

Monday, May 2

8:30 AM Opening Remarks
Michael Layden and Erin Davies, meeting co-organizers
Michelle Starz-Gaiano, Mid-Atlantic SDB Representative

Session 1: Developmental signaling across scale and systems

Moderators: Michael Boylan (NCI), Katharine Goodwin (Princeton University)

8:40 AM - 9:05 AM **Sarah Siegrist** (UVA)
Time is Up! Regulation of neurogenesis during development

9:05 – 9:20 AM **Timothy Hufford** (UMBC)
Metabolic Adaptation Through the Lens of the Anoxic Transcriptional Signature

9:20 – 9:35 AM **Mallika Bhattacharya** (UMBC)
Steroid hormone signaling regulates protrusive activity and cell-cell adhesion to time collective cell migration

9:35 – 9:50 AM **Jaeho Yoon** (NCI)
The WERDS protein complex regulates apical constriction

9:50 – 10:05 AM **Nathan Lord** (University of Pittsburgh School of Medicine) - *VIRTUAL*
Co-receptor expression shapes the pattern of Nodal morphogen signaling

10:05 – 10:20 AM *Break*

Session 2: Potency regulation, cell cycle control, and regeneration

Moderators: Kayla Titillii-Torres (NCI), Devin Shennard (Lehigh University)

10:20 – 10:45 AM **Timothy Dubuc** (Swarthmore College)
Germ cell induction of the non-bilaterian stem cell model, *Hydractinia*.

10:45 – 11:00 AM **Akshay Patel** (University of Maryland College Park)
Cytonemes are required to generate and maintain asymmetric signaling and cellular organization in the *Drosophila* muscle progenitor niche

11:00 – 11:15 AM **Brian Stevens and Clover Stubbart** (NCI)
Embryonic establishment of lifelong regenerative abilities in the planarian *Schmidtea mediterranea*

11:15 – 11:30 AM **Jia Song** (University of Delaware)
Post-transcriptional regulation during mitosis

11:30 – 11:45 AM **Adriana Alexander** (NIEHS) - *VIRTUAL*
Sex-specific regulatory networks prime primordial germ cell fate commitment

11:45 -12:30 PM *Lunch break*

Session 3: Stem cell biology and disease modeling in developmental systems

Moderators: Xiaofei Bai (NIDDK), **Govind Kunduri** (NCI)

- 12:30 – 12:55 PM **Nykia Walker** (UMBC)
The Awakening of Dormant Breast Cancer Cells by Mesenchymal Stem Cell Stimulation
- 12:55 – 1:10 PM **Tunde Berecz** (NIDCR)
Induced pluripotent stem cell-based modeling of neural crest defects in DiGeorge syndrome
- 1:10 – 1:25 PM **Kerry Larkin** (NIDDK)
copa-1 mutants experience heightened endoplasmic reticulum stress sensitivity in a *C. elegans* COPA Syndrome model
- 1:25 – 1:40 PM **Katarzyna Hussey** (Johns Hopkins University)
Foveolar cone subtype patterning in human retinal organoids
- 1:40 – 1:55 PM **Megan Ezeude** (NICHD) - *VIRTUAL*
Dissecting the roles of Rock1 and Rock2 in zebrafish vascular integrity and development
- 1:55- 2:05 PM *Break*
- 2:05 – 3:00 PM **Education Session: Elena Hernandez-Ramon** (NIH OITE) - *VIRTUAL*
Successful strategies for managing mentor-mentee relationships
- 3:00 – 3:20 PM **Anna Allen** (Howard University)
Updates from the Developmental Systems Cluster at NSF
- 3:30 – 6:30 PM **Poster Session** (in-person only)
Odd number presentations: 3:30 - 4:30, 5:30 - 6:00
Even number presentations: 4:30 - 5:30, 6:00 - 6:30

Tuesday, May 3

Session 4: Epigenetic regulation and gene regulatory networks in development

Moderators: **Kalin Konrad** (University of Delaware), **Isaac Skromne** (University of Richmond)

- 8:30 AM - 8:55 AM **Mark Zander** (Rutgers University)
Transcription factor-mediated epigenome reprogramming in plant stress responses
- 8:55 – 9:10 AM **Miranda Marvel** (NICHD)
Analyzing the roles of Kdm4ab as a novel epigenetic regulator of arterial specification and development in zebrafish
- 9:10 – 9:25 AM **Austen Barnett** (DeSalle University)
Where Did Eye Go? Exploring the Developmental Basis of Eye Loss in the Eyeless Arachnid, *Archegozetes longisetosus*
- 9:25 – 9:40 AM **Katharine Goodwin** (Princeton University)
An evolutionary toolbox for sculpting the vertebrate airways
- 9:40 – 9:55 AM **Dina Navon (Rutgers University) - VIRTUAL**
Quantifying phenotypic plasticity in response to diet with zebrafish
- 9:55 – 10:10 AM *Break*

Session 5: Neural patterning, development, and behavior

Moderators: **Marina Venero Galanternik** (NICHD), **Carolyn Remsburg** (University of Delaware)

- 10:10 – 10:35 AM **Andrew Gordus** (Johns Hopkins University)
Untangling the web of behaviors used in spider orb-weaving
- 10:35 – 10:50 AM **Jessica Sidisky** (Lehigh University)
Genome-Wide analysis reveals novel regulators of synaptic maintenance
- 10:50 – 11:05 AM **Isaac Skromne** (University of Richmond)
A signaling switch in zebrafish underlies the induction and maintenance of the spinal cord specification and patterning regulatory gene *cdx4*
- 11:05 – 11:20 AM **Dylan Faltine-Gonzalez** (Lehigh University)
Bilaterian brain patterning mechanisms predate evolution of the bilaterian brain
- 11:20 – 11:35 AM **Christopher Arnold** (Stowers Institute for Medical Research)
The Hox gene *Post2b* controls organismal size in the planarian *Schmidtea mediterranea*
- 11:35 – 11:50 PM *Break*
- 11:50 – 12:45 PM **Keynote Address: Coleen Murphy** (Princeton University)
Transgenerational epigenetic inheritance of learned pathogen avoidance
- 12:45 – 1:00 PM Award Ceremony and Closing Remarks

Program Abstracts: Oral Presentations

SESSION ONE: Developmental signaling across scale and systems

Program Abstract #1: Time is Up! Regulation of neurogenesis during development

Sarah Siegrist

University of Virginia

Our brains are composed of an immense diversity of neuron types. By far, most of the neurons in our brains are generated during development from the cell divisions of a heterogeneous population of neural stem cells and progenitors. Molecular differences exist among neural stem cells based on tissue location and neural stem cells themselves change their gene expression profile over time to produce different types of neurons early versus late. In the Siegrist lab, we use the genetically tractable model organism *Drosophila melanogaster* to better understand the genes and cell signaling pathways that regulate neural stem cell proliferation decisions during development (quiescence versus proliferation and termination). From a targeted RNAi screen aimed at identifying genes required for neurogenesis termination, we identified components of the evolutionarily conserved cell-cell signaling pathway Notch and E93, a steroid hormone induced transcription factor. I will present our lab's recent work (published and unpublished) on the roles of Notch signaling and E93 in regulating timing of neurogenesis during development.

Program Abstract #2: Metabolic Adaptation Through the Lens of the Anoxic Transcriptional Signature

Timothy Hufford, Nguyet Le, Jong Park, Rachel Brewster

University of Maryland Baltimore County

While cells harness most cellular energy (ATP) through oxidative phosphorylation, hypoxia, a known environmental stressor, limits this means of ATP production and causes cellular and organismal demise. Many organisms, including zebrafish, have evolved adaptive responses to withstand prolonged periods of hypoxia, which typically involve metabolic suppression of energy-demanding processes (e.g. transcription) to preserve ATP. Identifying novel signaling molecules that mediate such responses can provide insight on therapeutic approaches to mitigate hypoxic injury that occur in illnesses like chronic kidney disease. Previously, our lab has shown that N-Myc downstream regulated gene 1a (Ndr1a) is regulated and functions adaptively in response to hypoxia. Here, we performed an RNA-seq analysis to screen for genes differentially expressed in hypoxia (3% O₂) and anoxia (0% O₂). We hypothesized that hypoxia would result in widespread changes of metabolic genes implicated in glycolysis and oxidative phosphorylation, and that anoxia would result in increased expression of fewer genes essential for survival. Surprisingly, anoxia resulted in differential expression of over 2800 genes, only 87 of which were shared with hypoxia. Among the anoxia-induced genes were known hypoxia responsive genes, such as *ndrg1* and *hif3*, in addition to novel candidates such as cell cycle regulators and circadian genes. We also observed that anoxia resulted in a slight increase of intronic reads. Hypoxia-induced intron retention has been reported in the literature as a result of differential splicing. These findings suggest that metabolic suppression is an active rather than passive response that requires complex gene regulation. The work presented is funded by the Department of Defense (W81XWH-16-1-0466), National Institute of Health/NICHD (R21HD089476), National Institute of Health /NIGMS MARC U*STAR (T34 HHS 00001), CBI (NIGMS/NIH T32 GM066706), and IMSD (NIGMS/NIH T32 GM055036).

Program Abstract #3: Steroid hormone signaling regulates protrusive activity and cell-cell adhesion to time collective cell migration

Mallika Bhattacharya, Michelle Starz-Gaiano

University of Maryland Baltimore County

Cell migration plays a significant role in spatiotemporally-regulated physiological processes and its dysregulation has severe implications in birth defects and diseases such as cancer. The migratory border cell cluster is a genetically tractable model to investigate collective cell migration in vivo. Within the egg chamber, the reproductive unit of *Drosophila*, the cluster arises from the follicular epithelium and migrates in a temporally-regulated manner. Developmental timing in flies is largely controlled by the sole steroid hormone ecdysone, which complexes with a heterodimeric receptor to regulate transcription. Our work seeks to understand how signaling by the ecdysone receptor (EcR), a conserved nuclear hormone receptor, affects border cell migration. Downregulation of ecdysone signaling results in delayed migration due to late detachment from the epithelium and slow migration. The directional translocation of the cluster requires the extension of actin-rich protrusions that adhere to other cells in the migratory path. Live video analysis revealed that clusters expressing transcriptionally-inactive EcR extend fewer functional protrusions. Protrusion stabilization, cluster integrity, and migration require optimal distribution of the adhesion molecule, E-cadherin. Immunofluorescence characterization of E-cadherin showed abnormal levels and localization in border cells with reduced ecdysone signaling. Polarity of these clusters is also disrupted as assessed by Discs large localization. Lastly, transcriptionally-inactive EcR clusters have mislocalized activated myosin which normally promotes protrusion retraction and cluster motility. We propose that steroid hormone signaling coordinates cluster polarity with cytoskeletal dynamics within migratory cells to modulate detachment and directional protrusion extension. This novel role of steroid signaling will be useful to understanding nuclear hormone receptors in migratory contexts. Funding-NSF grant IOS-1656550.

Program Abstract #4: The WERDS protein complex regulates apical constriction

Jaeho Yoon, Ira O. Daar

National Cancer Institute Center for Cancer Research

Neurulation is the process of forming a neural tube from a neural plate. In the neurula embryo, both lateral sides of the neural plate fold toward the midline of the embryo and then eventually the two edges merge to make the neural tube. This process is essential for the embryonic development of the brain, spinal cord, and neural crest and is well conserved in fish, amphibians, birds, and mammals. During neural tube closure, the neuroepithelial sheet undergoes two major tissue morphogenesis: 1) Anteroposterior axis elongation by polarized cell division and mediolateral cell intercalation 2) Neural plate folding by apical constriction. Apical constriction is a polarized cell shape change along the apical-basal axis inducing tissue remodeling in many developmental processes such as neural tube closure. Contraction of the F-actin and the molecular motor Myosin Light Chain are key components driving the apical constriction. Shroom3 accumulates on the apical side of the epithelial cell layer and generates mechanical forces by producing contractile F-actin. Previously, we showed that ephrinB2 is necessary for neural tube closure. The loss of ephrinB2 caused apical constriction defects resulting in the incomplete neural tube closure. However, the molecular mechanism of how ephrinB2 regulates apical constriction was poorly understood. To study the role of ephrinB2 during apical constriction, we took advantage of an excellent model system, *Xenopus* embryos. *Xenopus* embryos are large, allowing easy surgical manipulation, external growth, and accessibility for live-cell imaging. We found a novel protein complex, consisting of Wnt4, ephrinB2, Ror2, Dsh2, and Shroom3 (WERDS). Loss- and gain-of-function analysis demonstrated that the WERDS signaling complex regulates contractile actin-myosin network formation during apical constriction. Our data also suggests that ephrinB2 modulates the conformational change of Dsh2 to facilitate the formation of the WERDS signaling complex.

Program Abstract #5: Co-receptor expression shapes the pattern of Nodal morphogen signaling

Nathan Lord, Adam Carte, Philip Abitua, Alexander Schier

University of Pittsburgh School of Medicine

Embryos often communicate instructions to their constituent cells using diffusible signaling molecules called morphogens. In textbook models, morphogens diffuse from a localized source to form a concentration gradient, and target cells select fates by measuring the local morphogen concentration. However, natural patterning systems often incorporate numerous co-factors and extensive signaling feedback, suggesting

that embryos require additional control to generate reliable patterns. This talk will present a recent study that illuminated how these additional regulatory features enable the formation of precise, robust patterns by the model morphogen Nodal during zebrafish embryogenesis. We found that Nodal signaling activity spans a normal range in the absence of signaling feedback, suggesting that diffusion is sufficient for Nodal gradient formation. We further showed that the range of endogenous Nodal ligands is set by the EGF-CFC co-receptor Oep: in the absence of Oep, Nodal activity spreads to form a nearly uniform distribution throughout the embryo. We recapitulate these experimental results with a computational model in which Oep regulates the diffusive spread of Nodal ligands by setting the rate of capture by target cells. Collectively, these results reveal that patterns of Nodal morphogen signaling are shaped by co-receptor-mediated restriction of ligand spread and sensitization of responding cells. Funding Sources: Arnold O. Beckman Foundation NIH NICHD.

SESSION 2: Potency regulation, cell cycle control, and regeneration

Program Abstract #6: Germ cell induction of the non-bilaterian stem cell model, *Hydractinia*.

Timothy Dubuc

Swarthmore College

Germ cells are undoubtedly the most important cells present in the body of an animal. Despite differences in developmental timing, animal germ cells share the unique biological connection that brings together the past, present and future of an individual. The genes that govern bilaterian germ cell induction are often found to be expressed in both multipotent stem cells and germ cells of non-bilaterians. Using RNA-seq, tissue-specific transcriptomics and CRISPR/Cas9 editing, we show that the transcription factor *Tfap2* regulates germ cell induction of the cnidarian, *Hydractinia*. Similar to mammalian germ cell induction, *Tfap2* is expressed during sexual development in a multipotent stem cell and induces this cell to migrate into the gonadal environment to begin the process of gametogenesis. *Tfap2*^{-/-} mutants are sterile but can be rescued through allogeneic transplantation. *Tfap2* is both necessary for germ cell induction and can sufficiently induce gamete formation when expressed in a stem cell context. These results suggest a conserved genetic pathway governs germ cell induction in diverse animal lineages. Ultimately, nature plays a part to facilitate genetic changes that allow for reproductive success. *Hydractinia* sexual reproduction is induced roughly 1-3 months after larval metamorphosis. Prior to this time, the animal grows exclusively through asexual reproduction. Using sterile mutants and transgenic reporters, we characterize how substrate shape and sterility influence animal patterning and reproduction. Together, these results help unite nature and the underlying genetics governing sexual reproduction.

Program Abstract #7: Cytonemes are required to generate and maintain asymmetric signaling and cellular organization in the *Drosophila* muscle progenitor niche

Akshay Patel, Yicong Wu, Xiaofei Han, Yijun Su, Tim Maugel, Hari Shroff, Sougata Roy

University of Maryland College Park

Tissue development and homeostasis rely on the ability of stem cells to efficiently balance between self-renewal and differentiation. Stem cell fate decisions are made in the context of the niche and are governed by asymmetric signaling and organization in the niche. Niche cells present self-renewal growth factors selectively only to stem cells, but not to their neighboring non-stem cell daughters, located just one cell diameter away. Understanding how this signaling asymmetry arises is critical to understand how stem cells determine their identity and prime differentiation in an organized pattern to generate/regenerate tissues. We investigated the basis of asymmetric signaling and cell organization using the *Drosophila* wing disc that creates an adult muscle progenitor (AMP) niche. We discovered that AMPs extend polarized actin-based filopodia, named cytonemes, to contact the wing-disc epithelial junctions and physically adhere themselves to the niche. AMP cytonemes localize FGF-receptor to selectively adhere to the FGF-producing niche and receive FGFs in a contact-dependent manner. Activation of FGF signaling in AMPs, in turn, reinforces the niche-specific cytoneme polarity/adhesion to maintain niche occupancy. We showed that this

interdependence between the cause and effect of cytoneme-mediated polarized signaling can self-organize diverse niche-specific asymmetric patterns. Furthermore, the extrinsic localized expression and restricted presentation patterns of FGFs in the wing-disc niche determine discrete AMP organization and diversification. Loss of cytoneme adhesion promotes AMPs to lose niche occupancy and FGF signaling, occupy a disc-distal position, and acquire morphological hallmarks of differentiation. These findings provide new insights into how contact-mediated polarized FGF signaling can play critical roles in generating and maintaining diverse niche-specific asymmetric signaling and organizations. Funding source: NIH R35GM124878 to SR.

Program Abstract #8: Embryonic establishment of lifelong regenerative abilities in the planarian *Schmidtea mediterranea*

Brian Stevens¹, Clover Stubbett¹, Alejandro Sánchez Alvarado², Erin L Davies^{1,2}

¹National Cancer Institute Center for Cancer Research, ²Stowers Institute for Medical Research.

Regeneration capacity is unlimited and position-independent in the planarian *Schmidtea mediterranea* (*Smed*): adult tissue fragments cut at any position along the anteroposterior (AP) axis reform complete individuals. We investigated the embryonic origin of whole-body regenerative abilities and show that regeneration competence emerges gradually in directly developing *Smed* embryos. Amputation assays demonstrated stage- and position-dependent effects on head regeneration. We are determining how the amputation response differs in regeneration-incompetent versus regeneration-competent *Smed* embryos. Though regeneration requires stem cells, the cellular source of new tissues, our results suggest that neither stem cell specification, proliferation, nor potency are rate-limiting for establishment of regenerative abilities. We report that regeneration-incompetent embryos showed a reduction or complete absence of expression of ERK-dependent, wound-induced transcripts previously shown to be expressed within the body wall musculature of adult regenerating fragments. Defects in the transcriptional wound response during regeneration initiation presage the failure of AP axis re-establishment in regeneration-incompetent posterior fragments. Our data suggest that AP axis resetting via Wnt pathway inhibition, a process likely linked to body wall musculature development, is rate-limiting for the acquisition of regenerative abilities during embryogenesis. Consistent with this hypothesis, knockdown of the Wnt pathway effector *β-catenin-1* induces precocious head formation in regeneration-incompetent embryos. Our results may suggest strategies for reconstituting self-organizing regenerative responses in vitro, and for reverse-engineering regeneration in systems maintained by adult stem cells. This Research was supported by the Center for Cancer Research, National Cancer Institute, National Institutes of Health Intramural Research Program project number ZIABC0120009.

Program Abstract #9: Post-transcriptional regulation during mitosis

Jia Song

University of Delaware

Translation is under tight spatial and temporal controls to ensure protein production at the right place and time in the embryo. The project goal is to examine how post-transcriptional regulation mediated by microRNAs (miRNAs) controls local translation to fine-tune mitosis. Results indicate that miR-31 and some of its target transcripts have cell cycle-dependent dynamic distribution: they are in the perinuclear region of non-dividing cells, and they are enriched on the mitotic spindles in dividing cells of sea urchin embryos. This novel and striking oscillating localization of miR-31 and its targets suggests that miR-31 and its RNA targets may regulate spindle assembly and/or cell cycle progression. The mitotic spindle is a highly dynamic structure consists of a large complex of microtubules and proteins that is regulated by actin and a complex set of proteins and RNAs. miR-31 inhibition results in defects in early developmental progression as well as cytoskeletal defects in cleavage stage embryos. In addition, miR-31 directly suppresses several cytoskeletal modulators, including Fascin, Rab35, and Gelsolin. miR-31 also regulates the translation of its target Fascin, which encodes an actin bundling protein that is capable of regulating microtubules. These results lead to the overarching hypothesis that miR-31 regulates local translation of its targets on mitotic spindles to

control cytoskeletal dynamics and impact mitosis. Our results will reveal for the first time a mechanistic understanding of the evolutionarily conserved regulatory role of miR-31 during mitosis. Discovering regulation of critical cytoskeletal components that mediate the segregation of chromosomes will be critical for understanding early cell division, genomic stability, and birth defects. This work is funded by the NSF Career Award #IOS-1553338 and MCB 2103453 to JLS; NIH/P20GM103653; NIH/NIGMS P20GM103446.

Program Abstract #10: Sex-specific regulatory networks prime primordial germ cell fate commitment

Adriana Alexander, Karina Rodriguez, Humphrey Yao
National Institute of Environmental Health Sciences

Male and female germ cells carry the hereditary information required for the generation of a new individual. Mature gametes share few morphological features yet develop from a common cellular origin, the primordial germ cell (PGC). PGCs are the bipotential stem cells of the germline that transit from pluripotency in early embryonic development to become highly specialized cell types, egg and sperm, in adulthood. Specification of PGCs is accompanied by extensive epigenetic modifications, including induction of germ cell genes, repression of the somatic program, and global changes in DNA methylation. PGC sex-determination commences in response to cues from the gonadal environment, during which PGCs commit to either a male or female lineage, characterized by mitotic arrest or meiotic entry, respectively. To establish the mechanisms that govern PGC sex-determination, we developed the first single-cell multi-omics map of chromatin accessibility and gene expression during murine PGC development in both male and female embryos. We resolved previously unreported PGC subpopulations and cataloged a multimodal reference atlas of differentiating PGCs. We discovered that regulatory element accessibility precedes gene expression during PGC reprogramming, suggesting that changes in chromatin accessibility may prime PGC lineage commitment prior to differentiation. Further, we mapped the cascade of transcription factors that regulate expression of sexually dimorphic genes in male and female PGCs. We also found that the timing of transcription factor expression is induced by receptor-ligand signaling events between PGCs and the somatic cells of the gonad. In summary, these results illustrate the temporal programming of sex-specific regulatory modules leading to PGC cell fate commitment. These data are an invaluable resource for the discovery of novel regulatory networks coordinating PGC differentiation and ensuring reproductive health. Funded by Intramural Research Program of NIH.

Session 3: Stem cell biology and disease modeling in developmental systems

Program Abstract #11: The Awakening of Dormant Breast Cancer Cells by Mesenchymal Stem Cell Stimulation

Nykia Walker
University of Maryland Baltimore County

Breast cancer malignancy is the second leading cause of death among women. Clinical evidence supports the notation that breast cancer cells can persist in the bone marrow for more than ten years when in a quiescent state. In the bone marrow, breast cancer stem cells (**B-CSCs**) are identified as a significant subset of cancer cells that are chemoresistance, can thrive in a low oxygen microenvironment, and undergo cycling quiescence. Methods by which B-CSCs adapt dormancy and reverse dormancy into metastatic cells are highly significant for developing new and safe therapies and is a major focus in our laboratory. Since the BM endosteum is one of the preferred sites for dormant B-CSCs, we focus a substantial amount of our research on this organ. Additionally, the bone marrow is a secondary site where breast cancer cells are associated with clinical relapse. Our lab has undertaken research efforts to develop strategies that can prevent or reverse cellular dormancy. Mesenchymal stem cells (**MSCs**) can recognize pathogens or danger signals, enabling them to communicate with immune cells to mount an immune response. Based on the chemical signals retrieved, MSCs can also suppress immune cell function allowing these cells to have profound immunomodulatory capabilities that aid in eliciting an immune response or aid in tissue repair. We

took advantage of this property to influence macrophage (MF) polarization. MF are classified into two subsets, either M1 or M2 type. M1 subtype are mostly pro-inflammatory (classically activated to kill microorganisms and tumor cells), while M2 macrophage are non-inflammatory (alternatively activated to promote tissue repair). Alongside MSCs, M2 MF are cells within the bone marrow stroma that supports the hematopoietic microenvironment.

We studied how activated MSCs influenced M1 or M2 polarization by co-culturing activated MSCs with either M1 or M2 MF and observed phenotypic changes in MF protein expression, resulting in the conversion of M2 MF into M1 type. To investigate whether M2 converted MF could influence CSC resurgence; we co-cultured M1 or M2 macrophages with Oct4^{hi} breast cancer cells that forced into cycling quiescence and examined changes in cell proliferation based on M1 or M2 converted macrophage influence. Breast cancer cells switched from G1/G0 into the S phase, after interactions with M2 converted to M1 MF. While B-CSCs exposed to M2 MF alone without MSC stimulation stayed in G1/G0 cycling phase. Suggesting that activated MSCs are involved in promoting M2 into M1 MF polarization whereas inactivated MSCs maintains M2 MF phenotype. In subsequent in-vivo experiments, M1 and M2 MF were labeled with a tracking dye and injected intravenously into immunodeficient mice. We observed that MFs home to the same area as the dormant B-CSCs. Once inside the endosteum, M2 MF were detected near the B-CSCs, while M1 MF were observed along the periphery and within the bone.

Program Abstract #12: Induced pluripotent stem cell-based modeling of neural crest defects in DiGeorge syndrome

Tunde Berecz, Agota Apati, Laura Kerosuo

National Institute of Dental and Craniofacial Development

Human disease models are crucial for understanding species-specific mechanisms, particularly if no appropriate animal models or human tissue samples are available. In a human induced pluripotent stem cell (hiPSC)-based in vitro system, we model DiGeorge syndrome, a congenital developmental disorder that affects the cardiovascular, nervous and immune systems, as well as the craniofacial skeleton. DiGeorge syndrome is caused by a heterozygous microdeletion on 22q11.2 chromosome region spanning approximately 100 genes. Surprisingly, despite the same genetic deletion, DiGeorge syndrome patients manifest a diverse range of symptoms from mild to severe. Many of the symptoms are caused by defects in neural crest derived tissues. The neural crest is a transient stem cell population that rises from the neural plate border and migrates to various locations in the developing embryo, contributing to the formation of multiple tissues including bones and cartilage of the face, mesenchyme of the thymus and the cardiac outflow tract. The family involved in our study consists of three patients manifesting the disease with different severity and two healthy controls, giving us the unique opportunity to study intrafamilial variability. We established hiPSC lines from all family members and differentiated them into neural crest cells (hiPSC-NCCs). Our preliminary results indicate the patient cells have an impaired capacity to form neural crest already at the premigratory stage. Furthermore, ongoing investigations address whether also migration is impacted, and if the DiGeorge patient cells can properly differentiate into chondrocytes, osteoblasts and vascular smooth muscle cells. Our research gives insight into the cellular and molecular mechanisms underlying DiGeorge syndrome and helps understand how these are linked to the severity of the disease. Funded by NIH DE000748-04, OTKA- K128369, NVKP_16-1-2016-0017, NAP 2017-1.2.1-NKP-2017-00002 and KTIA_NAP_13-2014-0011.

Program Abstract #13: *copa-1* mutants experience heightened endoplasmic reticulum stress sensitivity in a *C. elegans* COPA Syndrome model

Kerry Larkin, Isabella Zafra, Andy Golden

National Institute of Diabetes and Digestive and Kidney Diseases

COPA Syndrome is a rare autoimmune and autoinflammatory disease in which patients' immune cells erroneously attack their lungs, joints, and/or kidneys. The symptoms are caused by mutations in *COPA*, which encodes the alpha subunit of the COPI complex. COPI coats vesicles transporting protein cargo in retrograde from the Golgi apparatus to the endoplasmic reticulum (ER), and COPA Syndrome mutations prohibit COPI from properly identifying and binding cargo for transport. Like most rare diseases, treatments for COPA Syndrome remain limited due to a lack of understanding of the genetic mutations' molecular consequences. We aim to model the disease in *C. elegans* to further characterize the molecular mechanisms of the disease and identify novel genetic pathways affected by mutant *COPA*. Using CRISPR/Cas9 genome editing, we recreated patient alleles in the worm's orthologous gene to *COPA*, *copa-1*, and set out to characterize the resulting phenotypes. While the mutants appeared superficially wild-type, we hypothesized that they were experiencing heightened ER stress, as is observed in COPA Syndrome patients' cells. We showed that the mutant worms are more susceptible to ER stress, as demonstrated by a developmental delay when exposed to Tunicamycin, a drug that increases ER stress, compared to wild-type worms. This delay is accompanied by an increase in the ER Unfolded Protein Response (UPRER), as indicated by increased HSP-4::GFP expression, a marker turned on by a major branch of the UPRER in response to ER stress. Utilizing qRT-PCR, we are further characterizing which downstream pathways of the UPRER are affected in *copa-1* mutants. Ultimately, we aim to identify genetic mechanisms implicated in the *copa-1* mutants, and through this knowledge, potentially inspire novel treatment targets for COPA Syndrome patients. This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

Program Abstract #14: Foveolar cone subtype patterning in human retinal organoids

Katarzyna Hussey, Kiara Eldred, Clayton Santiago, Seth Blackshaw, Robert Johnston
Johns Hopkins University

The mechanisms that generate patterns of cell types unique to humans are poorly understood. In the central region of the human retina, the high-acuity foveola is notable for its dense packing of green (M) and red (L) cones and lack of blue (S) cones. To determine mechanisms that promote M/L and repress S cone patterning in the foveola, we examined human fetal retinas and differentiated human retinal organoids. During fetal development, the foveola initially contains a sparse population of cones that express S-opsin and later, a mix of cones that either co-express S-opsin and M-/L-opsin or express M-/L-opsin only. Two signaling pathway regulators are highly and continuously expressed in the central retina: *CYP26A1* that degrades retinoic acid (RA) and *deiodinase2* (*DIO2*) that promotes thyroid hormone signaling. *CYP26A1* null mutant organoids and high RA conditions independently increased S cones and suppressed M/L cones in human retinal organoids. Sustained thyroid hormone signaling promoted the generation of M/L-opsin expressing cones and induced M-/L-opsin expression in S cones in human retinal organoids, suggesting that cone subtype fate is plastic during development. Our data suggest that *CYP26A1* degrades RA to specify M/L cones and limit S cones and that continuous *DIO2* expression sustains high levels of thyroid hormone to convert S cones into M/L cones, yielding the cone subtype patterning of the foveola. As the foveola is highly susceptible to impairment in diseases such as macular degeneration, a leading cause of vision loss, our findings inform organoid design for potential therapeutic applications. This work was funded by NSF DGE-1746891 and National Eye Institute R01EY030872.

Program Abstract #15: Dissecting the roles of Rock1 and Rock2 in zebrafish vascular integrity and development

Megan Ezeude, Laura P. Pillay, Keith A. Barnes, Madeleine I. Kenton, Jong S. Park, Brant M. Weinstein
Eunice Kennedy Shriver National Institute of Child Health and Human Development

The small monomeric GTPase RHOA acts as a molecular switch, transducing upstream signals by activating effectors of cellular signaling, including the Rho-associated protein kinases ROCK1/2. RHOA/ROCK activity is elevated in hemorrhagic stroke-prone human patients with congenital vascular

cerebral cavernous malformations, and ROCK1 and ROCK2 are overexpressed in benign and malignant vascular tumors, sparking a substantial interest in ROCK1/2 as potential targets for vascular therapies. However, there is a lack of in vivo studies describing how ROCK1/2 regulate vascular integrity and patterning under normal developmental and physiological conditions. ROCK1 and ROCK2 share 92% amino acid identity within their kinase domains, but only 62% amino acid identity overall, and in vitro cell culture studies have identified unique functions and targets for ROCK 1 and ROCK2 in certain cellular and environmental contexts. ROCK1 and ROCK2 may therefore serve distinct functional roles in vascular development and disease. To determine how ROCK1/2 influence vascular development and integrity in vivo we treated zebrafish embryos with a pharmacological inhibitor of Rock1/2 kinase activity and used high-efficiency dgRNP-based CRISPR/Cas9 to distinguish between combined and individual roles of Rock1 and Rock2. Our results suggest that loss of Rock activity produces hemorrhage and cranial angiogenesis defects in developing zebrafish and that ROCK1 and ROCK2 may make distinct contributions in regulating vascular integrity and development in vivo. This work is supported by the Intramural Program of the NICHD (to BMW).

Session 4: Epigenetic regulation and gene regulatory networks in development

Program Abstract #16: Transcription factor-mediated epigenome reprogramming in plant stress responses

Mark Zander, Björn C. Willige, Chan Yul Yoo, Amy Phan, Yupeng He, Meng Chen, Joseph R. Ecker, Joanne Chory
Rutgers University

The pivotal interplay between light receptors and PHYTOCHROME INTERACTING FACTORS (PIFs) serves as an essential regulatory hub that perceives and integrates environmental cues into the plant's transcriptional networks. Although occupancy of the histone variant H2A.Z and acetylation of histone 3 emerged as critical regulators of environmentally-responsive gene networks, how these epigenomic features interface with PIF transcription factor activity is only poorly understood. Here, by taking advantage of the rapid and reversible light-mediated manipulation of PIF7 subnuclear localization and phosphorylation, we simultaneously assay both DNA binding properties of PIF7 as well as its impact on chromatin dynamics genome-wide. By these means, we found that PIFs act rapidly to reshape the H2A.Z and H3K9ac epigenetic landscape in response to a change in light quality. Furthermore, we discovered that PIFs achieve H2A.Z eviction through the direct interaction with EIN6 ENHANCER (EEN), the *Arabidopsis thaliana* homolog of the INO80 complex subunit INO80 Subunit 6 (les6). Thus, we describe a PIF-INO80 regulatory module that is a key intermediate step for allowing plants to change their growth trajectory in response to environmental changes.

Program Abstract #17: Analyzing the roles of Kdm4ab as a novel epigenetic regulator of arterial specification and development in zebrafish

Miranda Marvel, Jordyn Greenbaum, Kiyohito Taimatsu, Daniel Castranova, Andrew Davis, Aniket V. Gore, Brant M. Weinstein
Eunice Kennedy Shriver National Institute of Child Health and Human Development

Epigenetic mechanisms such as DNA and histone methylation play crucial roles in gene regulation and in cell and tissue differentiation during development, but most vertebrate tissue-specific epigenetic regulators are still unknown. Our lab recently developed a novel “EpiTag” transgenic epigenetic reporter zebrafish line that reliably reports tissue-specific epigenetic silencing and activation via the expression of a destabilized green fluorescent protein (GFPd2). Using this line, we performed the first ever large-scale vertebrate forward genetic screen for epigenetic-related mutants and identified a mutant in an uncharacterized histone demethylase, Kdm4ab, with defects in vascular sprouting and arterial development. Transcriptomic analysis of mutants identified a number of dysregulated angiogenic and arterial-specific genes, with many

arterial-related genes exhibiting downregulation and venous-related genes showing an unexpected upregulation in mutant tissues. Further *in situ* experiments revealed that these upregulated venous genes are ectopically expressed in arterial vessels of *kdm4ab* mutants. Additional analysis of histone methylation abundance on genetic loci of *kdm4ab* mutants via a novel CUT&RUN (ChIP-Seq) approach revealed many vascular-related genes also have aberrantly increased H3K9me3 abundance, signifying they may be targets of Kdm4ab demethylase activity during development. Our preliminary findings suggest that Kdm4ab is a novel critical epigenetic regulator of arterial specification and development, and we plan to further characterize the mechanisms of action and cell-autonomous roles of Kdm4ab in the future. This work is supported by the Intramural Program of the NICHD (to BMW).

Program Abstract #18: Where Did the Eye Go? Exploring the Developmental Basis of Eye Loss in the Eyeless Arachnid, *Archezogozetes longisetosus*

Austen Barnett

DeSalle University

The arthropod eye is an intensely studied organ in both developmental and evolutionary studies. Paleontological and neontological data suggest that the last common ancestor of extant arachnids possessed at least three pairs of eyes. This character state is relatively stable and exemplified in modern spiders. Non-arachnid arthropods possess a myriad of diverse visual systems. However, underlying this diversity is the observation that most studied arthropods utilize a conserved set of genes, termed the retinal determination genes (RDGs), to pattern their eyes during embryonic development. Eye loss is common amongst animals in cave or soil ecosystems. The mite *Archezogozetes longisetosus* is a tropical, soil dwelling arachnid that has second lost its eyes. Paradoxically, the recent sequencing of the genome of *A. longisetosus* has revealed that it has retained orthologs of the retinal determination genes. In an effort to interrogate the possible functions of these RDGs during embryonic development, we performed RNAseq on multiple life-stages as well as performed embryonic *in situ* hybridizations on *A. longisetosus* embryos. Our RNAseq results suggest that most of the RDGs are expressed during embryogenesis. To tie these expression data to positional information, we performed *in situ* hybridizations on of these RDGs on *A. longisetosus* embryos. These *in situ*s suggest that the RDGs are expressed in the ocular segment during embryogenesis in a manner comparable to other studied arachnids. However, we found that a subset of these genes are expressed in the developing appendages and the abdomen/opisthosoma, an observation thus far not reported for an arachnid. Our data suggest that 1) the repression of eyes likely occurs downstream of the deployment of the RDGs in *A. longisetosus*, and that 2) the RDGs are expressed in heterotopic regions in comparison to other arachnids. These data offer an explanation of how genes can be retained in a genome following the loss of the structure they originally specified. Funding for this study was provided by the Warren and Berg Endowments of DeSales University.

Program Abstract #19: An evolutionary toolbox for sculpting the vertebrate airways

Katharine Goodwin, Michael A. Palmer, Celeste M. Nelson

Princeton University

Evolution has produced a beautiful diversity of branched architectures in the lungs of terrestrial vertebrates, from the sac-like lungs of reptiles, to the looped airways of birds, to the tree-like lungs of mammals. The cellular and physical mechanisms that drive branching morphogenesis to produce airway epithelia of such different shapes are still being uncovered. During lung development in the anole, chick, and mouse, airway smooth muscle plays stage-dependent roles in shaping the epithelium. We hypothesized that smooth muscle sculpting of epithelia is a conserved morphogenetic tool that is repurposed in each species to achieve distinct airway morphologies. To investigate differences in airway smooth muscle patterning and function across terrestrial vertebrates, we generated scRNA-seq datasets from whole lungs of mouse, anole, and chicken embryos. Expression patterns of smooth muscle regulators are similar in mouse and anole lungs, which branch via smooth muscle sculpting, but not in chick lungs, which undergo

epithelial-driven branching. We found that smooth muscle arises from the mesenchyme in anole and mouse, but bioinformatic and experimental evidence point toward an alternate origin for airway smooth muscle in chick. Patterning of the pulmonary mesenchyme is tailored towards the specific needs of each species. For example, the highly contractile smooth muscle of the anole is densely innervated, and accordingly, its mesenchyme harbors cells that support nerve development. Our work sheds light on the diverse strategies used by multicellular organisms to generate complex tissue architecture, and especially how these feats of engineering are accomplished via cooperation between different tissue types. Funding: NSERC postgraduate scholarship; CFUW pre-doctoral fellowship; AHA predoctoral award; HHMI Faculty Scholar's Award; NIH/NICHD R01 HD0990300

Program Abstract #20: Quantifying phenotypic plasticity in response to diet with zebrafish

Dina Navon, Craig Albertson, Tetsuya Nakamura

Rutgers University

Phenotypic plasticity is the ability of an individual organism to transiently change its phenotype over the course of development in response to changes in the environment. While some studies, including ours, are beginning to untangle the developmental genetic mechanisms that facilitate plasticity in fish, the signaling cascades that convert environmental cues to phenotypic changes, and the extent to which these mechanisms are conserved across taxa, remain largely elusive. Many fish taxa exhibit evolution and plasticity of the craniofacial, fin, and body shape in response to different diets. Previously, we demonstrated that African cichlid fish show plasticity in all three suites of traits, and further identified *ptch1*, a key member of the Hedgehog signaling pathway, and *crocc2*, a ciliary gene, as putative candidate genes underlying cichlid craniofacial plasticity. To explore how these signaling pathways function and whether those functions have been conserved and or have diverged among teleost fish, we utilized zebrafish as a complementary model. We found that zebrafish can exhibit plasticity in response to diet, and that the morphological plastic changes to craniofacial, fin, and body shape across diets are strikingly similar to the patterns of plasticity we see in cichlids. Thus, these specific patterns of plasticity in response to diet may be a shared ancestral feature among teleost fish. Future genomic research on zebrafish and cichlid plasticity will reveal the degree to which this shared morphological response is maintained by a shared or a distinct genetic basis. Our funding sources include the NSF & NIH.

Session 5: Neural patterning, development, and behavior

Program Abstract #21: Untangling the web of behaviors used in spider orb-weaving

Andrew Gordus

Johns Hopkins University

Many innate behaviors are the result of multiple sensorimotor programs that are dynamically coordinated to produce higher-order behaviors such as courtship or architecture. Extended phenotypes such as architecture are especially useful for ethological study because the structure itself is a physical record of behavioral intent. A particularly elegant and easily quantifiable structure is the spider orb-web. The geometric symmetry and regularity of these webs have long generated interest in their behavioral origin. However, quantitative analyses of this behavior have been sparse due to the difficulty of recording web-making in real-time. To address this, we have developed a novel assay enabling high-resolution tracking of limb movements and web structure produced by the hackled orb-weaver *Uloborus diversus*. With a brain the size of a fly's, the spider *U. diversus* offers a tractable organism for the study of complex behaviors. Using machine vision algorithms for limb tracking, and unsupervised behavioral clustering methods, we have developed an atlas of stereotyped movements used in orb-web construction. The rules for how these movements are coordinated change during different phases of web construction, and we find that we can predict web-building stages based on these rules alone. Thus, the physical structures of the

web explicitly represent distinct phases of behavior. In addition to our behavioral efforts, we are also developing biological assays to investigate how this elegant behavior is encoded in the spider's brain.

Program Abstract #22: Genome-Wide analysis reveals novel regulators of synaptic maintenance

Jessica Sidisky, Danielle de Paula Moreira, Meryem Okumus, Russell Caratenuto, Bali Connors, Cassidy Drost, Sarrah Hussain, Steph Alkhatib, Daniel Babcock
Lehigh University

Maintaining synaptic structure and function is vital for overall nervous system function and survival. Recent evidence indicates that synaptic deficits are associated with both aging and neurodegenerative diseases. However, we do not yet have a strong understanding of the mechanisms responsible for how these structures are maintained. In order to identify genes responsible for sustaining synapses, we performed an unbiased forward genetic screen utilizing the *Drosophila* Genetic Reference Panel (DGRP) consisting of 200 lines that have been fully sequenced. We assessed neuromuscular junctions (NMJs) that innervate the indirect flight muscles utilizing a high throughput flight assay that provides a direct readout of synaptic integrity. We tested for the progressive loss of flight ability from early and late time points of all DGRP lines to identify Single Nucleotide Polymorphisms (SNPs) responsible for synaptic maintenance. Our screen generated a list of several candidate genes associated with the flight phenotype including Futsch, a microtubule binding protein required in motor neurons and MSP300, required in muscle tissue. We functionally validated the role of these genes through assaying the flight behavior of genetic mutants and tissue-specific RNAi. Our results implicate several novel genes not previously associated with synaptic maintenance. Thus, we seek to further characterize the nature of how these genes sustain synaptic integrity and the mechanisms responsible. This project is funded by the NINDS R01 NS110727 to Daniel T. Babcock.

Program Abstract #23: A signaling switch in zebrafish underlies the induction and maintenance of the spinal cord specification and patterning regulatory gene *cdx4*

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University of Richmond

Early central nervous system (CNS) specification, regionalization and patterning rely on a limited number of signaling factors used iteratively during development. How these signals –FGF, BMP, Wnt, Nodal, Retinoic Acid– direct different developmental programs in the CNS over time remains unknown. We investigated the activity of these signals on the regulation of the zebrafish *cdx4* gene. This gene encodes for a transcription factor involved in (1) the specification of hindbrain versus spinal cord territories and (2) the induction, establishment and maintenance of hox patterning genes. Using various loss and gain of function approaches we identified two phases of *cdx4* transcription, an early initiation and a later maintenance phase. These two transcriptional phases rely on different signaling inputs and correspond to different Cdx4 functions. During the initiation phase, *cdx4* expression domain along the gastrula margin depends on BMP ventrally and Nodal dorsally, with Wnts regulating the overall levels of transcription. In contrast to reports in *Xenopus*, we do not find evidence of FGF regulating *cdx4* during this early phase. This phase of expression coincides with Cdx4 function in the specification of spinal cord fates and the initiation of hox gene transcription. During the second phase, *cdx4* transcription in the spinal cord is under the control of an FGF-dependent autoregulatory loop that is required for the maintenance of hox gene transcription. At this stage, our previous work shows that Retinoic Acid limits the anterior extent of *cdx4* transcription to the spinal cord territory. Taken together, these results support a biphasic model of *cdx4* regulation that is dependent on different signaling factors and that correspond to Cdx4 functions in specifying and then patterning the spinal cord. This work was supported by the NSF grant IOS-1755386 and the University of Richmond School of Arts and Sciences.

Program Abstract #24: Bilaterian brain patterning mechanisms predate evolution of the bilaterian brain

Dylan Faltine-Gonzalez, Jamie Havrilak, Michael Layden
 Lehigh University

The evolution of the central nervous system (CNS) is long thought to be a crucial innovation which allowed for radiation of bilaterians. Similarities in regionalized expression of axial patterning (AP) genes within developing brains has been used as support for the hypothesis that the CNS evolved once during bilaterian evolution. However, these brain regionalization programs resemble those which generally pattern the AP axis. It is this similarity which led support to an alternative hypothesis in which AP patterning was convergently co-opted multiple times during bilaterian evolution to regionally pattern brains. To resolve these two hypotheses we wanted to identify whether axial genes also play a neurogenic role within an early metazoan which does not possess a brain, the sea anemone *Nematostella vectensis*. If neurogenic function can be identified within a cnidarian, sister taxa to the bilaterians, it would suggest that similarities in regionalization programs are not sufficient to infer homology across bilaterians, lending support to the idea that AP patterning mechanism were co-opted multiple times. To test this, we knocked down expression of the anterior marker, *Nvsix3/6*, which resulted in loss of aboral neurons. We then removed potential *Nvsix3/6* binding domains within two aboral neural transgenic lines, *Nvfoxq2d::mCherry* and *Nvserum amyloidA-like::mCherry*, and observed loss of mCherry+ cells. This suggests that *Nvsix3/6* actively promotes expression of these aboral neural markers. We then knocked down the trunk marker *Nvwnt2*, which resulted in expansion of aboral neurons orally and loss trunk neuronal markers. This suggest that *Nvwnt2* promotes trunk neural identity and repressed aboral neuronal markers. These data suggest that bilaterian brain patterning mechanisms existed prior to the cnidarian-bilaterian divergence. This research was supported by the NSF CAREER AWARD (1942777) and NIH National Institute for General Medical Sciences (R01GM127615).

Program Abstract #25: The Hox gene *Post2b* controls organismal size in the planarian *Schmidtea mediterranea*

Christopher Arnold, Alejandro Sánchez Alvarado
 Stowers Institute for Medical Research

Organisms with nearly identical body forms exist over a wide range of sizes, yet mechanisms controlling organismal size remain largely a mystery. We report functions for Hox genes in organismal size control in the planaria *Schmidtea mediterranea*. Planarian adult pluripotent stem cells enable dynamic adult body scaling in accordance with nutrient intake. Despite this seemingly unlimited growth potential, continuously fed planarian populations exist within specific size ranges indicating mechanisms restricting organismal size. We previously identified an interrelationship between planarian growth and asexual reproduction. After reaching a threshold size, planaria develop transverse fission behavior - separating posterior tissue that regenerates into clonal progeny. Therefore, planarian body size is established by a dynamic equilibrium of body mass gained from nutrient intake and body mass lost from fission. Given the roles of Hox genes in controlling axial growth and their recently identified functions in planarian asexual reproduction, we hypothesized that Hox genes might integrate these two processes to control body size. Long-term RNAi feeding experiments identified multiple Hox genes with roles in restricting organismal size. In particular, knockdown of the posterior Hox gene homolog *Post2b* eliminated fission activity and enabled adult animals to grow more than five times the maximal size of their wild-type counterparts. Knockdown of *Post2b* also increased the growth rate of sexually reproducing planaria, indicating additional roles in body size regulation independent of asexual reproduction. Interestingly, after *Post2b* RNAi animals exceeded their natural size limits, their tissues deteriorated in a TAK1 inflammatory pathway-dependent manner. Our work demonstrates novel roles for Hox genes in organismal size control and suggests a connection between unrestricted growth and aberrant inflammatory signaling. SIMR funded this work.

Program Abstract #26: Transgenerational epigenetic inheritance of learned pathogen avoidance

Coleen Murphy
 Princeton University

POSTER PRESENTATIONS

Program Abstract #27: Multivesicular bodies deliver sphingolipids to cleavage furrow during male meiotic cytokinesis

Govind Kunduri, Si-Hung Le, Valentina Baena, Nagampalli Vijaykrishna, Adam Harned, Kunio Nagashima, Daniel Blankenberg, Yoshihiro Izumi, Kedar Narayan, Takeshi Bamba, Usha Acharya, Jairaj Acharya
National Cancer Institute Center for Cancer Research

Cell division, wherein one cell divides into two daughter cells, is fundamental to all living organisms. Cytokinesis, the final step in cell division, begins with the formation of an actomyosin contractile ring, positioned midway between the segregated chromosomes. Constriction of the ring with concomitant membrane deposition in a spatiotemporal manner generates a cleavage furrow that physically separates the cytoplasm. Unique lipids with specific biophysical properties have been shown to localize to intercellular bridges; however, their biological roles and delivery mechanisms remain largely unknown. In this study, we show that Ceramide phosphoethanolamine (CPE), the structural analog of sphingomyelin, has unique acyl chain anchors in spermatocytes and is essential for meiotic cytokinesis. We demonstrate the enrichment of CPE in multivesicular bodies marked by Rab7, which in turn localize to cleavage furrow. Volume electron microscopy analysis using correlative light and focused ion beam scanning electron microscopy shows that CPE enriched Rab7 positive endosomes are juxtaposed on contractile ring material. Correlative light and transmission electron microscopy reveal Rab7 positive endosomes as a multivesicular body-like organelle that releases its intraluminal vesicles in the vicinity of ingressing furrows. Genetic ablation of *Rab7* alone results in significant meiotic cytokinesis defects. Our results imply that endosomal delivery of CPE to ingressing membranes is crucial for meiotic cytokinesis. This study was funded by the intramural division of the NCI, NIH (DHHS). The study was partly funded with Federal funds from the NCI, NIH, under Contract No. 75N91019D00024. The work was partially funded by the Grant-in-Aid for Scientific Research on Innovative Areas (17H06304) [T.B.] and a Grant-in-Aid for Scientific Research (B) (18H01800) [T.B.] from Japan Society for the Promotion of Science (JSPS).

Program Abstract #28: Modeling Zellweger Syndrome: the characterization of the deletion of *prx-10* in the nematode *Caenorhabditis elegans*

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National Institute of Diabetes and Digestive and Kidney Diseases

Zellweger Syndrome (ZS) is a rare genetic disease caused by a mutation in any one of the several nuclear encoded PEX genes, which are responsible for the biogenesis of peroxisomes. There are 14 PEX genes known to be involved in peroxisome biogenesis. Peroxisomes have functions that are highly diverse depending on the organism, but common functions of the organelle are lipid metabolism and neutralization of reactive oxygen species in the cell. Defects in peroxisome biogenesis are associated with the following phenotypes: hepatic and renal dysfunction, developmental delay, and neurological abnormalities, such as seizures, vision loss, hearing loss, and severe brain malformations. Mutations in PEX genes can disrupt the function of PEX proteins, leading to the improper formation of peroxisomes in the cell. Here, we examine how a deletion of the PEX-10 ortholog in *C. elegans*, *prx-10*, affects the organism to understand the effects of ZS in humans. When we deleted the gene, we saw that the majority of homozygous worms arrested at the L2 stage while a smaller number died as embryos with morphological defects. In the future, we plan to recreate ZS patient-specific missense alleles in the *C. elegans prx-10* gene, characterize them, and compare the effects of these missense alleles with the effects of the deletion allele. We will use peroxisome markers fused to GFP or mScarlet to visualize if these variants perturb peroxisome biogenesis. In the future, we hope to carry out genetic suppressor screens to identify extragenic suppressor mutations that restore function to our *prx-10* mutants. This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

Program Abstract #29: Conservation and Divergence in the Heterochronic Pathway of *C. elegans* and *C. briggsae*.

Maria Ivanova, Eric Moss
Rowan University

The nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* are close species that have similar morphology and share the same ecological niche. Their post-embryonic development consists of four larval stages separated by molts during which stage-specific proliferation, differentiation, and morphogenesis events occur. The heterochronic genes of *C. elegans* control developmental timing: mutations in these genes cause the skipping or reiteration of stage-specific post-embryonic events. *C. briggsae* possesses orthologs of the heterochronic genes, but their roles in development are not known. To begin to address the degree of developmental systems drift in the heterochronic pathway, we determined the phenotypes of the *C. briggsae* orthologs of the *C. elegans* heterochronic genes. *lin-14* is a well-studied heterochronic gene that exists only in the *Caenorhabditis* genus. *ce-lin-14* and *cbr-lin-14* mutants have identical precocious heterochronic phenotypes. Several other *C. briggsae* heterochronic genes orthologs, including *cbr-lin-4*, *cbr-lin-46*, *cbr-let-7*, and *cbr-hbl-1*, all display mutant phenotypes that either closely resemble those of *C. elegans*, but differ in severity, or diverge by affecting events of different larval stages. *lin-28* is a heterochronic gene that is conserved in many animals, including arthropods and vertebrates. Among other activities, it acts in stem cells and blocks the maturation of the *let-7* miRNA. It is often expressed early in a lineage and is downregulated as cells differentiate. *C. elegans* mutants of *lin-28* skip L2-specific events and execute adult cell fates precociously. *cbr-lin-28* mutants have a very different overall phenotype: they arrest their development at the end of L4 stage and develop a disorganized gonad in adulthood. In summary, the roles of several heterochronic genes differ between *C. elegans* and *C. briggsae*, but their involvement in the developmental timing and certain pairwise genetic relationships are preserved. Rowan University.

Program Abstract #30: Dissecting Vascular Reperfusion and Remodeling After Injury in Zebrafish

Leah Greenspan, Daniel Castranova, Brant Weinstein
Eunice Kennedy Shriver National Institute of Child Health and Human Development

The circulatory system is essential for life, and failed vessel reperfusion after tissue injury can cause life threatening complications such as heart attack and stroke, or for cutaneous injuries, defects in wound closure. Although the mechanisms regulating angiogenesis during development have been well studied, the molecular pathways driving vessel regrowth after injury are not well understood. Zebrafish provide an ideal model organism to visualize and study endothelial repair in vivo because of their optical clarity, experimental accessibility, conserved vertebrate vascular anatomy, and regenerative capacity. Using adult transgenic fish, I can monitor vessel reperfusion after cutaneous wounding through high-resolution confocal microscopy. I find that immediately after injury blood and lymphatic vessels are absent from the wound area but initiate sprouting by 2 days post wounding (dpw) and fully reperfuse the wound area by 9dpw. While some remodeling occurs, vessel patterning does not return to pre-injury conditions suggesting that signaling mechanisms that are required to set up vessel patterning initially may be absent during wound triggered angiogenesis. To better understand endothelial cell behavior in response to damage, I use the nitroreductase/metronidazole system in zebrafish larvae to mosaically ablate a small proportion of vessels throughout the fish. Most vessels reconnect and relumenize normally due to migrating endothelial cells that replace dying cells, but some vessels disconnect and regrow as the opposite vessel type. This change in arterial/venous identity suggests that vessels are plastic and can adapt to changes in flow after injury. I am now using TRAP RNAseq to probe endothelial gene expression changes upon damage and recovery. Together my studies will uncover the mechanisms that restore vascular networks in injured tissue, providing potential new targets for therapeutic approaches. Funding is from the NICHD and NIH Intramural Research Program.

Program Abstract #31: Deciphering the Cellular and Molecular Mechanisms of Congenital Generalized Lipodystrophy in *Caenorhabditis elegans*

Xiaofei Bai, Andy Golden

National Institute of Diabetes and Digestive and Kidney Diseases

The metabolic homeostasis of fatty acids is important for human health. At the cellular level, the cytosolic lipid-storage organelles, lipid droplets (LDs), serve as the major energy reservoirs to maintain fat homeostasis in nearly all cell types. Seipin, a conserved endoplasmic reticulum protein, plays pivotal roles during LDs biogenesis. Dysfunctional mutations in Seipin lead to Berardinelli-Seip congenital lipodystrophy type 2 (BSCL type 2). However, the cellular and molecular mechanisms of Seipin in these diseases are poorly understood. Here, we functionally characterized *seip-1*, the sole ortholog of SEIPIN in the nematode model *Caenorhabditis elegans*, using CRISPR/Cas9 gene editing, transcriptional assay, lipidomic analyses, and other genetic tools. We observed that SEIP-1 is associated with LDs and is crucial for controlling LDs size and lipid homeostasis. The depletion of *seip-1* disrupted fatty acid synthesis, and particularly reduced the ratio of polyunsaturated fatty acids (PUFAs) in the *C. elegans* embryonic fatty acid pool, suggesting an important role of SEIP-1 in maintaining fatty acid homeostasis. Of note, homozygous *seip-1* mutants displayed penetrant embryonic lethality, which is caused by the disruption of the lipid-rich permeability barrier, the innermost layer of the *C. elegans* embryonic eggshell. Intriguingly, dietary supplementation of selected PUFAs rescued the embryonic lethality and defective permeability barrier in *seip-1* deletion mutants. We also edited the gene to mimic a lipodystrophy allele of *seip-1*, which also resulted in embryonic lethality and could be rescued by PUFA supplementation. Lastly, we identified a set of candidate suppressor alleles in a chemical mutagen-based genetic suppressor screen, which restored embryonic lethality. These newly identified suppressing factors may lead to a new direction in LD biology and shed light on future therapies for lipodystrophies. This work was supported by the NIH Intramural Research Program.

Program Abstract #32: NDRG1 Protects the Kidney from Hypoxic Injury

Anya Viswanathan, Jong Park, Rachel Brewster

University of Maryland Baltimore County

Low oxygen disrupts homeostasis in human cells and can fatally damage vital organs. Studies show that hypoxia impairs kidney function and contributes to the development of kidney disease. Interestingly, zebrafish can withstand hypoxia by entering a hypometabolic state. Subsequently, the function of organs with high metabolic demand, including the kidney, declines. Because the pronephros (embryonic kidney) is functionally and structurally similar to the human nephron, the zebrafish is a valuable model to study human kidney disease. Our lab has identified a 9-fold upregulation of *n-myc downstream gene 1a* (*ndrg1a*) transcript in zebrafish exposed to prolonged hypoxia, suggesting this gene is crucial to the organism's survival and recovery from low oxygen. Furthermore, our lab found that Ndr1a does not regulate physiological processes under normal oxygen conditions. However, it remains unclear whether Ndr1a is required to maintain kidney homeostasis under hypoxia. Given that *ndrg1a* transcript is upregulated under hypoxia, we hypothesized that it may play an adaptive role in kidney function and survival. Here, we investigate the protective role of Ndr1a in maintaining kidney function after exposure to prolonged anoxia (0% oxygen) followed by a period of re-oxygenation, using a kidney clearance assay. The assay revealed that in *ndrg1a* *-/-* mutants, the filtering capability of the kidney is approximately 1.5x lower than that of WT embryos when exposed to 12 or 18 hours of anoxia. Further, we found that embryos with the most severe kidney malfunction had the lowest survival rate post-anoxia. Together, these observations suggest that Ndr1a protects the kidney from hypoxic damage. These findings are significant, given that NDRG1 function is likely to be conserved in humans, and modulation of its function could be exploited for therapeutic benefit. This research was funded by the Howard Hughes Medical Institute and the Department of Defense (RT# 150086).

Program Abstract #33: Transmembrane Protein 184A and Syndecan-4 function in the same pathway to promote angiogenesis

Leanna Altenburg, Rachel O'Toole, Grace Ciabattini, Isabella Papov, Leah Pasch, Brianna Maslonka, Linda Lowe-Krentz

Lehigh University

Morpholino (MO) knockdown (KD) of heparin receptor Transmembrane protein 184A (TMEM184A), increases endothelial cell (EC) proliferation rates, leading to abnormal or stunted vessel outgrowth in the regenerating zebrafish caudal fin. A similar phenotype is observed in the developing zebrafish embryo, where TMEM184A KD reduces the number and length of intersegmental vessels. The Heparan Sulfate (HS) Proteoglycan Syndecan-4 (Sdcn-4) is required for VEC internalization in angiogenesis and interacts with membrane proteins to promote cellular processes through interactions with its attached HS chains. Since HS is nearly identical to heparin in structure and both TMEM184A and Sdcn-4 appear to be required for stable angiogenesis, we asked whether TMEM184A and Sdcn-4 cooperate to promote vascular regeneration. Using TMEM184A and Sdcn-4 MO KD, we demonstrate that subthreshold concentrations of both MOs synergize to produce the same vascular defect in the caudal fin. In cells, we find immunostaining of TMEM184A, Sdcn-4, and VEC show trimeric colocalization at junctions, while in vesicle puncta they colocalize with the recycling marker Rab5a; and we show that TMEM184A precipitates Sdcn-4 in cell lysate. In cells transformed to overexpress TMEM or treated with heparin, VEC immunostaining shows increased adherens junction expression. These results suggest that TMEM184A and Sdcn-4 function in the same pathway to promote stable angiogenic repair, that TMEM184A interacts with both Syndecan-4 and VEC to form a complex that is recycled at the membrane surface, and that TMEM184A overexpression or activation stabilizes VEC in adherens junctions. Further study with current NIH funding HL54269 will determine whether TMEM184A and Syndecan-4 function together to regulate VEC trafficking and promote angiogenesis or whether TMEM184A expression impacts on VEC membrane stability are distinct from the TMEM184A–Sdcn-4 molecular mechanism that regulates angiogenic repair.

Program Abstract #34: Germ cell specification in the regenerative marine flatworm *Macrostomum lignano*

Xintao Fan, Erin Davies

National Cancer Institute Center for Cancer Research

Germline stem cell (GSC) lineages produce gametes and transduce genetic information across generations. In many animals, GSCs form once during development. Damage to GSCs often leads to GSC loss and infertility. Interestingly, some species can replace damaged or lost GSCs, from either germ cells or somatic cells. The marine flatworm *Macrostomum lignano* (*Mlig*) is a non-self-fertilizing hermaphrodite that regenerates GSCs and gonads de novo after amputation. Like the freshwater flatworm *Schmidtea mediterranea*, *Mlig* contains somatic pluripotent stem cells called neoblasts that self-renew and produce differentiating progeny during adult tissue homeostasis and regeneration. Somatic neoblasts are likely to be the cellular source of germ cells in *Mlig* adults but the GSC specification mechanism has not been determined. We are using volume electron microscopy, single-cell RNA-sequencing, and in situ RNA hybridization chain reaction to determine the cellular composition of *Mlig* testes and ovaries and staging for the male and female GSC lineages. Our goal immediate goal is to identify key cell states and candidate regulators of the neoblast to GSC transition(s) during adult regeneration. Finally, we seek to develop transgenic tools, including fluorescent fusion protein reporter strains engineered using CRISPR/Cas9, for visualization of germ cell dynamics using live imaging. This research was supported by the Center for Cancer Research, National Cancer Institute, National Institutes of Health Intramural Research Program project number ZIABC0120009.

Program Abstract #35: Shedding (blue) light on BMP signaling in zebrafish embryos

Catherine Rogers, Katherine W. Rogers

Eunice Kennedy Shriver National Institute of Child Health and Human Development

During embryogenesis, zygotes reliably generate cells expressing distinct sets of genes leading to different fates and functions. Signaling dynamics, levels, and combinations are thought to activate these different gene expression programs. Many contemporary signaling manipulation methods are inflexible and don't

provide the control needed to address how signaling is decoded. To address these limitations, we are developing optogenetic tools to precisely and reversibly activate signaling with blue or red light in zebrafish embryos, then observe how this impacts fate decisions. First, we have developed a tool, bOpto-BMP, to activate BMP signaling with blue light. In the presence of blue light, zebrafish expressing bOpto-BMP phenocopy BMP overexpression and show elevated levels of the BMP signaling effector pSmad1. We also found that bOpto-BMP is not activated by red light. This will allow us to use bOpto-BMP orthogonally with red light activators of other pathways in one embryo. We expect our tools can be applied to developmental and regenerative biology in different systems and will be useful for expanding our knowledge of how cells decode the dynamics of signals as a language for cell fate decisions.

Program Abstract #36: Physiological and functional implications of differentially enriched transcripts on eRpL22-family polysomes during development

Caroline Pritchard, Vassie Ware

Lehigh University

The *Drosophila melanogaster* eRpL22 ribosomal protein family contains two structurally divergent & developmentally essential paralogues: eRpL22 and eRpL22-like - the latter exhibits tissue-specific expression across development; the former is ubiquitously expressed. Multi-tissue co-localization comparison of eRpL22-like and core ribosomal components indicates eRpL22-like may have functional roles both within the ribosome itself and apart from ribosomal processes. Sequencing of RNAs enriched on eRpL22 and eRpL22-like polysomes in adult testes revealed differential enrichment of mRNAs suggesting that paralogue-specific “specialized ribosomes” translate specific mRNAs. Functional enrichment analysis guided investigation into specific tissues by physiology (where to look) and function (what processes, pathways, and programs function differently in Rp mutants). Transcripts differentially enriched on eRpL22-like polysomes were uniquely implicated in pathways not canonically associated with ribosomal functions, including WNT/PCP, JAK-STAT, and RAS-MAPK signaling. Some pathways were over-represented in both polysome types, but these were derived from unique transcripts. Transcripts of genes functionally implicated in human disease were over-represented on eRpL22-like polysomes. Investigation of the cellular and molecular impact of differential enrichment (via eRpL22-like conditional knock-out) revealed defective ovarian morphology, including stem cell niche disruption, ectopic rounded follicle cells, double-nucleated oocytes, anteriorized eggs, and specific spatiotemporal patterns of cell death & oogenesis arrest – perturbed cell polarity is a common feature. Taken together, these data broaden the context for essential roles of eRpL22-like across multiple developmental processes. Funding was provided in part by NIH grant 1R15GM-132889-01 to V.C.W.; Lehigh University Department of Biological Sciences Undergraduate Research Grant and Langer-Simon Scholar Award to C.P.

Program Abstract #37: Studying pharynx development using the zebrafish

Kiyohito Taimatsu, Aniket V. Gore, Miranda Marvel, Daniel Castranova, Andrew Davis, Madeleine Kenton, Gennady Margolin, Brant M. Weinstein

Eunice Kennedy Shriver National Institute of Child Health and Human Development

The pharynx and associated organs have important roles in breathing, eating, drinking, digesting, speaking and protecting the body from infection. Pharyngeal dysfunction can manifest as dysphagia, persistent palatal displacement, or exercise intolerance. Secondary complications are serious and life threatening and can include aspiration pneumonia, weight loss, and death. However, pharynx development is not well understood, in large part because of the inaccessibility of the developing pharynx to observation and experimental manipulation in most model organisms. I am establishing the optically clear zebrafish as a powerful model for observation and genetic and experimental analysis of pharynx development. I have used histology, microCT, and confocal imaging to describe adult and developing pharynx anatomy, and scRNAseq to identify resident cell types of the pharynx and their gene expression signatures. My anatomical and molecular findings show the zebrafish pharynx is a complex structure with numerous specialized cell types, many of which correspond well to cell types identified in the mammalian pharynx. I

have also used genetic screens that to identify pharynx-specific mutants that I am currently characterizing in detail. My ongoing work also includes careful staged analysis of the early development of the zebrafish pharynx, generation of cell type-specific transgenic lines to characterize the development and function of specific pharyngeal cell populations, and identification and functional work-up of genes required for the proper development of some of these cell types. My results thus far have established the fish as a valuable new experimental model for experimental and genetic analysis of the pharynx and a useful platform for functional and molecular characterization of the molecular basis for pharynx disorders. This work was supported by the JSPS (to KT) and the intramural program of the NICHD (to BMW).

Program Abstract #38: Developmental Determination of Central Auditory Physiology by the Inner Ear

Lashaka Jones, Catherine Weisz, Weise Chang, Matthew Kelley, R. Michael Burger
Lehigh University

Auditory nuclei express topographically distributed specializations enabling precise computations in frequency-specific channels. The degree to which expression of tonotopic features arises independently or in response to patterned input from the inner ear in development is unknown. Previous studies have established central neurons' dependence on cochlear afferents for survival, connectivity, and refinement by utilizing methods that profoundly disrupt hearing. Clearly, ablation and deafening studies demonstrate that peripheral input is required for central neuron survival and connectivity, but it is unknown how tonotopic refinement is regulated in the cochlear nucleus (CN) during the peri-hearing onset period. In particular, the influence of peripheral patterning on determination of intrinsic biophysical features in central neurons has not been investigated. We successfully induce molecular repatterning of peripheral tonotopy and evaluate the developmental outcomes of this treatment on patterning of central phenotypes in the avian CN.

Unilateral tonotopic rearrangement of the basilar papilla (BP) was induced through overexpression of Bmp7 in the developing ear prior to tonotopic patterning. We then investigated hallmarks of tonotopic identity in both hair cells and in CN using electrophysiological, molecular, and anatomical methods. Overexpression of Bmp7 in the BP resulted in disruption of the hair cell phenotypic gradient including ion channel distribution.

Centrally, CN neurons located in "high frequency" positions acquired low frequency phenotypes ipsilateral to treatment. These results strongly suggest that Bmp7 treatment generates BPs with overrepresentation of low frequencies and little to no representation of high frequencies. The repatterning of the periphery during a critical period induces tonotopic reorganization of the cochlear nucleus that is expressed at the ion channel level. Funding from R01DC018537 to RMB, and Intramural NIH funds to MK and CW.

Program Abstract #39: Analysis of zebrafish fin regeneration using an epigenetic reporter line

Allison Goldstein, Daniel Castranova, Aniket Gore, Brant Weinstein
Eunice Kennedy Shriver National Institute of Child Health and Human Development

In contrast to mammals, zebrafish have a remarkable ability to regenerate organs after injury. Zebrafish tail fin regeneration is an excellent model system to study how new cell types are specified from a group of undifferentiated cells called a blastema. Blastema formation, a critical step for proper regeneration, requires genome wide epigenetic reprogramming of differentiated cells at the wound site. Previous studies have shown several genetic pathways are required for proper blastema formation, but the role of epigenetics in this process is relatively unknown. We developed an "EpiTag" epigenetic reporter transgenic zebrafish line that reliably reports dynamic tissue specific epigenetic changes during development, gametogenesis, and regeneration. The transgenic construct used in this line contains a CpG island from the *dazl* gene, a germ cell-specific gene targeted for silencing in all somatic cells. The *dazl* CpG island is cloned next to a ubiquitously expressing *ef1a* promoter driving expression of destabilized GFP (GFPd2), allowing for dynamic visualization of rapid changes in GFP reporter expression in living animals. We are currently using this line to study how tail fin cells selectively undergo epigenetic reprogramming after injury. Our preliminary findings show GFPd2 expression is active within 1 day of injury, peaks between 1-2 days, begins to fade by 3 days and becomes almost undetectable by 9 days post injury. We are also analyzing the response of

epigenetic regulators during fin regeneration using RT-qPCR. Using in situ hybridization, immunohistochemistry and real time live imaging, we will precisely map the spatio-temporal identity of cells that selectively start and silence GFPd2 expression during tail fin regeneration. Using single cell genomics approaches, we plan to analyze gene expression and epigenomic changes taking place at the individual cell level during fin regeneration. This work is supported by the intramural program of the NICHD.

Program Abstract #40: From Wrappers to Drains: The diverse cellular identities of the Zebrafish Meninges

Marina Venero Galanternik, Ryan D. Gober, Daniel Castranova, Bakary Samasa, Tuyet Nguyen, Aniket Gore, Andrew E. Davis, Louis E. Dye 3rd, Gennady Margolin, Madeleine Kenton, Allison Goldstein, Avery Swearer, Van Pham, James Iben, Timothy S. Mulligan, Steven L. Coon, Jeffrey S. Mumm, Brant M. Weinstein

Eunice Kennedy Shriver National Institute of Child Health and Human Development

The vertebrate brain is an essential but surprisingly delicate vital organ protected by several anatomical components, including supportive and frequently disregarded protective tissues called the meninges. Located between the brain and the skull, meningeal tissue layers surround the brain and spinal cord shielding them from mechanical shock, providing blood supply, supporting brain buoyancy and maintaining homeostasis. Often thought of as “inert tissues,” the meninges remain poorly characterized, in part due to their thin morphology, their location immediately below the skull, and a lack of defined meningeal markers. To overcome these limitations, we are utilizing the zebrafish as a new model for studying the meninges. Our comprehensive teleost meningeal anatomical characterization using histology, electron microscopy and super-resolution confocal microscopy shows that like mammals, zebrafish have well-defined meningeal layers populated by a myriad of cellular components. Using scRNA-seq to comprehensively profile all of the resident zebrafish meningeal cell populations, we have identified approximately fifty unique cellular clusters, which include blood and lymphatic endothelial, pigment and immune cells, as well as a variety of uncharacterized cell populations. Among them, Ependymin-expressing cells (EPDs) represent a unique meningeal epithelial cell population that ensheaths other cells and delimits the boundaries of the meninges, allowing us to perform global or partial ablations of the meninges to better understand the importance of this tissue during development, adulthood and post injury regeneration. Our zebrafish studies are leading to a new understanding of the meninges, their developmental origins, their roles in protecting the brain and the effects of meningeal damage. This work is supported by an NICHD K99 Pathway to Independence Award (to MVG), and by the Intramural Program of the NICHD (to BMW).

Program Abstract #41: A possible feedback loop between Cx43 and β -catenin during fin regeneration

Victoria Hyland, M Kathryn Iovine

Lehigh University

How joints are correctly positioned in the skeleton is poorly understood. We use the zebrafish regenerating fin model to address this fundamental question. During fin regeneration, skeletal precursor cells commit to either osteoblasts or joint forming cells. The protein Connexin43 (Cx43) plays an important role in determining which cell fate will occur. Here we provide novel insight into how Cx43 is regulated during fin regeneration through a potential feedback loop between Cx43 and β -catenin. It has been shown that Cx43 and β -catenin function in a shared molecular pathway to influence joint location. Previous studies show that β -catenin synergistically works downstream of Cx43 to inhibit joint formation. Here we show that β -catenin may be involved in a feedback loop and can influence cx43 expression. We show that when β -catenin is inhibited, cx43 mRNA levels and protein expression are decreased. Interestingly, we show that when β -catenin is increased, cx43 mRNA levels are once again decreased and remarkably protein levels are increased. Continued studies are attempting to resolve this apparent paradox. If validated, these findings suggest tight coordination between Cx43 and β -catenin function and provide novel insights into skeletal patterning. This research is funded by R15-HD080507 from the NICHD.

Program Abstract #42: Reversing Metabolic Arrest: Downregulation and Return Of Na⁺-K⁺ ATPase (NKA)

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Oxygen plays a critical role in ATP synthesis during oxidative phosphorylation; thus it is not surprising that ischemic damage (i.e. stroke due to lack of oxygen) is a leading cause of death in the United States. By contrast, zebrafish embryos can survive up to fifty hours in a zero-oxygen (anoxic) environment by entering into a state of hypometabolism characterized by reversible metabolic arrest of ATP-demanding processes, such as Na⁺-K⁺ ATPase (NKA). The Brewster Lab has previously shown that the N-myc Downstream Regulated Gene (NDRG) mediates this transition, although exact molecular mechanisms remain unknown. My project specifically addresses the reversibility of zebrafish NKA downregulation to preserve cellular energy in anoxia. I hypothesize that if downregulation is an adaptive process, then normoxic (normal oxygen) NKA levels should be restored upon re-oxygenation, possibly via recycling of NKA stored in intracellular vesicles and/or via de novo protein synthesis. I tested this hypothesis by exposing 24-hour old zebrafish embryos to eighteen hours of anoxia (a duration of anoxia sufficient to cause severe downregulation of NKA), gradually reoxygenating them, then immunolabeling at five timepoints post-anoxia to observe if and when NKA levels in the plasma membrane return to their normoxic value. After three rounds of experiment and analysis, there appears to be a significant increase in NKA levels in the hours following anoxia. In the future, I plan on performing a proximity ligation assay to investigate whether NDRG1 and NKA interact during this reversal process, which would suggest that NDRG1 mediates both the down- and up-regulation of NKA in response to environmental fluctuations in oxygen. We anticipate that these studies will further our understanding of hypometabolism as a protective adaptation with great human therapeutic potential. This research was supported by Department of Defense: RT# 150086.

Program Abstract #43: Cohesinopathies Disturb *ddb1* Expression in Zebrafish Embryos

Annie C. Sanchez, Emma C. Anderson, M. Kathryn Iovine, Robert V. Skibbens
Lehigh University

Cohesins tether DNA together to promote enhancer-promoter interactions for proper gene expression during development. Cohesin pathway mutations lead to developmental syndromes called cohesinopathies, including Roberts Syndrome (RBS) and Cornelia de Lange Syndrome (CdLS), that exhibit severe organ malformations, craniofacial abnormalities, phocomelia, and intellectual disabilities. RBS is caused by mutations in *ESCO2*, an acetyltransferase that activates cohesins, while CdLS is caused by mutations in cohesin subunits or auxiliary factors, like NIPBL, which loads cohesins onto DNA. Similar developmental abnormalities can arise due to in utero exposure to teratogens. Thalidomide is a potent teratogen that led to tens of thousands of babies born with RBS/CdLS-like developmental conditions. Thalidomide teratogenicity is due to the direct binding of the drug to Cullin-4 Ring Ligase (CRL4). CRL4 is an E3 ubiquitin ligase comprised of CUL4, DDB1, ROC1, and a DDB1-CUL4 Associated factor, which function together to ubiquitinate proteins, targeting them for degradation. Previously, we used zebrafish to test a novel hypothesis that RBS/CdLS birth abnormalities may arise in part through dysregulation of CRL4 gene transcription. Our findings revealed that *ddb1* is downregulated in cohesinopathic (*Esco2* or cohesin subunit knockdown) zebrafish models compared to controls. We further found that increasing *ddb1* mRNA alters the phenotypes that occur in *Esco2* or cohesin subunit knockdown embryos, supporting our hypothesis that CRL4 and cohesin share a molecular pathway. Here, we report on new findings on Nipbl knockdown embryos that are consistent with this hypothesis. Together, our data support a link between thalidomide and cohesinopathies and provides a novel mechanism through which developmental maladies such as RBS and CdLS can be approached. This research is funded by Lehigh University Departmental Graduate Student Fellowship for ACS and Faculty Research Grant for RVS and MKI.

Program Abstract #44: Developmental trajectories and gene expression programs during zebrafish development

Abhinav Sur, Yiqun Wang, Gennady Margolin, Paulina Capar, Jeffrey Farrell
Eunice Kennedy Shriver National Institute of Child Health and Human Development

Animals consist of a collection of cells with beautifully diverse shapes, structures, and functions, and this diversity is rebuilt from scratch in every embryo. Understanding the genetic programs that direct this process is a central mystery of developmental biology. Single-cell RNAseq approaches provide the possibility of mapping the molecularly distinct populations of cells during development and inferring the changes in gene expression that occur as those cells acquire specific identities, functions, and morphologies. To investigate a broad range of cell types undergoing differentiation during wild-type development, we sequenced ~450,000 cells from closely spaced stages spanning late zebrafish embryogenesis and early larval development (14–120 hours post-fertilization) and integrated that with our published data from 3–12 hours post-fertilization. Using these data, we generated high-resolution cell atlases of individual tissues and reconstructed the developmental trajectories of gene expression changes leading to distinct cell types. The atlas and trajectories provide a unique framework to identify reuse of gene expression programs within and between cell types and enables discovery of previously unknown candidate regulators of fate decisions. Additionally, these atlases reveal unknown or poorly characterized molecular cell states in many tissues. For instance, our endodermal cell atlas contains a small population of intestinal enterocytes that express bestrophin-4 (best4) and otopterin-2 (otop2). Homologous cells were recently discovered in the human fetal colon and are depleted in inflamed colons, but their origin and specification remains unknown. Our approach suggests that these cells may arise from posterior intestinal progenitors and suggests the transcription factors that may define these cells, which we are testing using crispr approaches. Funding: NIH Intramural Program.

Program Abstract #45: miR-31 regulates cytoskeletal dynamics to impact formation of the mitotic spindle

Carolyn Remsburg, Kalin Konrad, Nadezda Stepicheva, Jia Song
University of Delaware

miR-31 is a highly conserved microRNA that is known to play a role in cell proliferation, migration, and differentiation. Using the sea urchin as a model organism, we observed that miR-31 inhibitor injected embryos are developmentally delayed throughout cleavage stages and have an increased lethality rate at blastula stage. We identified miR-31 to localize at the midzone of the mitotic spindle and perinuclearly in divided blastomeres. miR-31 inhibitor injected embryos display increased interpolar and kinetochore microtubules, occasional tri-polar spindles as well as increased filamentous actin compared to the control. To understand the mechanism of these cytoskeletal defects, we identified that miR-31 suppresses several actin remodeling transcripts, including *Rab35*, *Gelsolin* and *Fascin*. Interestingly, *Rab35*, *Gelsolin* and *Fascin* transcripts co-localize with miR-31. The 3'UTRs of *Gelsolin* and *Fascin* are sufficient for localization to the mitotic spindle, and mutation of the miR-31 binding site prevents localization of the transcript to the mitotic spindle. miR-31 inhibition results in an increase in Fascin protein at the mitotic spindle midzone and perinuclearly in blastomeres in interphase. Our research, as well as previous studies, indicate proper actin dynamics are critical for formation of the mitotic spindle. These findings lead to our hypothesis that miR-31 regulates local translation of transcripts that mediate actin to impact formation of the mitotic spindle. Since proper mitotic spindle formation is a prerequisite for faithful segregation of chromosomes, identifying how miRNAs mediate spindle formation and mitosis is critical for the fundamental understanding of early cell division, birth defects, and predisposition to cancer. NSF IOS & MCB grant to J.L.S. NIH/P20GM103653, NIH/NIGMS P20GM103446. University of Delaware fellowship to CR.

Program Abstract #46: MicroRNA-124 has a dual role to modulate neurogenesis and mesodermal development

Kalin Konrad, Santiago Suarez, Jia Song
University of Delaware

MicroRNA-124 (miR-124) plays a conserved role in regulating parts of neurogenesis; however, its additional function in various developmental processes is less defined. We use the purple sea urchin as a model to reveal the function of miR-124. The sea urchin has one of the most well-documented gene regulatory networks (GRN), making it an excellent model to integrate the role of miR-124 into its GRN. We observed that miR-124 inhibition resulted in decreased larval swimming velocity, indicating potential neuronal defects. miR-124 inhibition also led to neurons and increased neuronal progenitor cells. We identified miR-124 to directly suppress *NeuroD1*, *Notch*, *Nodal*, and a BC-specific transcription factor *Ese* to modulate neural differentiation, as well as mesodermal development, respectively. The removal of miR-124's suppression of *NeuroD1* is sufficient to induce similar swimming defects as miR-124 inhibition. Interestingly, by removing miR-124's suppression of both *Notch* and *NeuroD1*, we can recapitulate miR-124 inhibition-induced defects where the larvae had fewer Elav-expressing mature neurons cells and overall decreased neurons. In addition, we observed that miR-124 has a novel role in regulating the differentiation of mesodermally-derived immune cells. The sea urchin immune cells are blastocoelar cells (BCs) and pigment cells (PCs), which are derived from the same progenitor cells. miR-124 inhibition increased BC-specific gene expression and a concurrent decrease in differentiated PCs. Interestingly, when we remove miR-124's suppression of *Notch* and observe an increase in both BCs and PCs, suggesting that these cells become bipotential. In summary, we systematically examined the conserved function of miR-124 in neuronal development and identified miR-124's novel role in mesodermal differentiation. NSF IOS & MCB grant to J.L.S. NIH/P20GM103653, NIH/NIGMS P20GM103446. University of Delaware fellowship to K.K.

Program Abstract #47: Population density modulates asexual reproduction and affects gene expression in the cnidarian sea anemone *Nematostella vectensis*.

Layla Al-Shaer, Noor Baban, Michael Layden
Lehigh University

The decline in coral reefs is driving efforts to better understand coral biology in hopes of identifying strategies to mitigate loss. We are particularly interested in better understanding coral reproduction. The challenge is that experimentally tractable coral models are lacking. There is growing evidence that cnidarians (e.g., corals, anemones, hydras) use chemosensory communication to initiate and synchronize reproduction, thus we sought to investigate whether the sea anemone *Nematostella vectensis* may be an experimentally tractable model to interrogate the molecular mechanisms of chemical communication and reproduction in cnidarians. We tested if chemical communication is potentially utilized by *Nematostella* by testing if population density modulates *Nematostella* asexual reproduction. We compared the number of asexual reproduction events in both low- and high-density populations. As predicted, individuals from low-density populations had more clones than individuals from high-density populations. Additionally, we demonstrated that increased products of increased metabolism do not explain the change in fission rates. This suggests that *Nematostella* possess a previously undescribed chemical recognition system that allows individuals to sense population density, and that they use this sensory information to alter their reproductive strategy. To gain insights about the molecular basis of potential chemical communication mechanisms, we performed bulk RNA sequencing on individuals from high- and low-density populations and identified 239 genes that are differentially expressed between populations. We predict that genes with differential expression may be candidate genes that control asexual vs sexual reproduction, and we are currently screening changers for potential secreted molecules, potential receptors, or other factors that may be involved in chemosensory communication. Funding provided by Lehigh University's Faculty Innovation Grant and NIH RO1 GM127615.

Program Abstract #48: Characterizing the role of RHOA signaling in regulating vascular development and integrity

Laura Pillay, Megan Ezeude, Joseph Yano, Andrew Davis, Jong Park, Matthew Butler, Madeleine Kenton, Daniel Castranova, Gennady Margolin, Joshua Forgy, George Davis, Brant Weinstein
Eunice Kennedy Shriver National Institute of Child Health and Human Development

RHOA is a small, monomeric GTPase that transduces stimuli from hormones, growth factors, cytokines, and transmembrane signaling proteins to downstream effectors of cellular signaling via direct activation of target protein effectors. Previous *in vitro* cell culture studies suggest that RHOA can regulate many critical aspects of vascular endothelial cell (EC) biology, including junctional adhesion, focal adhesion, and actin stress fiber formation. However, the *in vivo* functions of RHOA in regulating blood vessel development and integrity are almost completely uncharacterized and, from a molecular perspective, it is unclear precisely how RHOA and its effectors regulate different EC biological processes. To study *in vivo* developmental consequences of RHOA gain- and loss-of-function, we generated an allelic series of zebrafish RHOA-ortholog (*rhoaa*) mutants and transgenic embryos expressing wild type or mutant forms of *rhoaa* specifically in ECs. Our combined data suggest that vascular development and integrity in zebrafish is exquisitely sensitive to RhoA gene dosage and/or activity, with too much or too little activity producing cranial hemorrhage and distinct blood vessel patterning defects. Activated RHOA targets frequently exhibit distinct phosphorylation states. To further elucidate the downstream molecular mechanisms by which RHOA regulates vascular integrity and development, we conducted quantitative phospho- and total proteomic profiling of cultured human ECs with increased or decreased RHOA activity. We identified previously established as well as several new members of the vascular RHOA signaling network. Mutating some of these RHOA pathway targets in zebrafish embryos produces cranial hemorrhage and/or angiogenesis defects, suggesting that our proteomics dataset includes novel regulators of vascular development and integrity that we are now characterizing in-depth. This work was supported by the Intramural Program of the NICHD (to BMW).

Program Abstract #49: Modified DNA damage response during early development and its interaction with cell specification

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DNA damage occurs naturally during normal development, and to develop normally, embryos must respond properly. We have observed that zebrafish blastulae express a modified transcriptional response to DNA damage; this response combines canonical DNA damage response genes and some genes only associated (so far) with other developmental processes. Computational analysis of scRNAseq data shows this response is expressed unusually frequently in non-neural ectodermal progenitors and rarely in dorsal organizer cells. These observations led us to question: (1) do the blastula-specific response genes have a previously undocumented role in the DNA damage response, (2) if cells' location or progenitor type influences their likelihood of experiencing damage or triggering a response to it, and (3) whether expression of this response influences cell fate decisions. To these ends, we generated mutants for many of the genes in this transcriptional response and are testing their role in cell cycle arrest and apoptosis, two common responses to DNA damage. Via *in situ* hybridization, we found that this response occurred more often on the ventral side of the embryo, which suggests that non-neural ectoderm's location or developmental program may increase the frequency of this response. Finally, to test whether damage affects cell fate, we are co-transplanting damaged and undamaged cells into unlabeled hosts to determine whether damaged cells transplanted into the presumptive dorsal organizer region exhibit different tissue contribution patterns than healthy cells. These early results illustrate a possible interaction between the DNA damage response and cell fate in normal development, which could provide valuable insights into the robustness of embryonic tissue patterning and into how proper cell identity is sometimes lost when cells' genomic stability is compromised. Funding: NICHD Intramural Program.

Program Abstract #50: Characterizing the effects of *wee-1.3* knockdown on spermatogenesis in *C. elegans*

Shannon Pfeiffer, Aimee Jaramillo-Lambert
University of Delaware

Meiosis is a specialized type of cell division that results in the production of haploid gametes. This can take the form of oogenesis, which produces oocytes, or spermatogenesis, which produces sperm. In order for

meiosis to occur, a protein complex known as maturation promoting factor (MPF) must be activated. In the model organism *Caenorhabditis elegans*, the inhibitory kinase WEE-1.3 regulates meiotic entry by preventing MPF from being activated until the appropriate stage of the cell cycle is reached. Previous work has shown that abnormal WEE-1.3 activity can cause various meiotic defects. Knockdown of *wee-1.3* via RNA interference (RNAi) in hermaphrodites resulted in sterility caused by precocious oocyte maturation. The goal of this project is to characterize the effects of *wee-1.3* knockdown on spermatogenesis in *C. elegans*. Previous to this study, little was known about the role of WEE-1.3 in spermatogenesis except that gain-of-function mutations in the *wee-1.3* gene cause primary spermatocyte arrest during spermatogenesis. We found that knockdown of *wee-1.3* via RNAi was found to negatively impact male fertility and cause severe germline defects. Upon exposure to *wee-1.3* RNAi, some male *C. elegans* completely fail to develop a germline. To further examine the role of *wee-1.3* during spermatogenesis, we plan to use auxin-inducible degradation. Unlike RNAi, auxin-inducible degradation can be used to deplete the target protein in a specific tissue and with relative rapidity. We have generated a *C. elegans* strain that will allow us to deplete WEE-1.3 in the germline. In order to study WEE-1.3 expression and localization in the male germline, we are also generating eGFP-tagged WEE-1.3 strains. This project is funded by the Jaramillo-Lambert lab.

Program Abstract #51: Investigating the Impacts of Iron Supplementation and Chelation on Steroid Hormone Signaling in *Drosophila melanogaster* Oogenesis

Kaylia Edwards, Michelle Starz-Gaiano
University of Maryland Baltimore County

The fruit fly, *Drosophila melanogaster*, acts as an advantageous model of study due to its genetic homology to other animals, including humans, and abundantly available genetic techniques. *Drosophila*'s ovaries are composed of egg chambers, each consisting of somatic and germline cells. Although the developmental regulation of oogenesis is well studied, the role of metal ions in the homeostasis of this process is not well understood. Our lab's previous research identified *ferritin 1 heavy chain homologue (fer1HCH)* as a target regulated by ecdysone signaling. Ecdysone, the primary steroid hormone in flies, regulates the timing of crucial transitions of development. *Drosophila*'s *fer1HCH* gene encodes a subunit of the ferritin molecule, responsible for sequestering iron. In this study, we are investigating the role that iron homeostasis has on development and corresponding ecdysone receptor presence in ovaries. We grew the flies in food supplemented with or depleted of nutritional iron by the use of Ferric ammonium citrate (FAC) and Bathophenanthrolinedisulfonic acid (BPS), respectively. We assayed the timing of eclosion in pupal development, phenotypic germline cell viability and morphology, and relative ecdysone receptor presence. Results of this investigation provided strong preliminary data indicating delays in pupal eclosion times as well as morphological defects in germline cells at higher concentrations of iron. We continue to analyze how iron chelation impacts the discussed factors and cellular development by mutant analysis. This work was funded by a National Science Foundation Award IOS-1656550 to M.S.G.

Program Abstract #52: Characterizing the Role of Sperm Proteins SPE-11 and F07A5.2 during Spermatogenesis and Early Development in *C.elegans*

Nancy Duker, Ji Kent Kwah, Frank Doring, Aimee Jaramillo-Lambert
University of Delaware

Haploid genomes from sperm and oocytes are required to form a diploid zygote during fertilization. In addition to haploid genomes other components from these specialized cells are essential for the success of both molecular and physiological events from fertilization to embryogenesis. So far *spe-11* is the only known sperm-specific gene required for the early events of embryonic development in *C.elegans*. Embryos fertilized by *spe-11* mutant sperm do not complete meiosis, form a weak eggshell and do not orient the first mitotic spindle correctly. SPE-11 localizes as a fenestrated ring around the DNA in nuclei undergoing spermatogenesis. We have recently identified another sperm protein, F07A5.2, that interacts with SPE-11. We have found that a deletion allele of F07A5.2 reduces embryonic viability in hermaphrodites; however this reduction is rescued when fertilized by wildtype sperm. Through the creation of the GFP::F07A5.2 line, we

found that F07A5.2 has a similar localization pattern to SPE-11 where proteins appear as a fenestrated ring around the DNA of nuclei during spermatogenesis. In the future, I will determine if F07A5.2 and SPE-11 colocalize during spermatogenesis and whether they are dependent on each other for localization in sperm meiosis. This will help determine the potential roles these sperm genes play in the events required for successful embryogenesis in *C.elegans*.

Program Abstract #53: Investigating microRNA *let-7* in cell migration in *Drosophila melanogaster*

Chase Andre, Mallika Bhattacharya, Michelle Starz-Gaiano
University of Maryland Baltimore County

Cell migration is an essential process in animal tissue development. The coordinated movement of a group of cells - collective migration - requires proper regulation of molecular signaling at multiple levels. Our research seeks to characterize these signaling regulators in *Drosophila melanogaster* border cells within the ovaries. *Drosophila* is a useful model for studying cell migration, as it shares homology with humans in developmental signaling pathways, can be manipulated using genetic tools, and their small size makes them ideal for direct tissue imaging. In both flies and humans, cell migration is well-studied on the protein level, but less is known about the roles of non-coding RNAs in this process. Our bioinformatics analysis suggests that several miRNAs may be regulated by signaling pathways necessary for border cell migration, such as the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. Among these is microRNA *let-7* (*mir-let7*), implicated in neuronal integrity and lifespan in flies. We are currently investigating the requirement of *let-7* in border cell migration. We manipulated *let-7* expression and evaluated defects using immunofluorescence. Notably we found that overexpression of *let-7* resulted in lethality. Our preliminary data indicates altered *let-7* expression results in cell migration delays. Using bioinformatics, we have identified potential *let-7* target genes associated with migration. Moving forward, we will assess the effects of altered *let-7* levels on the expression of predicted target genes associated with migration. Studying miRNA involvement in migration will elucidate conserved roles in human development, disease, and wound healing. This work was funded by a UMBC Undergraduate Research Award to C.A. and NSF Award IOS-1656550 to M.S.G.

Program Abstract #54: Characterization of functionalized carbon dots and their interactions with bone: toward the development of bone-specific nanocarriers for drug delivery

Quan Chau, Lesly Corado-Santiago, Rachel DuMez, Esmail Miyanji, Shannon Jones, Jonathan Dattelbaum, Isaac Skromne
University of Richmond

Osteoporosis treatments focus on preventing bone erosion instead of restoring bone mass because drugs that could promote cell proliferation in bone could promote cancer in other tissues. Thus, methods that can bypass these adverse effects by directly delivering drugs to bones are highly sought after. Carbon nanodots (C-dots) have emerged as novel therapeutic and diagnostic biomaterials due to their unique physicochemical properties such as small size (<10 nm), high carbon content, robust photostability and bright fluorescence. Our previous work has shown that particular preparations of C-dots bind to high affinity and specificity to larvae and adult zebrafish bones undergoing homeostatic turnover, repair and regeneration. Here we report further experiments characterizing C-dot's physicochemical properties and show their potential as drug-nanocarriers. We have derivatized C-dots in the presence of different concentrations of the infrared fluorophore Cy5, and characterized their bone-binding properties, immunogenicity and bio-tolerance. Spectrophotometric analysis confirmed that Cy5 fluorescence at 650nm increased proportionally to the level of Cy5 bound to the C-dots. Cell culture assays using mouse macrophages indicate that unconjugated and Cy5-conjugated C-dots do not trigger inflammation. Finally, similar to controls, Cy5-labeled C-dots injected into adult zebrafish undergoing caudal fin regeneration were observed to bind to bones, at sites of appositional growth, without interfering with bone regeneration and growth processes. We did observe, however, a dose-dependent decrease in C-dots deposition in bones as the amount of conjugated Cy5 increased. Together, these results support the further development of C-dots

as therapeutic agents for the highly specific delivery of drugs to bones. (Funding support NIH-NIAMS R21AR072226 and NSF-DMR 1809419).

Program Abstract #55: *Fgfr1* and *Fgfr2* are redundantly required for proper somite differentiation in the mouse

Michael Boylan, Mark Lewandoski

National Cancer Institute Center for Cancer Research

In vertebrate development, somites are periodically added to the embryonic axis during posterior axis extension. Once formed, somites mature, so that “older”, more rostral somites are more differentiated, into lineages of axial skeleton, tendon and muscle. Though the role of FGF signaling in the presomitic mesoderm is established for somite generation, its role in somite differentiation is less clear. To address this, we inactivated *Fgfr1* and *Fgfr2* only within differentiating somites with the *Meox1^{Cre}* mouse line. We assessed mutant gene expression by Hybridization Chain Reaction, which provides a quantitative assay of multiple markers. Loss of somitic FGF signaling by embryonic day (E)9.5 was evident by a severe downregulation of FGF response genes (e.g. *Spry1*, *Etv4*). We then examined gene expression needed for skeleton, tendon, and muscle development. Myotome (muscle lineage) markers indicated abnormal migration of the myotomal lineage, and specific muscle layers are absent in neonates. Syndetome (tendon lineage) and sclerotome (skeletal lineage) markers are downregulated at E9.5 and E10.5, suggestive of tissue identity loss. Especially intriguing are changes in *Tbx18* and *Uncx4.1* expression, which label the rostral and caudal halves of the somite, respectively. Mutant expression of *Uncx4.1* is reduced in rostral (more mature) somites at E9.5; at E10.5 it is nearly extinguished while *Tbx18* expression is expanded. Loss of *Uncx4.1* expression is correlated with increased apoptosis in the caudal portion of the sclerotome. Consistent with aberrant sclerotomal development, the ribs and neural arches are malformed in mutant neonates. Future work will establish whether loss of somitic rostral-caudal identity precedes or is preceded by increased cell death. This research reveals an unexpected role for FGF signaling in maintaining somite rostral-caudal identity after somite segmentation has occurred, and in promoting sclerotome cell survival. Funded by intramural NIH funds.

Program Abstract #56: Determining the Role of Ferritin in Collective Cell Migration in *Drosophila melanogaster*

Maria Bieberich, Mallika Bhattacharya, Michelle Starz-Gaiano

University of Maryland Baltimore County

Collective cell migration is a vital aspect of a number of biological processes, including immune response, wound healing, embryonic development, and tumor metastasis. Border cell migration that occurs during *Drosophila* oogenesis is a unique model system that is amenable to genetic manipulation while allowing in vivo investigation of collective cell migration. Additionally, *Drosophila* signaling mechanisms are largely conserved with humans, granting the potential identification of key regulators of migration. A microarray identified the iron-carrier protein Ferritin as a transcriptional target of the steroid hormone signaling pathway, which regulates the timing of border cell migration. Ferritin's role in iron storage and transport makes it indispensable to general homeostasis and stress response mechanisms within the cell. Previous work in our lab has identified enriched Ferritin expression within the border cells. Because of this, we hypothesize that Ferritin may play a role in regulating border cell migration and here, we present Ferritin's requirement in border cell migration. Upon knockdown of Ferritin subunits within the border cells and follicular epithelium of the egg chamber, we observed delays in the early stages of migration. Expressing a mutated Ferritin subunit within the egg chamber resulted in mild defects in border cell migration. To determine if Ferritin mediates cell adhesion within the egg chamber, we evaluated β -catenin and E-cadherin distribution. In the future, we will continue to characterize interactions with other cell adhesion proteins and examine Ferritin's interactions with changes in dietary iron. Identifying Ferritin's role within the context of border cell migration will contribute to better understanding the role of iron homeostasis in migration in development and disease.

This work was funded by a UMBC Undergraduate Research Award to M.B. and NSF Award IOS-1656550 to M.S.G.

Program Abstract #57: Investigating the Role of *foxD3*-like during *Nematostella* Neurogenesis

Devin Shennard, Dylan Faltine-Gonzalez, Michael J Layden

Lehigh University

Investigating neuronal development in the sea anemone *Nematostella vectensis* provides insights into the origin and evolution of bilaterian nervous systems including complex centralized nervous systems. We recently identified ~20 putatively neurogenic transcription factors expressed at the earliest stages of neurogenesis, including the fox family homolog *foxD3*. Characterization of *foxD3* expression via in situ hybridization demonstrated a salt and pepper pattern characteristic of neural cells in *Nematostella*, and *foxD3* homologs play a role in vertebrate neurogenesis. Together these findings led us to speculate that *foxD3* regulates *Nematostella* neurogenesis. To characterize the role of *foxD3* in *Nematostella* we have investigated how loss of *foxD3* impacts other known neural progenitor genes and known neuronal markers. shRNA mediated knockdown of *foxD3* decreased expression of cnidocyte markers such as *PaxA* and *NCoI-3* and revealed that *foxD3* is not downstream of the putatively pan neural progenitor genes *soxB2* and *ath-like*. The loss of cnidocyte cells due to *foxD3* knockdown has been seen via in situ hybridization and DAPI staining to visualize mature cnidocyte capsules. However, we were unable to replicate the loss of *paxA*, thus it is not clear where *foxD3* functions to cnidocytes differentiation pathway. Moving forward we are investigating if *foxD3* is expressed in progenitor cells or differentiating cnidocytes and whether other neuronal fates require *foxD3*. Additionally, we will seek to elaborate the cnidocyte role of *foxD3*. Understanding where *foxD3* falls within *Nematostella* neurogenesis is important as cnidocytes are an evolutionarily novel neuronal fate and identifying how they arose will provide insights on mechanisms that drive nervous system evolution. This project is funded by NIH R01 GM127615 and NSF CAREER 1942777 grants.

Program Abstract #58: *met-2* and *set-25* Have Sexually Dimorphic Effects on Chromosome Structure in *C. elegans*

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University of Delaware

Meiosis is a highly conserved cellular division, which allows for the formation of haploid gametes. During meiosis chromosomes undergo two successive segregation events. Homologous chromosomes segregate in meiosis I and the sister chromatids subsequently segregate in meiosis II, leading to the production of egg and sperm with half the number of chromosomes of the parent cells. Although many similarities exist between meiosis in males and females, there are also meiotic mechanisms that differ between oogenesis and spermatogenesis. In *C. elegans*, we found that the bivalent length of diakinesis oocytes is significantly longer than spermatocytes. In addition, we found that mutations in different genes involved in histone post-translational modifications have distinct impacts on oocyte and spermatocyte bivalent length. A mutation of *set-25(n5021Δ)*, a histone methyltransferase converting histone H3 lysine 9 dimethylation (H3K9me2) to trimethylation (H3K9me3), reduces the bivalent length in oocytes. On the other hand, *met-2(n4256Δ)*, a histone methyltransferase depositing H3K9me2 in the germ line decreases the bivalent length in spermatocytes. Although bivalent length is altered in these mutants, I discovered that there is no significant impact on embryonic viability. Because the epigenome is complex there could be redundant mechanisms to prevent germline dysfunction. Analysis of a *met-2(n4256Δ); set-25(n5021Δ)* double mutant, which have previously been found to interact genetically, resulted in a significant increase in diakinesis bivalent length compared to wild type and the single mutants. I am currently analyzing the localization of histone modifications in oogenesis and spermatogenesis in both wild type and mutant animals to determine if there are any differences between oogenesis and spermatogenesis and whether there is any impact on meiotic processes in the mutants. This work is supported by R35GM142524 to AJL.

Program Abstract #59: Anatomy and Development of the Pectoral Fin Vascular Network in the Zebrafish

Scott Paulissen, Brant Weinstein

Eunice Kennedy Shriver National Institute of Child Health and Human Development

In this work, we describe the assembly of the vascular network supplying blood to the zebrafish pectoral fins, analogous structures to the forelimb of tetrapods. The superficial location of the pectoral fin and its developing vessels make it ideal for observing and studying a broad timespan of vascular development and vascular network assembly. Our analysis of this process spans from initial sprout into the limb bud through the first adult-like vascular network forms at approximately 3-4 weeks post fertilization. The blood vessels that feed the pectoral fins begin via a highly stereotyped process that starts with invasion of dorsal and ventral sprouts from the common cardinal vein into the fin bud. These sprouts grow around the endoskeletal disk of the pectoral fin and converge to become the primary arc of the pectoral fin vasculature. Completion of the flowpath and perfusion of the primary arc with blood only occurs after a sprout from the base of the ventral arm of the primary arc migrates to and attaches to the dorsal aorta; this attachment follows a reproducible path in the trunk to supply an arterial feed near the base of the second trunk intersegmental vessel. After the initial embryonic flowpath through the fin is assembled, we then show additional, and fascinating, reorganization events occur to bring about an adult-like vascular structure. The primary arc of the pectoral vessel remodels to form a distal vascular plexus in the fin fold that subsequently remodels a second time and sprouts separate arterial and venous networks that give rise to the vasculature that feeds the fin rays. We also show early data showing that notch signaling contributes to the patterning of specific branches of the initial circulatory circuit. Funded by the NICHD Intramural Research Program.

Program Abstract #60: miR-1 regulates mitotic spindle arrangement and cytokinesis

Kayla Hammond, Nina Faye Samplio, Jia L. Song

University of Delaware

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by binding to the 3'UTR and silencing translation. In vertebrates, miR-1 is known to regulate cardiac development, skeletal muscle proliferation and differentiation, and blood vessel formation. In the sea urchin embryo, miR-1 overexpression (OE) significantly delayed development, leading to a high percentage of embryos arrested at the cleavage stage and embryonic lethality. Preliminary results show that miR-1 OE results in misaligned mitotic spindles and defective cytokinesis, potentially leading to embryonic arrest. The functional role of miR-1 in mitosis and cell division is unknown. The LGN-Gai-NuMA complex is involved in anchoring astral microtubules to the cell cortex. Cdc42 has been known to play a part in the binding of microtubules to the kinetochores of chromosomes, as well as to play a role in actin polymerization, crucial for proper cytokinesis. Prior studies showed that perturbation of either the LGN-Gai-NuMA complex or Cdc42 leads to misaligned mitotic spindles and cytokinesis failure, respectively. Based on their role in mitosis and that we bioinformatically identified miR-1 binding sites within LGN-Gai-NuMA complex and Cdc42 transcripts, we hypothesize that miR-1 suppresses these transcripts to mediate mitosis. Using dual luciferase assay and site-directed mutagenesis, we demonstrated that miR-1 directly suppresses Cdc42. This work will provide a deeper understanding of post-transcriptional regulation of an evolutionarily conserved miRNA and its role in mitosis. The understanding of how miRNAs regulate mitosis will contribute to our knowledge of this highly conserved and regulated process that results in proper and precise cell division. Funding: NSF IOS CAREER, NSF MCB, NIH Neurobiology COBRE.

Program Abstract #61: Studies on the role of pluripotency factors in neural crest development

Hannah Geller, Karla Barbosa-Sabanero, Jenny Hsin, Laura Kerosuo

National Institute of Dental and Craniofacial Development

The neural crest is a transitory population of stem cells that emerge at the neural plate border after gastrulation and eventually migrate out from the neural epithelium to form various cell types throughout the

body in vertebrate embryos. Originating from the ectodermal germ layer, they form peripheral nervous system components and melanocytes, but they also differentiate into mesodermal- and endodermal-like cells such as craniofacial bone and cartilage, as well as chromaffin cells. Determining the basis of the neural crest's exceptionally high stem cell potency is central to understanding neurocristopathies, neural crest-derived cancers, and even the origin of vertebrates. We have previously shown that a subset of premigratory neural crest cells co-express pluripotency genes in the chick embryo, but the functional role of these stem cell factors remains unknown. To address this question in the context of human neural crest development, we utilize an *in vitro* embryonic stem cell-derived 3D ectodermal organoid model that forms migrating neural crest cells during a ten-day protocol. By analyzing the premigratory stages of this protocol, we have confirmed that these *in vitro* organoids have an equivalent of the Nanog/Oct4-positive *in vivo* neural crest stem cell niche. Immunostainings show a higher proliferative capacity and expression of E-cadherin as characteristics of this niche as compared to the neural (future central nervous system) and non-pluripotent neural crest cells surrounding it. Preliminary data using JQ1, a pharmacological inhibitor of Nanog, effectively abolishes the premigratory organoid's capacity to form neural crest. Ongoing knockdown studies will further elucidate the role of Nanog and Oct4 and determine whether these pluripotency genes are also essential for the fate decisions of differentiating migratory neural crest cells. Funding by NIH DE000748-04.

Program Abstract #62: Intrinsic regulation of thyroid hormone signaling in human retinal organoids
Christina McNerney, Katarzyna Hussey, Kiara Eldred, Clayton Santiago, Seth Blackshaw, Robert Johnston
Johns Hopkins University

Thyroid hormone (TH) signaling is critical for normal metabolism and development. Endocrine regulation of TH on an organismal scale promotes metabolic health. In contrast, TH levels are regulated in individual developing tissues to control cell fate specification. How TH signaling is regulated locally during human development is poorly understood. To address this question, I studied how dynamic TH signaling specifies cone photoreceptor subtypes in human retinal organoids. TH signaling promotes red/green cone fate and suppresses blue cone fate. My data suggest that DIO3, a TH-degrading enzyme, is expressed in retinal precursor cells (RPCs) to ensure low TH signaling and generate blue cones early. Later, as RPCs differentiate and turn off DIO3, DIO2 (a TH-activating enzyme) is expressed in photoreceptor precursors and blue cones to increase TH signaling and promote red/green cone fate. In addition to these spatiotemporal dynamics, a negative autoregulatory feedback loop controls levels of TH signaling pathway genes within the retina. Genetically or pharmacologically elevating TH levels led to the activation of genes that degrade TH and repression of genes that activate, transport, and mediate signaling of TH. These perturbations of the local TH negative autoregulatory feedback loop altered the ratios of photoreceptor types and generated mixed photoreceptor fates. These data suggest that, in addition to negative autoregulation of TH levels at the organismal scale, homeostatic regulation of TH levels is required locally in developing tissues for cell fate decisions during human development. Funding sources: NIH NEI R01EY030872, F31EY033187

Program Abstract #63: Mechanisms controlling skeletal pattern formation in the Zebrafish fin
Alexander Seaver, M. Kathryn Iovine
Lehigh University

Proper patterning of the vertebrate skeleton is a complex and sensitive process regulated by many expression profiles and small molecules, with improper patterning leading to developmental disorders such as achondroplasia. We utilize zebrafish (*D. rerio*) as a model system due to its robust ability to regenerate the bony structures in the caudal fin to better understand the pathways responsible for skeletal patterning. Prior research revealed that depletion of Connexin43 (Cx43), as well as blocking of gap junctional intercellular communication (GJIC) by small peptides, leads to premature joint formation and the shortening of bone segments. These findings indicate that Cx43-GJIC suppresses joint formation. Interestingly, levels of *cx43* mRNA oscillate during regeneration and the lowest levels coincide with the initiation of joint

morphogenesis, yet pathways that control this oscillation are poorly understood. In understanding the upstream pathway of *cx43* expression we can come closer to getting a complete picture of patterning the skeletal structures of the regenerating fin. Likely candidates include the phosphatase calcineurin and the morphogen retinoic acid (RA). Data using the calcineurin inhibitor FK506 suggest that calcineurin may be upstream of RA and that both are active in the distal tip of the regenerate. Here we aim to investigate the relationship of calcineurin and RA pathways with the regulation of *cx43* oscillations. Preliminary data suggest that aldehyde dehydrogenase1a2 (*aldh1a2*)—responsible for the synthesis of RA—is reduced preceding the decrease in *cx43* during regeneration, suggesting that RA may positively control *cx43* expression. These data represent the first steps in elucidating the upstream pathway of *cx43* oscillations. This and future studies will increase the number of possible targets for therapies to screen for and mitigate disorders arising from the improper patterning of the skeletal system. Funding: NICHD - R15-HD080507

Program Abstract #64: Gene expression characterization of retinal development and degeneration in *Astyanax mexicanus*

Albert Wakim, Avery Kaye, Brooke Pileggi, Rachel Moran, Suzanne McGaugh, Jeff Trimarchi, Johanna Kowalko
Lehigh University

Formation of the eye during development is critical to normal vision. By studying natural variation in visual function, we aim to identify genetic and developmental factors that contribute to defects in the development of the visual system in vertebrates. To do so, we utilize an emerging model system, the blind Mexican tetra *Astyanax mexicanus*. *A. mexicanus* is a small freshwater fish that exists in two forms, a sighted surface-dwelling form and a blind, cave-dwelling form. These fish are a powerful model system for studying eye development. While they share many of the advantages of zebrafish, including optical clarity of eggs and genetic access, cavefish have evolved changes to the eye that recapitulate aspects of human developmental eye defects. Surface fish develop a large eye, whereas cavefish initiate eye development, but then eye growth arrests and subsequently degenerates. Transcriptomics comparing the developing eye in surface fish and Pachón cavefish led to the discovery of genes that are differentially regulated between cave and surface fish. Whole genome sequencing of wild-caught individuals from multiple cave and surface populations has further led to the identification of genes which are putatively under selection. We have combined these to identify a set of genes that are significantly altered in expression in cavefish versus surface eyes during stages critical for eye development and are putatively under selection. Using in situ hybridization, we have further confirmed that our approach can identify new genes potentially involved in the dramatic differences in eye development and morphology in cavefish. In the future we will employ CRISPR/Cas9 editing to explore how these genes functionally regulate the natural variation in vision. AK and BP thank Lehigh University for a forum student research grant that partially funded this project. SEM and JEK were partially funded by NSF 2202359.

Program Abstract #65: Tagging the *C. elegans* 19S Lid Proteasomal Subunit rpn-6.1 with the epitope OLLAS results in a significant fertility defect.

Destiny Minns, Lourds M. Fernando, Anna Allen
Howard University

The 26S proteasome is best known for its role in protein degradation. It is comprised of a 20S core particle, responsible for the breakdown of a protein, and two 19S regulatory particles (RP), which function in recognizing and translocating ubiquitin-tagged proteins. The 19S RP is comprised of multiple subunits necessary to perform their overall function, yet, there is little known about many of these subunits' specific roles. Previous research in *C. elegans* showed that depletion of specific 26S proteasomal subunits leads to defects such as embryonic lethality and germline integrity defects. Our lab is studying the potential role of individual proteasome subunits in the reproductive process of *C. elegans* hermaphrodites. We have started to do this by using CRISPR/Cas9 endogenous genome editing to tag proteasomal subunits with fluorescent proteins or small epitope tags to learn about a subunit's spatiotemporal location, tissue-specific functions,

and interactions with other germline-specific proteins. This poster will discuss the resulting effects of tagging two 19S lid subunits: rpn-6.1 and rpn-8. When tagged with the small epitope OLLAS, rpn-6.1::ollas animals display a 12% reduction in lifetime brood size compared to wild-type worms. In comparison, GFP::rpn-8 animals show no significant differences in lifetime brood. Interestingly, rpn-6.1 and rpn-8 tagged strains also showed an altered lifespan compared to control animals. Characterization of these two lines is essential before we further investigate individual 19S lid subunit function during *C. elegans* reproduction. Our work is expanding our knowledge of the proteasome's role during reproduction. Funding: Department of Defense Grants W911NF1810465 and 64684-RT-REP.

Program Abstract #66: Escape From the Epithelium: A Role for Trol in Collective Border Cell Migration

Mackenzie Primrose, Alexander George, Michelle Starz-Gaiano
University of Maryland Baltimore County

Cells coordinate their motility in a process called collective migration, which is imperative for development, wound healing, and disease progression in humans. We utilize the border cells, which collectively migrate in the ovary of *Drosophila melanogaster*, as a model system to identify novel genetic regulation of cell motility. Border cells are excellent for investigating cell migration because they are easily visualized live within fly tissues, and the molecular mechanisms that they are known to use are well conserved. Previous research has found that the extracellular matrix (ECM) plays an important role in cell migration and ovary development, although many individual ECM components remain untested. Here we elucidate the potential role of Trol, a secreted heparan sulfate proteoglycan homologous to the human ECM component perlecan, in collective migration. Using the GAL4/UAS binary expression system, we induced overexpression and knockdown of *trol* in the border and epithelial cells and visualized cell migration patterns. Migration delays were seen in both overexpression and knockdown conditions when compared to controls. We are investigating if these delays are due to clustering and detachment defects, or slower cluster movement. Furthermore, we are evaluating the possibility that Trol impacts border cell migration via dystroglycan, a non-integrin ECM receptor shown to directly bind with Trol. Additionally, as Trol has been implicated as a regulator in cytokine diffusion, we are determining if specification by JAK/STAT signaling is affected. Together, this study will provide further insight into how ECM components like Trol mechanically mediate detachment of the border cell cluster from the epithelium and generally impact collective cell migration, cytokine diffusion, and developmental patterning. This work was funded by a UMBC Undergraduate Research Award to M.P. and NSF Award IOS-1656550 to M.S.G.

Program Abstract #67: Investigating the role of *NvashA* during *Nematostella* neurogenesis

Minghe Cheng, Michael Layden
Lehigh University

Most studies of neurogenesis during *Nematostella* development focus on gastrula stages, but how neurons are generated at planula stages is less understood. Thus, understanding larval neurogenesis will help us better understand how the nerve net is built in *Nematostella*. Here we focus on the achaete-scute homolog *NvashA* during neural development. Previous study found that *NvashA* is necessary and sufficient to promote neurogenesis in gastrula stages. However, its role at planula stages has not been studied. We investigated the role of *NvashA* at larval stages using RNA interference. Despite a requirement for *NvashA* to specify *NvLWamide*⁺ neurons and *Nvserum amyloid A*⁺ neurons in gastrulae, loss of *NvashA* had no detectable impact on later born *NvLWamide*-like or *Nvserum amyloid A* expressing neurons, suggesting those neurons are regulated independently by *NvashA* in planulae. To gain a better insight about whether *NvashA* still participates in neurogenesis at larval stages, we screened previously identified neuronal markers for changes in expression following loss of *NvashA* in planulae. *Nvcoup-like2* is reduced, suggesting that *NvashA* regulates these neurons in larval stages. Interestingly, *Nvcoup-like2* is not a target of *NvashA* at the gastrula stage. These data suggest that *NvashA* differentially regulates neuronal subtype marker expression during *Nematostella* development. We are currently developing a dominant negative

mutant for NvashA and optogenetic misexpression alleles to improve our understanding of NvashA functions during neurogenesis and during regeneration. This project is funded by NSF CAREER 1942777 and NIH R01 GM127615.

Program Abstract #68: Defining the substrate specificity and signaling function of lysine methyltransferase SMYD3

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University of Maryland Baltimore County

SMYD3, a member of the evolutionary conserved SET- and MYND- domain family of lysine methyltransferases (KMTs), is predominantly expressed in early embryonic development and modulates lineage specific gene expression. SMYD3 is involved in myogenesis and muscle differentiation of cardiac and skeletal tissue. It is also overexpressed in multiple cancers including prostate cancer, where high expression levels correlate with poor patient survival. SMYD3 targets both histone and non-histone proteins for methylation including histone H4 at K5 and MAP3K2, however a clear link between SMYD3 methylation activity and its proto-oncogenic functions has not been defined. In multiple prostate cancer cell lines and in vivo, we show that SMYD3 promotes tumorigenesis by contributing to multiple hallmarks of cancer including migration, invasion, and anchorage-independent growth, which are also crucial properties during development. Similar to other cancer cells, SMYD3 is predominantly cytoplasmic in prostate cancer cells, suggesting that methylation of a non-histone substrate by SMYD3 is likely promoting malignancy in prostate tissue. We are pursuing multiple avenues to identify the key non-histone substrate(s) of SMYD3 which links its activity to tumorigenic phenotypes in prostate cancer, with a particular focus on the regulation of proteins involved in epithelial-mesenchymal transition (EMT). Our data provide insights into the substrate specificity of SMYD3 in prostate cancer and enhance our existing understanding about SMYD3-dependent signaling pathways potentially revealing new therapeutic opportunities in prostate cancer. Funding source: NIH R01GM124342

Program Abstract #69: ETR-1 isoforms containing exon 8 are important for reproduction and development in *C. elegans*

Benedict Quagraine, Sarai Sales, Sydney Bell, Anna Allen
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The CUG-BP, Elav-like family (CELF) of RNA Binding Proteins (RBPs) regulates several RNA processing steps and dysregulation of CELF-mediated processes is implicated in numerous human diseases. In the nematode *Caenorhabditis elegans* the ELAV-Type RNA-binding protein, ETR-1, is one of two CELF RBPs and is essential with genetic null animals being embryonic lethal. Previous studies demonstrated that ETR-1 is required for developmental processes including muscle development, nervous system development, and reproduction. RNAi depletion of ETR-1 from the 4th larval stage results in a reduced reproductive capacity of the treated mother and an increase in the number of apoptotic germ cells. The ETR-1 pre-mRNA is highly alternatively spliced to form 19 isoforms and we hypothesize that ETR-1 plays its diverse roles through isoform-specific manners. Here we will show that heterozygous *etr-1(tm6221)/+* mothers exhibit a 23% reduction in brood and further depletion of ETR-1 via RNAi in *etr-1(tm6221)/+* hermaphrodites results in a 36% reduction in brood. Using a new viable mutant with a premature stop codon in exon 8, *etr-1(lq61)*, we show that ETR-1 isoforms containing exon 8 play significant roles in development. *etr-1(lq61)* animals have a 66.7% brood reduction, a 21.5% reduced lifespan, and a delay throughout embryogenesis and larval development compared to controls. We will use the *etr-1(tm6221)/+*, *etr-1(lq61)* and other mutants to investigate ETR-1's role in germline apoptosis. Finally, we have endogenously tagged the *etr-1* genomic locus with the OLLAS epitope tag by CRISPR/Cas9. This strain exhibits wild-type character, germline ETR-1::OLLAS expression comparable to ETR-1 antibody staining, and will be essential in future investigation of ETR-1's germline functions. Our research adds new knowledge to the role of this essential RBP in germ line function. Funding: NIH 1R15HD084253-01A1, Department of Defense Grants W911NF1810465 and 64684-RT-REP.

Program Abstract #70: Not all axolotls grow the same

Natalie Ellis, Moshe Khurge

Bridgewater College

We investigated individual growth trajectories in axolotls during the first 6 months post-hatching. Sibling animals were randomly divided into groups that were housed at diverse water temperatures or maintained under different diet protocols. Mass and body lengths were obtained on a weekly/biweekly schedules. We observed a substantial increase in the variance of growth measures for animals within each group of animals.

Program Abstract #71: Investigating the Role of the Zinc-finger Transcription Factor Sp8 in the Vertebrate Trunk-to-Tail Transition

Samuel J. Kuo, Robert J. Garriock, Mark W. Kennedy, Ravindra B. Chalamalasetty, Terry P. Yamaguchi

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Sp8 is a zinc-finger transcription factor and Wnt target gene with a well-characterized role in limb development. *Sp8* null mice also exhibit many primary axis defects including spina bifida and axial truncation at the level of the tail that remain largely uncharacterized. Vertebrate tail development exhibits significant differences from trunk elongation, including a transition from primary to secondary neurulation and relocation of axial progenitors, particularly the bipotent neuromesodermal progenitors, from the primitive streak to the tail bud. How Sp8 regulates this trunk-to-tail transition and its broader role in primary axis patterning, however, remains largely unexplored. Transcriptional profiling of *Sp5;Sp8* double mutant embryos, which exhibit severe caudal truncations, reveals differentially expressed components of many signaling pathways including the retinoic acid (RA) pathway – a potent activator of neural differentiation. To explore the potential role of RA in the development of the caudal axial defects, we have begun to investigate RA signaling activity in *Sp8* mutants. Preliminary analysis of *Cyp26a1*, a catalytic enzyme that degrades RA, reveals a dramatic loss of *Cyp26a1* RNA in *Sp8* null tail buds at E9.5. As *Cyp26a1* null mice exhibit axial phenotypes strikingly similar to those in *Sp8* mutants – including tail loss and neuropore closure failure, our work suggests that *Cyp26a1* is an important effector of Sp8 activity. *Cyp26a1* is also a known target of T/Brachyury (T/Bra), a critical mesodermal determinant and regulatory target of Sp8, raising the possibility that *Cyp26a1* could be indirectly regulated by Sp8 through T/Bra. Indeed, T/Bra expression is also lost in *Sp8* null tail buds. We hypothesize that Wnts, signaling via Sp8 and T/Bra, protect axial progenitors from excessive RA during the trunk-to-tail transition by inducing *Cyp26a1*. This work was supported by funding from the Intramural Research Program, NIH, NCI, CCR project #ZIA BC 010345

Program Abstract #72: Investigating the Role of Testicular Vasculature in Fertility

Keith Ameyaw, Leah Greenspan, Brant Weinstein

Eunice Kennedy Shriver National Institute of Child Health and Human Development

Blood vessels play a crucial role in the testis by providing oxygen, nutrients, and gonadotropin signals. Lack of an adequate blood supply can result in various malformations such as varicocele, testicular torsion, hormonal imbalances, testicular cancer, and infertility. In the context of spermatogenesis, the role of the vasculature in spermatogonia stem cell renewal and differentiation is unknown. Blood vessels reside in the interstitial space between the seminiferous tubules which contain developing spermatogonium and gonadal somatic cells. Previous work in mice has shown that spermatogonia stem cells show biased localization to the blood vessel network suggesting possible interactions between the blood vessels and spermatogenesis. To further investigate the role that the testis vasculature plays in spermatogenesis and fertility, we are using zebrafish as a model organism due to its conserved vascular and testicular anatomy, and genetic and experimental accessibility. Using a combination of tissue clearing, immunohistochemistry, and high-resolution confocal microscopy we found that the adult testis has two major blood vessels that run through with smaller vessels sprouting off to encircling each seminiferous tubule. In addition, we are using Trap RNAseq to profile endothelial cell gene expression in the testis to understand how testicular

endothelial cells differ from other endothelial cell populations, and to uncover the role they play in spermatogenesis. Since about 25% of the causes of infertility are unexplained, better understanding how the testis vasculature contributes to spermatogenesis may shed light on these unexplained causes and help promote better fertility outcomes. This work is supported by the Intramural Program of the NICHD.

Program Abstract #73: The Wnt-dependent Transcription factors, Sp5 and Sp8, regulate the survival of axial progenitors

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The conserved Wnt/b-catenin signaling pathway controls posterior identity throughout the animal kingdom. During mouse gastrulation, Wnt3a emanating from the primitive streak (PS) induces multipotent neuromesodermal progenitor (NMPs) from the adjacent pluripotent caudal epiblast (CE). NMPs sequentially generate the spinal cord and somitic mesoderm of the neck, trunk and tail. Transcriptional profiling of *Wnt3a* mutants led to the identification of the Zn-finger transcription factors, Sp5 and Sp8 (Sp5/8), as potential effectors of Wnt3a. We have previously shown that *Sp5/8* double knockouts (DKO) exhibit severe axial truncations that closely resemble *Wnt3a* mutants however, NMPs were not characterized. We have combined genetic lineage tracing of the CE using *Nkx1-2CreERT2* mice with IF analysis of NMP markers (T/Bra and Sox2), to examine the fate of the CE and NMPs in *Sp5/8* DKO. This approach revealed that NMPs failed to differentiate from CE due to down-regulation of T/Bra. Instead of ingressing through the PS and forming caudal mesoderm, the progeny of *Nkx1-2+* CE largely remained in the PS epiblast and differentiated into neural progenitors. This retention of cells in the epiblast resulted in elongation of the PS, illustrating how changes in progenitor homeostasis can regulate caudal morphology. Significant apoptosis was also observed in the PS, which was confirmed through in vitro differentiation of *Sp5/8* DKO ESCs. RNA-seq and ChIP-seq analysis demonstrated that Sp5/8 directly regulated genes in the Wnt, Fgf, Nodal and RA signaling pathways suggesting that cell death arises due to alterations of the signaling environment. We suggest that Sp5/8 are crucial mediators of Wnt3a signaling that function in the niche to establish a sustained signaling network for the maintenance of NMPs. This work was supported by funding from the Intramural Research Program, NIH, NCI, CCR project #ZIA BC 010345.

Program Abstract #74: Variables Impacting Asexual Reproduction in *Nematostella vectensis*

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We investigated the variables affecting asexual reproduction in *Nematostella vectensis*. *N. vectensis* can reproduce both sexually and asexually, with the asexual process involving a clone fissioning off of the parent anenome. Following fissioning, the parent and clone have the ability to regenerate missing structures leading to two functional anemones. We tracked individuals from a *NvLWamide-like::mCherry+* transgenic population to test if the rate of asexual reproduction is related to initial animal size as well as the size of clones generated. The *LWamide::mCherry* transgene labels a subset of well-characterized neural subtypes in the *Nematostella* nerve net. Using the *LWamide::mCherry* transgene, we are able to locate and visualize longitudinal neurons, which have been shown to scale with animal length and provide a reliable method for measuring animal size based on the number present. We found that ~20% of adults asexually reproduced and that this behavior occurred after ~8 days on average, and was not related to the animal's nerve net size. Additionally, we tested how much of the adult nervous system was partitioned to the clone following the asexual event. Adults lost between 10-30% of their longitudinal nerve next to their clones. Further, we are currently analyzing neural subtype counts from adults and clones following the fissioning event so that changes in neural subtype numbers can be tracked as the animals regenerate missing structures. We are

looking to compare this data to previously published neuronal subtype data from work characterizing nerve net changes following whole-body axis regeneration. This investigation was funded by the Lehigh University Undergraduate Research Grant.

Program Abstract #75: Msgn1 regulates ingression, migration, and differentiation of mesodermal progenitors to coordinate cell fate decisions and axial extension

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National Cancer Institute Center for Cancer Research

Neuromesodermal progenitors (NMPs) are bipotent progenitor cells that contribute to presomitic mesoderm (PSM) and spinal cord and drive posterior axial extension in mouse development. The basic helix-loop-helix transcription factor *Msgn1* acts downstream of Wnt signaling as a master regulator of posterior mesoderm development and may play a role in directing NMP differentiation toward PSM fates. Embryos lacking *Msgn1* expression are characterized by a mass of immature mesodermal progenitor cells in the tailbud region and a resulting inability to form differentiated posterior trunk and tail tissue. While *Msgn1* mutant mice display a phenotype similar to *Tbx6* mutants, mutant mice lack the ectopic neural tissue seen in other mutants of Wnt pathway genes and targets of Wnt signaling. Previous work has shown that *Msgn1* is required for correct differentiation, ingression and migration of mesodermal progenitors, but the mechanisms by which *Msgn1* regulates and coordinates NMP differentiation and axial extension remain unclear. Here we investigate the hypothesis that *Msgn1* is required for progenitor cells to undergo a complete epithelial-to-mesenchymal transition (EMT) and ingression through the primitive streak, thereby moving away from multipotency-promoting factors and differentiating to form PSM. We use bioinformatic analysis to demonstrate that *Msgn1* regulates genes involved in ingression and migration, such as *Hox* genes and *Ap1nr*, and show that the expression of several *Hoxb* genes is downregulated in *Msgn1* mutant mice using in vivo approaches. Single cell level analyses are consistent with a model wherein mutant cells display a partial EMT. Taken together, these data indicate that *Msgn1* may be involved in multiple interconnected but distinct aspects of axial extension and trunk development and underscores its role as a master regulator of mesodermal fate. This work was supported by funding from the Intramural Research Program, NIH, NCI, CCR project #ZIA BC 010345.

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We are grateful for the dedicated support and assistance provided by our on-site coordinators at Lehigh University and the Zoellner Arts Center. A special thank you goes out to **Mandy Fraley**, who has worked tirelessly on our behalf to make accommodations and to ensure the event runs smoothly. We appreciate you!

We thank the Society for Developmental Biology for its support, particularly **Laura Hilliker**, **Marsha Lucas**, Mid-Atlantic Representative **Michelle Starz-Gaiano**, and **Ida Chow** for their logistical assistance, financial support, and feedback.

We are grateful to **Nikon Instruments** for their sponsorship of the meeting.

We thank **Stephanie Nowotarski** for creating the striking meeting artwork featured on our website and promotional materials. Apparel, mugs, and prints with the meeting design can be purchased online, with proceeds benefitting the Society for Developmental Biology:

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Many thanks to our community volunteers for donating their time, talents, and expertise to this event:

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