distributed across the *Eip74EF* locus. In addition to furthering an understanding of the evolution and development of a fruit fly trait, the results and future studies may shed light on the expression control of regulatory genes and its evolution. This work is supported by the NSF award IOS-2211833 to TMW.

Program Abstract #447

Connecting the *trans*-regulators of an evo-devo trait to their direct target genes through genetic, genomic, and biochemical approaches

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Gene Regulatory Networks (GRNs) control the orchestrated spatial and temporal patterns of gene expression that are responsible for trait development. The gain, modification, and deconstruction of GRNs logically must be major causes of trait evolution. Despite this perceived importance, the evolution of few if any traits are thoroughly understood at the scale of a GRN. This shortcoming has several causes. One is the difficulty of finding the breadth of GRN transcription factors and mapping these to their binding sites in *cis*-regulatory sequences of their downstream realizator genes. Another is the need for GRN studies to occur in experimentally tractable species for which closely related species exist that possess ancestral, modified, and secondarily lost phenotypes. One suitable model trait is the gain, modification, and loss of sexually dimorphic abdomen pigmentation in the lineage of *Drosophila melanogaster* and its close relatives. We will share updates from our genetic, genomic, and biochemical studies that are mapping the regulatory connections between the key transcription factors of a pigmentation GRN and their realizator genes that comprise a pigment metabolic pathway. Findings will also be shared from genome sequence comparisons that are teasing out how this GRN has evolved at the level of transcription factor binding sites. This work is supported by the NSF award IOS-2211833 to TMW.

Program Abstract #448

The regulation of the *Drosophila melanogaster pale* gene and its evolution during the origin of a dimorphic pigmentation trait

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Impressive outcomes of the genetic and molecular mechanisms of morphological evolution are the coordinated expressions of trait building, realizator, genes in the appropriate cell types and developmental stages. For each realizator gene, its pattern of expression is anticipated to result from the interaction of a combination of transcription factors to binding sites within one or more *cis*-regulatory element (CRE). A question that remains murky is to what extent these similar patterns of realizator expression require their CREs to be bound by similar combinations of transcription factors. One model trait that may shed light on this question is the sexually dimorphic pattern abdomen pigmentation that evolved in the lineage of *Drosophila* (*D.*) *melanogaster* fruit flies. This male-specific pattern of melanic

pigmentation on the posterior abdomen results from the spatial-, temporal-, and sex-specific deployment of a pathway of metabolic genes. While the CREs for several of these pathway genes have been well-studied, the first acting gene, known as *pale*, has received little attention. Here, we will share insights from studies on the regulation of the *D. melanogaster pale* gene and how this regulation and *pale* expression evolved during the origin and diversification of this pigmentation trait. This work is supported by the NSF award IOS-2211833 to TMW.

Program Abstract #449

Insights on the role of the Hr4 transcription factor during the development and evolution of a sexually dimorphic fruit fly pigmentation trait

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Animal morphological traits are patterned by Gene Regulatory Networks (GRNs) which include regulatory genes that pattern the expression of the trait-building realizator genes. The earliest- and most well-studied GRNs have shown how GRNs can include dozens or more transcription factor encoding genes. The study of GRNs has moved to "evo-devo" model traits in recent years, for which the identify and function of obvious candidate genes has more or less been resolved. These successes moved these models to a challenging state, where the remainder of their GRNs need to be characterized but the candidate genes have been exhausted. If trait evolution is going to be understood at the scale of GRNs, then a more complete characterization of these GRNs is an essential goal to be reached. One such evo-devo trait is the male-specific pattern of black pigmentation that develops on the posterior abdomen segments of *Drosophila melanogaster* and which evolved in the *Sophophora* subgenus of fruit flies. Several novel transcription factors have been identified whose loss-of-function perturbed pigmentation development. Here, we share our findings on the regulation, function, and evolution of the *Hormone receptor 4* (*Hr4*) transcription factor gene. This work highlights the potential and challenges to an expansive understanding of GRNs and their evolution. This work is supported by the NSF award IOS-2211833 to TMW.

Program Abstract #450

Changes in the mode of transcription factor action in the diversification of insect wings

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Insect wings are an intriguing subject to study due to their vast morphological and functional diversity. While changes in the wing gene network (WGN) likely facilitated the diversification in wing structure, detailed molecular mechanisms underlying the evolution and diversification of WGN remain elusive. *Ultrabithorax* (*Ubx*), a Hox gene involved in body plan patterning, has been identified as a central factor in producing a variety of diverse morphologies in insect wings. Ubx regulates the expression of various wing patterning genes, including *spalt*, a gene that plays a major role in determining the position of wing veins. Interestingly, Ubx promotes wing development by activating *spalt* expression in some insects such as *Tribolium castaneum* (red flour beetle), while inhibiting wing formation by repressing *spalt* in other insects such as *Drosophila melanogaster* (fruit fly). Furthermore, Ubx appears to be neutral to wing development in yet other insects such as *Apis mellifera* (honeybee). From these observations, we hypothesize that Ubx was ancestrally neutral to *spalt* expression, and changes in the mode of Ubx action as an activator or repressor facilitated the diversification of wing morphology between flies and beetles.Comparing how Ubx regulates wing enhancers of *spalt* between beetles and flies will evaluate this hypothesis and reveal how changes in the mode of transcription factor action contributed to the evolution and diversification of related structures among insect species. We will present our current attempt of utilizing cross-species reporter assays to identify the enhancer that controls the *spalt* wing expression in *Tribolium castaneum*. This project is funded by NSF IOS 0950964.

Program Abstract #451

A comprehensive examination of elytron mutants in the red flour beetle, *Tribolium castaneum*

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Insect wings are evolutionarily prominent structures that have sparked interest into their origins and

diversification. Even among winged insects, there are many different forms and functions of wings. In beetles, order Coleoptera, the second thoracic segment (T2) contains a pair of modified wings called elytra that act as a protective shield, while the third thoracic segment (T3) contains a pair of membranous flight wings. Investigation into how elytra evolved while hindwings maintained their relatively ancestral wing morphology will help further our understanding of the genetic basis underlying morphological evolution. We use *Tribolium castaneum*, the red flour beetle, as a model system to investigate the evolution and diversification of insect wings. *Tribolium* offers a fully sequenced and annotated genome, as well as well-established functional genomics and gene loss-of-functions techniques, including highly efficient RNA interference. Genetics studies can be classified into forward and reverse genetics: researchers start their investigation with a mutant(s) in the former, while with a gene(s) in the latter. With the advancement of sequencing technology, the general trend in genetics has shifted from forward to reverse genetics. However, due to its unbiased nature, traditional forward genetics can offer new insights into gene functions and interactions that have not previously been identified. We recently obtained eight previously undocumented mutant lines with obvious elytra abnormalities. We will present our detailed morphological analysis of these mutants and their epistatic relationships, which will help us gain further insight into the evolution and development of insect wings. This project is funded by the NSF IOS 0950964.

Program Abstract #452
Regulatory evolution at the locus-wide scale
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Transcriptional enhancers are essential genomic elements that control the time and location of gene expression. Changes in gene regulation are a major driver of evolution. While considerable attention has been paid to the function and evolution of individual enhancers, little is known about how an entire regulatory locus changes over evolutionary time. In diverged species, important regulatory genes are frequently conserved in terms of function and expression. However, when comparing the complete regulatory landscape of such conserved orthologs, many important questions remain unanswered, including: What is the balance between conserved and novel enhancers? Do individual enhancers maintain their locations and relative positions? Are functionally equivalent enhancers maintained by descent or generated by convergence? We have been addressing these questions through an analysis of the *single-minded (sim)* locus in several insects including *Drosophila melanogaster* and the mosquito *Aedes aegypti. sim* encodes a conserved transcription factor that mediates development of the arthropod embryonic ventral midline. Regulation of *D. melanogaster sim* has been explored in detail over many years. Recent work from our laboratory has investigated *A. aegypti sim* regulation, identifying two midline-specific enhancers that function equivalently in transgenic flies and mosquitoes. One *A. aegypti* enhancer is highly similar to known *Drosophila* enhancers in its activity, location, and autoregulatory capability. The other differs from any known *Drosophila sim* enhancers with a novel location, failure to autoregulate, and regulation of expression in a unique subset of midline cells. These results suggest that the conserved pattern of *sim* expression in the two species results from both conserved and novel regulatory sequences. We will report on our ongoing analysis of the *sim* locus in *D. melanogaster, A. aegypti*, and the scuttle fly, *Megaselia abdita*. Funded by NSF grant IOS-1911723.

Program Abstract #453
Species-specific rhythmic yolk contractions and their role in teleost gastrulation
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Biological processes during embryonic development and patterning are regulated by rhythms. One example is the rhythmical contractile waves reported in teleost species, such as goldfish (*C. auratus*), pike (*E. lucius*) and medaka (*O. latipes*). This movement is the first macroscopic rhythm in teleost development and it propagates across the periderm of the embryo from mid-epiboly on. In our study, we explore the function of yolk contractions during early development in various species within the *Oryzias genus*. During early stages of development *O. latipes* shows rhythmic yolk contractions, known as "Tsunami". Because these actomyosin contractions precede epiboly and embryo axis formation, we hypothesize that the Tsunami are necessary for gastrulation. To investigate this possibility we blocked rhythmic contractions at

different stages of development using the gap-junction uncoupling agent n-heptanol. Our study showed that blocking the Tsunami before stage 14 (20% epiboly), was sufficient to arrest epiboly progression and axis formation. We showed that developmental arrest was reversible up to 8-hour treatment, as embryo development resumed after n-heptanol removal. Interestingly, we were able to rescue both epiboly progression and axis formation in n-heptanol inhibited embryos by applying external rhythmic compressions: i.e. provided by a high-precision mechanical tester mimicking the Tsunami. We further analyzed different *Oryzias* species to determine the evolutionary conservation of the Tsunami. We confirmed that all *Oryzias* species undergo Tsunami at comparable developmental stages, however the frequency and force applied varied between species; suggesting species-specific adaptations within the *Oryzias genus*. By understanding the importance of the rhythmic yolk contractions, we hope to shed light on the fundamental role that mechanical forces play during gastrulation in teleosts. This work was supported by the Company of Biologists [Grant DEVTF23101253].

Program Abstract #454

Chondrichthyan epithelial appendages: developmental diversity of tooth-like structures in sharks

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Sharks and their cartilaginous relatives (rays, skates and chimaeras) offer an incredible diversity of tooth-like appendages, associated with both the oral apparatus and embedded in the skin as unique dermal units. This array of mineralized epithelial appendages offers further diversity in both the developmental and regenerative aspects of their biology. Importantly, modern sharks and their relatives occupy an important position within the vertebrate phylogenetic tree and possess a set of characters that might have existed at the dawn of dental evolution. Here we compare three characters of chondrichthyan dental diversity that offers a unique insight into the evolution of vertebrate teeth. We ask why such a conserved morphology "the tooth" can vary so widely in related chondrichthyan groups. We analyze the genetic and regenerative components of these elements to find both common developmental trajectories and novelty in these unconventional models of dental development. We also introduce a rare model in developmental biology, the chimaera (holocephalan), with a unique set of dental characters that reflects its evolutionary separation from the rest of chondrichthyan lineage. Our work defines the opportunities offered by the use of rare and unconventional models in developmental biology, which allows us to appreciate how these odd models develop away from the limits of standard models. Funding: National Science Foundation (NSF) IOS #2128032.

Program Abstract #455

***Cis*-regulatory evolution during neo-functionalization of a master sex determination gene**

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The differentiation into males and females is a fundamental process in sexually reproducing organisms. For such an important process, the genes that initiate sex determination are remarkably plastic, with over a dozen different master sex determining (MSD) genes having been identified thus far. Some of these genes have repeatedly become MSD genes. How do some genes repeatedly acquire the role of determining sex? How do MSD genes evolve and integrate with existing male and female differentiation pathways? To answer these questions, we are using stickleback fish, where anti-mullerian hormone (*amh*) has convergently evolved as the MSD gene in multiple species. The threespine stickleback fish (*Gasterosteus aculeatus*) has a Y-linked duplication ofa-mullerian hormone (*amhy*) that evolved approximately 20 million years ago. Functional genetic work in our lab has established that this duplicate copy of *amh* is both necessary and sufficient for male development. It remains unknown what regulatory mutations were necessary to allow amhy to neo-functionalize into a novel role as MSD gene. To characterize novel expression domains of *amhy*, I am using *in situ* hybridization to understand how the spatial and temporal expression of *amhy* differs from its ancestral paralog on the autosomes (*amh*). We have narrowed a 5 kb region upstream of *amhy* that is sufficient to drive *amhy* expression, inducing male development. This suggests there are enhancer elements within this region that are critical for neo-functionalization of *amhy*. Ongoing work will dissect this region further, using reporter constructs to see which subsections can drive GFP expression. Our work will provide insight into the role of *cis*-regulatory

evolution in how novel MSD genes can activate a conserved sex differentiation network. This work is supported by NIH R01GM147312.

Program Abstract #456

The function of β-catenin during development in the ctenophore *Mnemiopsis leidyi*

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β-catenin (β-cat) is a structurally and functionally highly conserved metazoan protein. In its role as a key nuclear signal transducer in the canonical Wnt (cWnt) pathway, β-cat regulates many developmental processes such as embryonic axis determination, and mesendodermal germ layer specification in many metazoans. Additionally, it is a critical regulator of cell-to-cell adhesion within the cadherin-catenin-complex in adherens junctions. The cytoplasmic availability of β-cat is tightly regulated by both the β-cat degradation complex (itself regulated by the cWnt pathway), and cadherins at the plasma membrane. Despite the importance of β-cat in all studied metazoans, no investigations have been done in the earliest lineage, the ctenophores. Genome sequencing in ctenophore species revealed that many components of both the Wnt signaling pathway and the cadherin-catenin-complex are conserved. Nevertheless, a previous bioinformatic study showed that *Mnemiopsis leidyi* cadherins don't possess a β-cat binding site, casting doubt on the conservation of the β-cat function in cell-to-cell adhesion in ctenophores. However, using a polyclonal antibody raised against *M. leidyi* β-cat (*Ml*-βcat), we show that *Ml*-βcat is localized at the cell-cell junctions during *M. leidyi* embryonic development, which suggests that the role of *Ml*-βcat in cell-to-cell adhesion is conserved. Moreover, to show a potential role in mesendodermal cell fate, we used the GSK-3 inhibitor CHIR99021, to up-regulate the cWnt pathway from one-cell to the juvenile stage. Treatments during early cleavage stages, but not post-gastrulation stages, revealed a disruption of the pharynx and an ectopic development of endodermal structures (e.g. endodermal canals). Further experiments are necessary to fully understand the ancestral β-cat functions in the ctenophore *M. leidyi*. This work was supported by the National Science Foundation, and the National Aeronautics and Space Administration.

AUTHOR INDEX

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