Schedule of Events

Day 1 – Wednesday, May 15th

1:00 - 5:00 pm: **Registration and poster setup**
Registration: Ballrooms foyer
Posters: Ballrooms A and B

2:00 - 2:15 pm: **Welcome address in Alumni Theater**

2:15 - 3:45 pm: **Session 1 - Differentiation and Cell Fate**
Alumni Theater

2:15 - 2:45 pm: **Scott Dougan**, University of Georgia (**session chair**)
Transcriptional control of extraembryonic signals during vertebrate development
2:45 - 3:05 pm: **Elyse Christensen**, Kennesaw State University
Investigating the proneural transcription factor NGN-1 via comparative transcriptomics
3:05 - 3:25 pm: **Brandon Carpenter**, Emory University
SPR-5; MET-2 maternal reprogramming antagonizes H3K36me3 in the control of germline versus soma
3:25 - 3:45 pm: **Ceri Weber**, Duke University
Temperature-dependent activation of STAT3 regulates expression of Kdm6b during sex determination in a turtle species

3:45 - 4:00 pm: **Break**
Alumni Theater foyer

4:00 - 5:30 pm: **Session 2 - Development in Cancer and Disease**
Alumni Theater

4:00 - 4:30 pm: **John Parant**, University of Alabama Birmingham (**session chair**)
Roberts Syndrome: developmental disorder and cancer predisposition
4:30 - 4:50 pm: **Mackenzie Davenport**, University of Alabama Birmingham
Targeting oncogenic non-coding RNA in lung cancer
4:50 - 5:10 pm: **Kelsey Lewis**, University of Florida
The endocrine disruptor vinclozolin induces penile malformations by modulating apoptosis in the genital tubercle
5:10 - 5:30 pm: **Zhongyao Ma**, University of Georgia
Reprogramming MEFs into thymic epithelial cells

5:45 - 6:45 pm: **Keynote Address #1**

Alumni Theater

Dr. Cecilia Lo
F. Sargent Cheever Chair and Professor, Department of Developmental Biology, University of Pittsburgh School of Medicine
Modeling the complex genetics of congenital heart disease with mouse forward genetics

7:00 - 8:00 pm: **Dinner**
Ballrooms C and D

8:30 - 10:00 pm: **Poster session #1 and reception**
Odd numbered presented
Ballrooms A and B

Day 2- Thursday, May 16th

7:30 - 9:00 am: **Registration and breakfast**
Registration: foyer outside of ballrooms
Breakfast: Ballrooms B and C

9:15 - 10:45 am: **Session 3 - Morphogenesis and Organogenesis**
Alumni Theater

9:15 - 9:45 am: **Shuyi Nie**, Georgia Institute of Technology (**session chair**)
Protein glycosylation in neural crest EMT and migration
9:45 - 10:05 am: **Corey Bunce**, Duke University
Contextualizing development of the mouse gonad through whole embryo clearing
10:05 - 10:25 am: **Sarah Peters**, University of Alabama Birmingham
TGF beta regulates neurotropic signals in developing teeth
10:25 - 10:45 am: **Hayley Milner**, Kennesaw State University
Analysis of defective heart patterning in akirin mutants

10:45 - 11:00 am: **Break**
Alumni Theater foyer

11:00 - 12:30 pm: **Session 4 - Cell-Cell Communication and Signaling**
Alumni Theater
11:00 - 11:30 am: **Tessa Burch-Smith**, University of Tennessee Knoxville (**session chair**)
Chloroplast development and signaling control intercellular communication in plants

11:30 - 11:50 am: **Joanna Wardwell-Ozgo**, Emory University
A rheostatic switch between transcriptional repressor Smrter and the steroid hormone ecdysone patterns growth and development of wing imaginal discs
11:50 - 12:10 pm: **Marina Martinez-Bartolome**, Auburn University
A bi-phasic role for non-canonical Wnt16 signaling during early anterior-posterior patterning and morphogenesis of the sea urchin embryo
12:10 - 12:30 pm: **Alissa Armstrong**, University of South Carolina
Adipocyte nutrient sensing controls the germline stem cell lineage in Drosophila melanogaster
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12:30 - 1:30 pm: **Lunch**
Ballrooms C and D

1:30 – 2:00 pm: **Faculty "quick fire" session**
Alumni Theater

2:00 - 3:30 pm: **Education session – Community Outreach**
Alumni Theater

2:00 – 3:00 pm **Part 1: How to leverage the knowledge, expertise and resources of our universities to impact the surrounding communities**

3:00 – 3:30 pm **Part 2: Tips for improving our "lay science" language during community interactions**

3:30 - 5:00 pm: **Poster session #2**
Even numbered posters presented
Ballrooms A and B

5:15 - 6:15 pm: **Keynote Address #2**
Alumni Theater

Dr. Philip Benfey
Paul Kramer Professor of Biology, Trinity College of Arts and Sciences, Duke University
Underground signaling

6:30 - 9:30 pm: **Banquet dinner**
Ballrooms C and D

Day 3 – Friday, May 17th

7:00 - 8:30 am: **Breakfast**
Ballrooms foyer

9:00 - 10:30 am: **Session 5 - Evolution and Development**
Ballroom C and D

9:00 - 9:30 am: **John Yoder**, University of Alabama Tuscaloosa (**session chair**)
Intersection and cross-regulation of Homeotic and Sex Determination Pathways in the Drosophila abdomen – a model for morphological evolution and stasis

9:30 - 9:50 am: **Hongyan Sun**, University of Miami
Evolution of Axin function in the Wnt/b-catenin signaling pathway in metazoans: insights from sea urchins and sea anemones

9:50 - 10:10 am: **Alexis Lanza**, Whitney Laboratory for Marine Bioscience
The road less traveled: Activin/Nodal signaling functions in Capitella teleta dorsal-ventral axis formation

10:10 - 10:30 am: **April DeLaurier**, University of South Carolina Aiken
Understanding the role of histone deacetylases (Hdacs) in pharyngeal skeletal development

10:30 - 10:45 am: **Break**
Ballrooms foyer

10:45 - 12:15 pm: **Session 6 - Stem and Progenitor Cell Biology**
Ballrooms C and D

10:45 - 11:15 am: **Elizabeth Ables**, East Carolina University (**session chair**)
Stem cells on steroids: connecting physiological signals with stem cell proliferation and differentiation

11:15 - 11:35 am: **Teresa Lee**, Emory University
Repressive H3K9me2 protects lifespan against the transgenerational burden of germline transcription in C. elegans

11:35 - 11:55 am: **Ann Foley**, Clemson University
The role of Map kinases in differentiation of the SAN

11:55 - 12:15 pm: **Julio Belmonte**, North Carolina State University
A self-organized mesenchymal-to-epithelial model of somitogenesis

12:30 pm: **Awards and goodbye**
Ballrooms C and D

Session # 1- Differentiation and Cell Fate

Abstract #1

Transcriptional control of extraembryonic signals during vertebrate development

Scott Dougan
University of Georgia, USA

The process of germ layer formation is a universal feature of animal development. The germ layers separate the cells that produce the internal organs and tissues from those that produce the nervous system and outer tissues. Most vertebrate embryos contain extraembryonic tissues such as visceral endoderm (VE) in mammals, hypoblast in avian and yolk syncytial layer (YSL) in teleost fish. These tissues serve as signaling centers that produce and secrete morphogens, diffusible molecules that form concentration gradients in the embryo and instruct cell fates in a dosage dependent manner. The signals produced by extra-embryonic tissues are under transcriptional control, but little is known about how expression of these signals is controlled. In teleost fish, YSL formation occurs when cells at the margin of blastomeres collapse and release their cell contents into nearby yolk cell to form a multinuclear cell syncytium. We have established that members of the TGF-β superfamily Nodal and Bone Morphogenetic Protein (BMP) are required in the YSL to pattern the embryo. Here, we identify a unique transcription factor, Max's Giant Associated Protein (MGA), as a key component of a transcriptional network that controls the expression of key signaling molecules in the YSL. We show that this protein has an independent role in the embryo, where it regulates Myc activity. Thus, MGA regulates factors that specify cell fate as well as those that control pluripotency. This suggests a new mechanism for controlling the developmental potential of embryonic cells.

Abstract #2

Investigating the proneural transcription factor NGN-1 via comparative transcriptomics

Elyse Christensen, Alexandra Beasley, Jessica Radchuk, Martin L. Hudson
Kennesaw State University, USA

Errors in transcription factor regulation are implicated in multiple neurodevelopmental disorders, and genome-wide association studies indicate that polymorphisms linked to schizophrenia and autism spectrum disorder frequently map to non-coding regions of the genome. Neurogenins, a family of highly conserved basic helix-loop-helix transcription factors, are required for fate specification of the developing nervous system. The nematode *Caenorhabditis elegans* has a single neurogenin ortholog, *ngn-1*. Given its transparent body, short reproductive cycle, and invariant cell lineage, *C. elegans* is ideal for investigating the downstream regulatory control of *ngn-1*. While some of its targets have been identified in other organisms, primarily NeuroD1, a large disparity in phenotypes between these two mutants suggests additional downstream targets of *ngn-1*. We used two approaches to identify targets of *ngn-1*: candidate gene analysis and comparative transcriptomics. Using gene expression and loss-of-function analyses of candidate genes, we found that *ngn-1* has complex genetic interactions with cell surface receptors (*sax-3*/Robo, ephrin), guidance cues (semaphorin), and cell-surface HSPGs (syndecan). We also used transcriptomics to compare gene expression between wild-type and *ngn-1(ok2200)* mutant embryos. We found that *ngn-1* controls neurogenesis primarily through gene repression, as ~85% of differentially expressed genes were upregulated in the *ngn-1* mutant transcriptome. In addition, we demonstrate that *ngn-1* controls the expression of multiple homeodomain and bHLH transcription factors with known roles in neural differentiation. Taken together, these results support the hypothesis that proneural genes function near the top of regulatory

cascade of numerous downstream transcription factors, and speak to the power of comparative transcriptomics as a tool for identifying novel gene interactions.

Abstract #3

SPR-5; MET-2 maternal reprogramming antagonizes H3K36me3 in the control of germline versus soma

Brandon Carpenter
Emory University, USA

In *C. elegans,* the H3K4me2 demethylase, SPR-5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte where they cooperate to reprogram histone methylation, and thereby reestablish the epigenetic ground state of the newly formed zygote. Here, we show that the progeny of *spr-5; met-2* mutants display a severe developmental delay that is associated with the ectopic expression of MES-4 germline genes. Previous work from the Strome and Kelly Labs demonstrates that maternally deposited MES-4 transgenerationally maintains H3K36me2/3 at ~200 germline genes in a transcription-independent manner, and is required to reactivate germline genes in the subsequent generation. We hypothesized that SPR-5; MET-2reprogramming may be required to prevent MES-4 from ectopically maintaining H3K36me2/3 at germline genes in somatic tissues. By performing ChIP-seq on L1 progeny from *spr-5; met-2* mutants, we find that MES-4 germline genes ectopically accumulate H3K36me3 in somatic tissues. Additionally, knocking down MES-4 suppresses the ectopic expression of MES-4 germline genes and rescues the developmental delay. These data suggest a model where SPR-5; MET-2 maternal reprogramming antagonizes H3K36me2/3 to enable the proper transgenerational control of germline versus somatic cell fates. In the absence of this reprogramming, somatic cells struggle to specify their proper cell fate amongst the noise of inappropriate germline gene transcription, leading to developmental delay. A similar mechanism may underlie the developmental delay of Soto and Kabuki Syndrome patients who have mutations in histone modifying enzymes.

Abstract #4

Temperature-dependent activation of STAT3 regulates expression of Kdm6b during sex determination in a turtle species

Ceri Weber[1], Chutian Ge[2], Jong Gwan Lee[1], Loren Looger[3], Guoying Qian[2], Blanche Capel[1]
[1]Duke University, USA; [2]Zhejiang Wanli University, China; [3]Janelia Research Campus, USA

Many reptiles undergo a fascinating mode of sex determination that is contingent upon the temperature of the nest. This mode of sex determination, called temperature-dependent sex determination, is observed in many turtles, including the red-eared slider turtle *Trachemys scripta elegans* (*T. scripta*). However, in the 50 years since temperature-dependent sex determination was first reported in reptiles, the mechanisms underlying how temperature governs gonad development have remained elusive. In *T. scripta*, the gonad arises as a bipotential tissue that will develop as an ovary at warmer temperatures (31?C) or as a testis at cooler temperatures (26?C). Recently, we showed that knockdown of a male-biased epigenetic regulator, *Kdm6b*, at 26?C resulted in male-to-female sex reversal. In the absence of *Kdm6b*, male genes are not activated and the female pathway is instead initiated, leading to formation of an ovary. A negative regulator of *Kdm6b*, STAT3, is only phosphorylated at 31?C during early gonad development. When pSTAT3 is inhibited at 31?C, *Kdm6b* and its downstream target *Dmrt1* are upregulated. ChIP-qPCR analysis revealed enrichment of pSTAT3 at the *Kdm6b* locus at 31?C and the effects of pSTAT3 inhibition can be rescued by simultaneously knocking down expression of *Kdm6b.* STAT3 is known to be activated by

signaling pathways that respond to environmental signals, including temperature. We propose that activation of these pathways at warmer temperatures promotes phosphorylation of STAT3, blocking expression of *Kdm6b*, thus preventing activation of the male-pathway.

Session #2 – Development in Cancer and Disease

Abstract #5

Roberts Syndrome: developmental disorder and cancer predisposition

John Parant
University of Alabama at Birmingham, Department of Pharmacology and Toxicology, USA

Roberts syndrome (RBS) is a rare developmental disorder, due exclusively to recessive inactivation mutation in the establishment of sister chromatid cohesion gene ESCO2; placing it in a class of diseases called cohesinopathies. RBS patients have a wide range of severities, from embryonic lethal to viable but potentially cancer predisposed. Phenotypes include microcephaly, mental retardation, limb deformities, growth retardation and craniofacial defects; with less penetrant phenotypes like heart disease and cataracts being observed. To better understanding this disease, we have identified a zebrafish *Esco2* null allele. Homozygous loss of *Esco2* in zebrafish is embryonic lethal, resulting in phenotypes consistent with the more severe RBS phenotypes such as microcephaly and growth retardation, partially due to *p53* dependent neuronal tube apoptosis. At the cytogenetic level, homozygous loss of Esco2 results in non-paired chromatids due to sister chromatid cohesion loss. In-embryo single cell live imaging revealed extensive chromosome segregation defects, spindle rotation, and micronuclei formation. Most surprising is that while heterozygous loss of *Esco2* is viable, they display mild cohesion defects (appearing cytological similar to RBS patients) and a predisposition to tumor formation (described in a few RBS patients). Our data suggest that these heterozygous animals have mild genomic instability, which enhances the rate of tumor suppressor gene loss. Further analysis of this and other mutants involved in sister chromatid cohesion have established a "gradient of cohesion dysfunction model" which indicates that the severity of disease is proportional to the degree of SCC loss and the amount of chromosome missegregation. In this ESCO2 deficient patients we hypothesize that subtle genetic alterations in the complex cohesion network will create differing degrees of cohesion dysfunction and variability in patient severity. Understanding this complex genetic network will be important for understanding the variation in human disease. We have generated zebrafish frameshift mutations in the esco2 homolog esco1, as well as antiestablishment factors *Pds5a* and b, *Wapal*, and *Hdac8*, to determine if they modify the *Esco2* loss phenotypes.

Abstract #6

Targeting oncogenic non-coding RNA in lung cancer

Mackenzie Davenport
University of Alabama at Birmingham, USA

Lung Cancer is the leading cause of cancer related death in the world. While great strides have been made in understanding the genetic events necessary to drive the development of many epithelial cancers, the molecular mechanisms which allow for lung cells to initiate carcinoma growth, disseminate, and metastasize remain unclear. Our lab has previously shown that the microRNA miR-31 is overexpressed in patient lung adenocarcinoma compared to normal lung, that increased miR-31 correlates to decreased survival, and using a transgenic mouse model, overexpression of miR-31 alone in the mouse lung epithelia initiates lung tumorigenesis and eventual adenocarcinoma development. This indicates that miR-31 could be a potentially important therapeutic target in lung cancer. While all of the aforementioned studies were performed overexpressing miR-31, we've been able to genetically engineer loss of miR-31 in human lung adenocarcinoma cell lines using CRISPR/Cas9 genome editing. Loss of miR-31 in these cancer cell lines results in decreased cell

growth and colony formation. In addition, preliminary in vivo studies demonstrate that miR-31 loss suppresses tumor growth in a subcutaneous xenograft model. Given that miR-31 expression significantly correlates to increasing pathological stage and that elevated miR-31 correlates to overall patient survival, we have begun to examine whether miR-31 promotes lung cancer dissemination. Our preliminary studies suggest miR-31 regulates cellular processes such as migration and invasion that are involved in lung cancer metastasis. We hypothesize this gene is necessary for the growth and progression of lung tumors, in lung adenocarcinoma and other histological subtypes of lung cancer, and may serve as a potentially important therapeutic target across lung cancer subtypes.

Abstract #7

The endocrine disruptor vinclozolin induces penile malformations by modulating apoptosis in the genital tubercle

Kelsey Lewis[1], Zhengui Zheng[1,3], Lillian Svete[1,4], Marianne Kozuch[1], Mohammed-Zaman Nouri[1], Saunders Ching[2], Ophir Klein[2], Nancy Denslow[1], Martin Cohn[1]
[1]University of Florida, USA; [2]University of California, San Francisco, USA; [3]Southern Illinois University, USA; [4]University of Colorado, USA

In recent decades, there has been a rise of endocrine-related diseases and disorders, including an increased incidence of genital anomalies, low semen quality, adverse pregnancy outcomes, neurobehavioral disruption, endocrine-related cancers, earlier onset of breast development, obesity, and type 2 diabetes (UNEP and WHO, 2013). An example of increased genital anomalies is seen with congenital penile anomaly (CPA) frequency, which has increased to a rate of 1 in 120, or 0.83%, of male newborns (Nelson et al., 2005). The most commonly reported CPA is hypospadias, which is characterized by an atypical urethral opening along the penile shaft, within the scrotum, or in the perineum. It was previously established that by specifying cell type and timing of deletion of the androgen receptor gene, a range of CPAs can be induced in the mouse that mimic the spectrum of human CPAs (Zheng et al., 2015). Male mouse embryos exposed to the endocrine disruptor vinclozolin develop external genital anomalies, and these mimic mouse androgen receptor-deletion defects and human CPAs. Quantification of sex steroid hormones and their respective receptors indicated that they are affected by vinclozolin treatment. Examination of cell death and proliferation demonstrated that these genital anomalies result from disruption of sexually dimorphic cellular processes that normally pattern the genital tubercle.

Abstract #8

Reprogramming MEFs into Thymic epithelial cells

Zhongyao Ma
University of Gerogia, USA

Thymus is one of the primary lymphoid organs. However, through the aging, thymus involution leads to the decrease of immune function, which significantly increases the risk of diseases. Thus, finding a sufficient method to recover the thymus function caused by thymus involution & thymus abnormalities is really significant. One way to recover thymus function after involution is transplant neonatal thymus, but this methods is highly limited by its resources. In order to solve these problems, we are trying to use cell reprogramming to replace lost thymus epithelial cells (TECs, one of the major cell type in thymus which support T cell development and mature. The transcription factor FOXN1 plays a crucial role in thymus development. Previous study successfully reprogramed Mouse Embryonic fibroblast (MEF) into induced functional thymus epithelial cells (iTECs) by using exogenous Foxn1 expression. In this study, we further analyze the gene expression profile of the iTECs as well as the reprogramming pathway by using whole cell population RNA-seq and qRT-pcr.

Our qRT-PCR data shows that iTECs gene expression profile is similar to Foxn1-high thymus epithelial cells, but still missing some important function gene such as Aire. Also, Our RNA-seq data shows that the reprogramming pathway acts in a step-by-step manner, in which the early stage cell population and the late stage cell population is very distinct. In this setp-by-step reprogramming process, several Foxn1-pathway gene and non-Foxn1-pathway gene is involved. In this study, we are also developing a non-transgenic method of iTEC generation for future clinical application. Our data shows by using dCAS9-vpr, we can successfully activate Endogenous Foxn1 gene expression from Foxn1 promoter region and potential enhancer region. Further experiments still need to done to further analyze the reprogramming completeness and efficiency.

Keynote Speaker #1

Dr. Cecilia Lo
F. Sargent Cheever Chair and Professor, Department of Developmental Biology
University of Pittsburgh School of Medicine

Modeling the complex genetics of congenital heart disease with mouse forward genetics

Session # 3 – Morphogenesis and Organogenesis

Abstract #9

Protein glycosylation in neural crest EMT and migration

Shuyi Nie
Georgia Institute of Technology, USA

Neural crest epithelial-mesenchymal transition (EMT) and migration involves coordinated remodeling of cell-cell and cell-matrix adhesions. During this remodeling event, interaction between adhesive molecules needs to be dynamically regulated. We recently explored the involvement of protein glycosylation in regulating cell adhesions and consequently controlling neural crest EMT and migration. Through the employment of chemical inhibitors for protein glycosylation, we demonstrated that glycosylation is required for neural crest EMT and migration. To determine how glycosylation mediates this cell behavior, we performed a mass spectrometry-based glycoproteomic analysis with embryos at different stages of neural crest migration. The analysis identified multiple proteins that are differentially glycosylated at different stages, including E-cadherin. E-cadherin is a transmembrane glycoprotein and a major epithelial cell-cell adhesion molecule. Through lectin blot and proximity ligation assay, we showed that E-cadherin is dynamically glycosylated in neural crest cells. E-cadherin was glycosylated at two linkage sites in frog embryos. We further studied how specific glycosylation at these sites mediate the activity of E-cadherin in neural crest EMT and migration. These glycosylation sites were mutated individually or in combination, and the function of mutant E-cadherin was compared with that of wild type E-cadherin. Our results showed that mutant E-cadherin was less effective in promoting neural crest cell migration and this was partly due to changes in their adhesive properties.

Abstract #10

Contextualizing development of the mouse gonad through whole embryo clearing

Corey Bunce, Jennifer McKey, Blanche Capel
Duke University, USA

The mouse gonad undergoes profound morphological changes during its development. It arises at embryonic day 9.5 (E9.5) as a ridge of cells on the surface of the mesonephros spanning much of the posterior half of the embryo and develops into either a testis or ovary. By E12.5 the testis and ovary are anatomically distinct, with the testis partitioning germ cells into cord structures, while the ovary tissue remains largely homogenous. At E15.5 the testis is substantially larger than the ovary and has moved significantly posteriorly. While the general anatomical changes that the gonads undergo have been well documented, the morphological process of gonadogenesis has not been understood in a holistic sense. Until now, the decontextualization of the gonad from the embryo due to the invasive nature of dissection and culture techniques has limited our ability to accurately assess system level dynamics. Investigating the relationship between the gonad and the surrounding tissue requires methods that maintain the complete structure. We took advantage of recent advances in tissue clearing and light sheet microscopy to visualize the developing gonad within the embryo and newly address systems level questions. Using iDISCO tissue clearing and fluorescent immunostaining on whole mouse embryos, we generated a time course of gonad development from E9.5 to E15.5. This includes markers of the genital ridge and mesonephric tubules, as well as sex specific factors. This collection of high resolution light sheet images of gonad development in situ, which we hope will

provide a valuable resource for the field, allows us to analyze the dimensions of the gonad through its formation and morphogenesis and its relation to the adjacent ducts, kidney, adrenal glands, and the mouse embryo as a whole. Our initial analyses address the relationship between the developing mesonephric tubules and the genital ridge as well as extension and compaction of the gonad during early growth.

Abstract #11

TGF beta regulates neurotropic signals in developing teeth

Sarah Peters, Courtney Barkley, Rosa Serra
University of Alabama at Birmingham, USA

Transforming growth factor β (TGFβ) is known to play an important role in tooth morphogenesis and differentiation. Our laboratory established a mouse model in which TGFβ receptor 2 (Tgfbr2) was conditionally deleted in odontoblast-producing mesenchyme using an Osterix promoter driven Cre recombinase (Tgfbr2cko). These mice survived postnatally with significant tooth defects, including reduced mineralization and root lengths. We performed an mRNA-Seq analysis using RNA from postnatal day 7 (P7) dental pulp (DP) from control and mutant mice to investigate the pathways involved in tooth development. We found significant regulation of neuronal genes in mutant DP suggesting TGFβ signaling in the DP regulates tooth innervation. Immunofluorescent staining (IF) for neuronal marker, β3 tubulin (β3T), on P7 and P24 molars verified reduced innervation in Tgfbr2cko molars as compared to controls. Gene ontology analysis indicated several mineralization genes were transcriptionally regulated by Tgfbr2 deletion in the DP, including Osteopontin (OPN). Interestingly, OPN is also reported to evoke neurite outgrowth. The objective of this study was to investigate the role of Tgfbr2 in regulating paracrine signals from the DP that could regulate axonal outgrowth. We utilized a co-culture assay with trigeminal neurons cultured on transwell filters overlying primary Tgfbr2f/f or OPN-/- DP cells. Tgfbr2 was deleted in the Tgfbr2f/f DP with Adenovirus-Cre. Confocal imaging of axonal extensions was used to quantify neurite outgrowth in response to co-culture conditions. We found increased axonal sprouting when TG neurons were cultured in the presence of DP, which was reduced with Tgfbr2 knockdown in the DP. Co-culture experiments with OPN-/- DP cells did not induce neurite outgrowth. Proteomics analysis on co-culture media confirmed reduction of OPN with Tgfbr2 deletion. These results indicate Tgfbr2-guided paracrine signals, including OPN, from the DP guide tooth innervation.

Abstract #12

Analysis of Defective Heart Patterning in akirin Mutants

Hayley Milner[1], Madison Hupp[1], Austin Howard[1,2], Scott Nowak[1,2]
[1]Department of Molecular and Cellular Biology, Kennesaw State University, USA; [2]Master of Science in Integrative Biology Program, Kennesaw State University, USA

Among the metazoans the heart is one of the ealiest discrete organ structures to form during embryogenesis, in a process highly conserved across the phyla. Heart development is controlled by a cascade of factors beginning with the emergence of cardiac progenitors known as cardiomyoblasts. In Drosophila melanogaster the specification of cardiac progenitors from mesoderm, differentiation and patterning of the cardioblasts, and ensuing heart formation is controlled by the recursive action of the Tinman/Nkx2-5 transcription factor, which is itself initiated by the activity of the Twist bHLH transcription factor. We have identified Akirin as a highly conserved cofactor that works with Twist to selectively regulate expression of Twist target enhancers, such as mef2 and tinman. akirin mutants have profoundly abnormal hearts displaying defects heart patterning, with disrupted organization and

reduced numbers of Tinman-positive cardioblasts. To investigate the nature of the heart defects observed in akirin mutants, we developed a rapid live imaging assay to visualize contractions in pre-hatching embryos. Our analysis indicates that akirin mutant hearts that do in fact form either display profoundly uncoordinated contractions, or completely lack contractions in stage 17 embryos. Taken together, these data indicate that Akirin represents a new co-regulator of the cardiac developmental pathway, and is critical for heart patterning and formation.

Session # 4 – Cell-Cell Communication and Signaling

Abstract #13

Chloroplast development and signaling control intercellular communication in plants

Tessa Burch-Smith
University of Tennessee Knoxville, USA

Cell-to-cell communication in plants is essential for growth and development but remains poorly understood. Cytoplasmic pores called plasmodesmata (PD) are conduits for the intercellular trafficking of signaling and developmental molecules like transcription factors, and metabolites including products of chloroplast metabolism. In order to coordinate expression of the nuclear and chloroplast genomes for production of functional organelles, chloroplasts generate signals that are transduced to the nucleus to fine tune expression of nuclear genes. This phenomenon is called chloroplast-to-nucleus retrograde signaling (CRS). The Arabidopsis ise2 mutant, identified in a genetic screen for mutants with defects in PD-mediated intercellular trafficking, had increased intercellular trafficking concomitant with increased numbers of PD openings. Gene expression analysis of the ise2 mutant revealed altered expression of numerous nuclear genes, and implied that changes in nuclear gene expression in response to CRS from defective chloroplasts result in altered PD function and biogenesis, a process we termed organelle-nucleus-PD signaling (ONPS). According to the ONPS model chloroplasts control the partitioning of metabolites and signaling molecules among tissues via control of PD function. ISE2 is a conserved chloroplast RNA helicase and we have found it has critical roles in expression of the chloroplast genome and hence chloroplast development. Using plants with varying levels of ISE2 expression, we are testing the hypothesis that multiple chloroplast-initiated signaling pathways are integrated in the nucleus to modify PD function and/or structure to modulate intercellular trafficking of chloroplast products. We will also present evidence that light-regulated chloroplast development and physiology influence intercellular trafficking.

Abstract #14

A rheostatic switch between transcriptional repressor Smrter and the steroid hormone ecdysone patterns growth and development of wing imaginal discs.

Joanna Wardwell-Ozgo
Emory, USA

Precisely timed developmental transitions are fundamental to many aspects of organism patterning and development. In Drosophila, systemic pulses of the steroid hormone ecdysone (Ec) trigger the larval-to-pupal transition and control rates of organism growth. Ec is converted from dietary cholesterol in the prothoracic gland and is locally converted into its bioactive form (20-hydroxyecdysone; 20E) in peripheral tissues. Binding of 20E to its cognate nuclear hormone receptor, Ecdysone Receptor (EcR), activates EcR-driven expression of genes involved in molting and metamorphosis. In absence of 20E, the transcriptional corepressor SMRTER binds EcR and represses transcription of EcR-regulated loci. To examine the functional requirement for 20E signaling in control of growth and patterning genes in the larval imaginal discs (L3) during the larval-to-pupal pulses of 20E, we created a Gal4/UAS-regulated “20E sponge” transgene composed of the ligand binding domain of EcR (EcRLBD). As expected, 20E sponge expression represses EcR

activity in wing cells when 20E titers are high (late L3), but unexpectedly, 20E sponge expression activates EcR activity in these same cells during early L3 when 20E titers are low. The transcriptional repressor Smrter (NCoR) has been shown to bind EcR through the ECRLBD, and in younger L3 wing cells, Smrter loss phenocopies 20E sponge, suggesting that 20E sponge blocks 20E activation but also relieves Smrter repression. Consistent with this hypothesis, a version of the 20E sponge unable to bind Smrter (termed Dumber) repressed EcR activity regardless of developmental age. Together these data suggest that EcR switches from acting primarily as a transcriptional repressor (via Smrter) in early L3 wing cells to acting as an activator in late L3 when 20E titers rise. Current work is focused on further defining how this Ec rheostat is balanced between Smrter repression and 20E activation on specific EcR target genes in the wing imaginal disc.

Abstract #15

A bi-phasic role for non-canonical Wnt16 signaling during early anterior-posterior patterning and morphogenesis of the sea urchin embryo

Marina Martinez-Bartolome, Ryan Range
Auburn University, USA

Anterior-posterior (AP) patterning of the deuterostome sea urchin early embryo depends on integrated information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways, forming an interconnected Wnt signaling network. We have previously shown that a non-canonical signaling pathway involving the Wnt receptor Fzl1/2/7 antagonizes the progressive posterior-to-anterior downregulation of the anterior neuroectoderm (ANE) gene regulatory network (GRN) by canonical Wnt/β-catenin and non-canonical Wnt1/Wnt8-Fzl5/8-JNK signaling. This interaction is critical to establish the spatial expression of the early GRNs along the AP axis. Here, we show that maternal wnt16 is expressed ubiquitously during cleavage stages and zygotic expression is concentrated in the endomesoderm as early as the mid-blastula stage. We used morpholino and dominant negative interference approaches to analyze Wnt16 function during early AP specification and patterning. Our results indicate that during cleavage and early blastula stages Wnt16 antagonizes ANE restriction mediated by Wnt/β-catenin and Wnt1/Wnt8-Fzl5/8-JNK signaling and that this activity depends on a functional Fzl1/2/7 receptor. In addition, our data reveal that both the Fzl5/8-JNK and Wnt/β-catenin signaling are necessary for zygotic wnt16 expression in posterior endomesoderm cells during early stages of gastrulation. We demonstrate that Wnt16 is necessary for a specific set of endomesoderm GRN transcription factors in posterior blastomeres and is essential for gastrulation. Interestingly, Wnt16 signaling is also necessary to maintain the correct number of skeletogenic and non-skeletogenic mesoderm cells. Together, our data suggest that Wnt16 has two functions during early AP specification, patterning and morphogenesis: an early role in activating the Fzl1/2/7 pathway that antagonizes the ANE restriction mechanism mediated by Wnt/β-catenin and Fzl5/8-JNK signaling as well as a later function in the morphogenetic movements of gastrulation.

Abstract #16

Adipocyte nutrient sensing controls the germline stem cell lineage in Drosophila melanogaster

Alissa Armstrong
University of South Carolina, USA

An organism's physiology, particularly nutritional status, can positively or negatively impact reproduction by affecting gamete production. The stem cell-supported ovary in Drosophila melanogaster sustains robust reproductive capacity of adult females. Female flies fed a protein-poor diet lay significantly fewer eggs than those fed a protein-rich diet. This response to diet is mediated by the activity of nutrient-sensing pathways in controlling germline stem cells and their progeny. It is well-established that target of rapamycin (TOR)-mediated and insulin/insulin-like growth factor

signaling within the ovary control GSC maintenance and proliferation, germline survival, vitellogenesis and ovulation. Our previous studies show that the ovary receives dietary cues from other tissues. More specifically, distinct nutrient sensing pathways function within adipocytes, the major cellular component of Drosophila fat tissue, to remotely control the GSC lineage. We find that insulin signaling within adipocytes influences GSC maintenance as well as germline survival using distinct downstream signaling effectors. We have also shown that amino acid sensing by adipocytes controls GSC maintenance and ovulation of mature oocytes via activity of the amino acid response pathway and TOR, respectively. Currently, we are using genetic and cell biological tools to decipher the complex molecular mechanisms of adipocyte insulin signaling and amino acid sensing that control oocyte development. This work will illuminate how inter-organ communication regulates the germline to match an organism's nutritional status to an investment in egg production.

Education Session

"Community Outreach"

Our education session will focus on how we can leverage the knowledge, expertise and resources of our universities to impact the surrounding communities. The session will take place in two parts that are designed to be interactive discussions with the audience. Part 1 will comprise of a panel discussion with several UAB faculty and graduate students who are involved in outreach programs in Birmingham. They will discuss how to create programs that bring the university in contact with public schools and more broadly within the community. Part 2 will involve two members of the UAB Graduate School who will provide tips on how we can improve our "lay science" language during our community interactions.

Session Moderator:

Melissa Bentley, UAB Graduate Student, Graduate Biomedical Sciences Program, President of Graduate Biomedical Student Outreach (GBSO), CSHL Mouse Development, Stem Cells and Cancer Alumni Social Media Coordinator

Session Panelists:

Part 1:

Lori McMahon, Ph.D. Dean, UAB Graduate School, Jarman F. Lowder Professor of Neuroscience, Director of the Comprehensive Neuroscience Center, Co-Director of the Roadmap Scholars Program

Mike Wyss, Ph.D. Professor, Department of Cell Development and Integrative Biology, Director of UAB Center for Community Outreach Development (K-12, CORD)

Carmel McNicholas, Ph.D. Associate Professor, Department of Cell Development and Integrative Biology, Director of the CDIB Science Outreach Program (K-6)

Jazmine Benjamin, UAB Graduate Student, Graduate Biomedical Sciences Program, ASBMB Hill Day Student Representative, Women in STEM mentor, Graduate Student Outreach mentor, NSF AL LSAMP student mentor, Letter to a pre-scientists/pen pal

Part 2:

Kimberley Eaton, Communications and Events Specialist UAB Graduate School, Coordinator for Discoveries in the Making, Three Minute Thesis, and Say it in 6 outreach competitions

Jeff Walker, Ph.D. Director for Research Communication certificate program, Lead Academic Writing and Communications Instructor UAB Graduate School

Keynote Speaker #2

Dr. Philip Benfey
Paul Kramer Professor of Biology
Duke University
Howard Hughes Medical Institute
Hi Fidelity Genetics

Underground Signaling

To understand the progression from stem cells to differentiated tissues we are exploiting the simplifying aspects of root development. We have developed new experimental, analytical and imaging methods to identify networks functioning within different cell types and developmental stages of the root. We are particularly interested in a subnetwork that regulates a key asymmetric cell division of a stem cell and the regulatory networks that control differentiation of the stem cell's progeny. To quantify dynamic aspects of these networks, we are employing light-sheet microscopy to image accumulation of their different components. How roots explore their soil environment determines their ability to acquire nutrients and water. Very few genes controlling root growth in soil are known, primarily because of the difficulty of observing the underground environment. From mutagenized populations of rice, we have identified a gene that controls the circular movement of the root tip known as circumnutation. In collaboration with Dan Goldman (Physics, Georgia Tech) we have shown that circumnutation facilitates the root's ability to find biopores in soil horizons. We have also identified novel signaling compounds that modulate root system architecture in dicots and monocots. Finally, at Hi Fidelity Genetics we have developed an inexpensive device, the RootTracker, that can monitor maize root growth in soil over time.

Session #5 – Evolution and Development

Abstract #17

Intersection and cross-regulation of Homeotic and Sex Determination Pathways in the Drosophila abdomen – a model for morphological evolution and stasis

John H. Yoder
University of Alabama, Tuscaloosa, AL, USA

The developing adult abdomen of *Drosophila melanogaster* is a powerful system for exploring molecular bases of morphological evolution. Studies of characters under strong sexual selection, like pigmentation, have provided critical insight into mechanisms of *cis*-regulatory evolution in existing gene regulatory networks (GRNs). The *Drosophila* abdomen also bears more deeply evolved characters, useful for exploring the evolutionary origins of more complex morphological traits, as well as factors that contribute to their evolutionary stasis. We study a deeply fixed trait - sexually dimorphic abdominal segment number - to address these latter phenomena. Within the Cyclorrhapha, a monophyletic clade that diverged an estimated 150 mya, adult abdominal segment number differs between the sexes. Posterior female segments are modified as part of the ovipositor, while, during metamorphosis, the corresponding male segments are eliminated. I will present work exploring the structure of the GRN responsible for male-specific segment elimination in *Drosophila*. Specifically, we find the posterior-most Hox protein, Abdominal-B, through transcriptional regulation of the sex-determining gene *doublesex,* establishes a dimorphic cascade that differentially regulates abdominal expression of the morphogen *wingless*, as well as *doublesex* itself. This dimorphic cascade is enhanced through reciprocal feed-back regulation of *Abdominal-B* by the sex-specific *Drosophila* Doublesex isoforms.

Abstract #18

Evolution of Axin function in the Wnt/β-catenin signaling pathway in metazoans: Insights from sea urchins and sea anemones

Hongyan Sun, Athula Wikramanayake
University of Miami, USA

Axin is a critical scaffolding protein in the Wnt/β-catenin (cWnt) signaling pathwaywhich binds many components of a “destruction complex” to regulate β-catenin stability. BilaterianAxin has four main domains; APC- (RGS), GSK- (GID), β-catenin-(βcBD) and Dishevelled- (DAX) binding domains. In some bilaterians, Axin interacts with β-catenin through the βcBD to target it for degradation. Intriguingly, all non-bilaterian Axins lack the βcBD, questioning a conserved role for this critical negative regulator in regulating β-catenin stability across taxa. To begin to address this question, I asked if Axin function in cWnt signaling is conserved in sea urchins and the cnidarian *Nematostella vectensis (Nv)*. Overexpression of Axin in sea urchin embryos anteriorized embryos and inhibited endomesoderm (EMS) formation. Knockdown of Axin using a morpholino posteriorized embryos. Interestingly, ectopic induction of EMS was observed when Axin was knocked down in animal pole blastomeres, indicating that all early blastomeres have the potential to form EMS, but this is suppressed by Axin. To determine the importance of the four Axin domains in regulating β-catenin stability in sea urchins, I overexpressed each of the single domain deletion Axin constructs. Overexpression of full length Axin, βcBD, and DAX deletion constructs all anteriorized embryos. Overexpression of Axin DRGS blocked gastrulation but did not affect EMS gene expression. However, overexpression of Axin DGID produced normal embryos indicating that the GID domain was critical for regulating β-catenin stability in sea urchins. To determine if a

non-bilaterian Axin is functionally active in bilaterians, I overexpressed *Nv*Axin in sea urchin embryos and saw no effect on EMS specification. This indicated that NvAxin cannot downregulate cWnt in sea urchins and I am currently investigating if Axin regulates EMS specification in *Nematostella.*My work will provide insight into the evolution of the destruction complex.

Abstract #19

The road less traveled: Activin/Nodal signaling functions in *Capitella teleta* dorsal-ventral axis formation.

Alexis Lanza
Whitney Laboratory for Marine Bioscience, University of Florida, USA

The TGF-beta superfamily is comprised of two distinct branches: the Activin/Nodal and the BMP pathways. During development, this superfamily regulates a variety of embryological cellular processes and has a conserved role in patterning the dorsal-ventral body axis. Recent studies in some spiralian species have suggested the BMP pathway plays a crucial role in dorsal-ventral axis patterning. However, previous pharmacological inhibition studies in the spiralian annelid *Capitella teleta* suggests that signaling via the ALK4/5/7 receptor patterns the dorsal-ventral axis, implicating the Activin/Nodal pathway. In this study, we further determine the role of the Activin/Nodal pathway as it functions in *C. teleta* axis patterning. Morpholino Oligos were designed to target *Ct-Smad2/3* and *Ct-Smad1/5/8*, transcription factors specific to the Activin/Nodal and BMP pathways, respectively. Morphants were raised to larval stages and scored for axial phenotypic anomalies. Our results suggest that signaling via the TGF-beta superfamily functions via a different road in the annelid, *C. teleta,* than in their spiralian counterparts, the mollusks. These investigations will contribute to our understanding of how changes in developmental programs lead to the evolution of spiralian body plans.

Abstract #20

Understanding the role of histone deacetylases (Hdacs) in pharyngeal skeletal development in zebrafish

April DeLaurier, Cynthia Lizzet Alvarez, Terence Willoner, Kali J Wiggins
University of South Carolina Aiken, USA

Histone deacetylases modify chromatin by removing acetyl groups from the N-terminal regions of histone lysines, leading to chromatin compaction, and in most cases, repression of gene transcription. In mice, *Hdac4* functions to regulate the timing of chondrocyte hypertrophy. Using CRISPR-Cas9, we have generated zebrafish mutants for *hdac4*. Zygotic mutants show precocious ossification of pharyngeal skeletal elements, including increased expression of *runx2* paralogs, reproducing the phenotype and pattern of gene expression observed in mice. This phenotype is enhanced in maternal-zygotic mutants, indicating a maternal contribution of *hdac4* to larval skeletal development. A subset of maternal-zygotic mutants and heterozygotes have deficiencies of the anterior region of the facial skeleton. We hypothesize that loss of these structures is due to a defect in cranial neural crest cell specification or migration, and that maternal *hdac4* contributes to patterning of these structures. *Hdac1* has previously been shown to function in development of the craniofacial cartilage, pectoral fins, retina, and pigmentation in zebrafish (*colgate/hdac1* mutant). Using valproic acid (VPA), we reproduce aspects of the *hdac1* mutant phenotype, including severe loss or reduction of pharyngeal skeletal structures by 6 days post-fertilization. Intriguingly, the timing of initial exposure to VPA influences the severity of the defect, where exposure from cleavage stages onwards produces much more severe losses than exposure from gastrulation and embryonic stages onwards. These

observations have led us to hypothesize a function for *hdac1* having an early role in specification of cells that contribute to the pharyngeal skeleton, and also a later role in development of cranial neural crest-derived elements. Together, our studies indicate distinct functional and temporal requirements for Hdacs during zebrafish craniofacial development, including a role for maternal factors influencing patterning.

Session #6 – Stem and Progenitor Cell Biology

Abstract #21

Stem cells on steroids: connecting physiological signals with stem cell proliferation and differentiation

Elizabeth T. Ables
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Stem cells incorporate intrinsic and extrinsic cues to maintain their fate and proliferative capacity. In developing and established tissues, steroid hormones facilitate communication between stem cells and their environment to promote tissue regeneration and remodeling. Yet despite the pervasive actions of steroid hormones in development and physiology, it remains largely unclear how steroid hormones regulate stem cell proliferation and differentiation. My lab uses the well-characterized germline stem cells of the *Drosophila* ovary to study how a steroid hormone-induced gene regulatory network can control stem cell fate and function. Many aspects of *Drosophila* oogenesis, including germline stem cell proliferation, germ cell differentiation, and follicle survival are regulated by the steroid hormone ecdysone; however, relatively little is known about the molecular targets of this pathway in ovarian cells. Using reverse genetic and transcriptomic screens, we identified a network of ecdysone-regulated target genes essential for germline stem cell maintenance and proliferation. While key targets included nuclear hormone receptors, which are well-known to mediate physiological responses, we also identified a subset of genes with fundamental roles in nucleocytoplasmic transport and cell cycle regulation. We are currently investigating whether and how nuclear hormone receptors might cross-regulate each other to impact cell differentiation and proliferation. We postulate that induction of specific target genes in specific cells or developmental stages likely functions to fine-tune responses to physiologically-regulated steroid signals, culminating in tissue regeneration.

Abstract #22

Repressive H3K9me2 protects lifespan against the transgenerational burden of germline transcription in C. elegans

Teresa W. Lee, Heidi S. David, Amanda K. Engstrom, David J. Katz
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Across metazoans, lifespan is limited by the germline. One cost of the germline may lie in germline transcription, but mechanisms remain elusive. During transcription, the COMPASS complex methylates histone H3 at lysine 4 (H3K4me). The Brunet lab has shown that deficiencies in COMPASS subunits, including WDR-5, extend *C. elegans* lifespan in a germline-dependent manner. Here we demonstrate that longevity in *wdr-5* mutants is a transgenerational trait that only manifests after populations lack COMPASS for eighteen generations. Surprisingly, the full lifespan increase requires both an increase in *wdr-5* mutants and a decrease in wild type recovering from freezing or starvation. We have previously found that levels of repressive H3K9me2 increase at some genes in *wdr-5* mutants, implicating H3K9me2 in COMPASS mutant longevity. We find that genomic H3K9me2 enrichment correlates with transgenerational changes in longevity in both wild-type and

wdr-5 mutants. Furthermore, *wdr-5* mutant longevity requires the H3K9 methyltransferase MET-2 and is phenocopied by a mutation in the putative H3K9 demethylase JHDM-1. Strikingly, the Brunet lab showed that COMPASS mutant longevity can be inherited by their wild-type descendants. Our finding that H3K9me2 is heritable between generations suggests that it may also function in the inheritance of longevity. We find that the longevity of *jhdm-1* mutants is inherited by their wild-type descendants, and that MET-2 is required for the inheritance of longevity in wild-type descendants of *wdr-5* mutant. Our findings strongly implicate H3K9me2 in the epigenetic inheritance of longevity. We present a model in which germline transcription-coupled H3K4me encroaches upon regions of H3K9me2, gradually reducing repressive chromatin to limit lifespan. The loss of COMPASS alleviates this burden, extending lifespan and enabling its inheritance. Thus, we propose that germlines limit lifespan in part because germline transcription reduces heterochromatin.

Abstract #23

The role of Map kinases in differentiation of the SAN.

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Heart rate is determined by pacemaker cells residing in the sinoatrial node (SAN) of mammalian hearts. SAN damage has serious impact on quality of life by causing a slow heart rate/bradycardia. Mechanical pacemakers can improve quality of life but do not prolong life, due to various causes, including battery failure and lead slippage. The goal of this work is to elucidate the molecular mechanisms that direct the differentiation and maturation of SAN cells. Activation of the TGFbeta-activated Map kinase (TAK1/Map3k7), directs myocardial differentiation from pluripotent stem cells to the SAN fate. Our data also shows that adoption of the transcriptional program that directs SAN differentiation is separable from the adoption of mature SAN electrophysiologies. Embryoid bodies overexpressing TAK1 show marked downregulation of several known downstream targets. Using small molecule inhibitors of these kinases, we have preliminary data that suggests that blockade of downstream targets with small molecules is sufficient for cells to adopt a rapid beat rate and other characteristics of SAN cells, suggesting that differentiation of electrophysiologically mature SAN can be accomplished by manipulating Map kinase signaling.

Abstract #24

A self-organized mesenchymal-to-epithelial model of somitogenesis

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It is generally believed that the formation of body segments (somites) in vertebrate embryos is solely determined by molecular oscillations waves traveling along the body axis. Recent work, however, suggests that somite formation may be a self-organized mechanical process emerging from a mesenchymal-to-epithelial transition. Here we show experimental evidence of a wave of dorsal epithelialization of the pre-somitic mesoderm that precedes somite segmentation in chicken embryos. The first signs of somite formation happen in this layer, when its anterior-most segment arches dorsally and creates a cleft indicating the position of the next somite boundary. We propose a

mechanical model of somitogenesis in which epithelialization and apical constriction waves play major roles in the self-organization of somites. First we built a computational model to explore the contributions of cell polarization, adhesion and elongation to the formation of the dorsal epithelial layer, and show that the sequence in which these events take place is crucial to proper monolayer formation. Next we simulate a wave of apical tension build up and show how it can create spatially uniform and temporally periodic dorsal tissue segments, similar to somite formation seen *in vivo*. We show that this mechanical segmentation process shares some of the scaling properties of the clock-and-wavefront model and discuss the limits of a purely mechanical model of somite formation.

Poster Abstracts

Differentiation and Cell Fate

Abstract #25

Transcriptome approaches to understanding C. elegans nervous system development

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Correct specification of neuronal fate is crucial to an organism's nervous system development and function, with multiple neurodevelopmental disorders associated with pro-neural transcription factor mutations. For instance, mutations in human NeuroD1, which codes for a basic-helix-loop-helix transcription factor required for interneuron cell fate specification, have been associated with mental retardation, visual impairment, and also type I diabetes. In addition, heterologous expression of NeuroD1 is sufficient to directly reprogram microglia to neurons, demonstrating a pioneering role for this gene in neurogenesis. NeuroD1 is deeply conserved across phyla, and has an ortholog, *cnd-1*, in the nematode *Caenorhabditis elegans*. The worm, with its invariant cell lineage and simple nervous system of 302 neurons, is an ideal organism to explore the earliest stages of neural development and the pioneering role of *cnd-1*/NeuroD1 in this process. We used a comparative transcriptome approach to investigate transcription changes in wild type and *cnd-1(ju29)* mutant embryos. We find that *cnd-1* partially represses its own expression during embryogenesis. In addition, we show that *cnd-1* controls the expression of *ceh-5*, a Vax2-like homeobox class transcription factor expressed in the nervous system and in head muscles. We also demonstrate a role for the Hox gene *ceh-13*/labial in defining the fate of *cnd-1*-expressing motorneurons. Finally, we show that *cnd-1* controls the expression of genes required for post-mitotic function, including the RING-finger ubiquitin ligase *rpm-1*. These data highlight the utility of comparative transcriptomes for understanding the nuances of transcription factor control, and establish a paradigm for future transcriptome-based studies.

Abstract #26

FGF signaling pathway is important for Xenopus pharyngeal development

Elizabeth Wilke
Northern Kentucky University, USA

During vertebrate embryonic development, the pharynx give rise to several structures including parts of the heart, lower jaw, muscles and nerves of the face, craniofacial cartilage, and the thyroid and parathyroid glands. *Xenopus laevis* is genetically and structurally similar to humans, making it a useful model for studying the development of the pharynx. The pharynx contains pouches and arches

and contributions from all three germ layers as well as neural crest cells. These different layers use many genetic signaling pathways to communicate with one another including the focus of our study, the Fibroblast Growth Factor (FGF) pathway. The FGF pathway is known to be important for pharyngeal development as the absence or mutations of FGF proteins can cause craniofacial abnormalities and health complications. In our research, we analyzed the role of the Fibroblast Growth Factor (FGF) genetic signaling pathway in the development of the *Xenopus* pharynx. To determine the importance of FGF signaling, we inhibited the pathway during different stages of embryonic development with a small molecule inhibitor. We used *in-situ* hybridization to detect changes in gene expression patterns in the pharynx of manipulated embryos and found that genes such as *cyp26a1* are downregulated with FGF inhibition. Additionally, to verify that FGF signaling was inhibited in our experimental embryos, proteins extracts from embryos were analyzed with western blotting and compared to control, sibling embryos. By identifying downstream targets of the FGF pathway, we will be able to start to understand the different ways the FGF signaling pathway guides pharyngeal development. In the future, this research may help us understand how different pharyngeal birth defects occur.

Abstract #27

Runx2 activity in hypertrophic chondrocytes regulate homeostasis of both cartilage and bone tissue

Harunur Rashid, Haiyan Chen, Robert Koski , Amjad Javed
Department of Oral and Maxillofacial Surgery, School of Dentistry, University of Alabama at Birmingham, United States

Global deletion of the Runx2 gene results in the total absence of bone tissue. Deletion of Runx2 in resting chondrocytes leads to failed chondrocyte hypertrophy and endochondral ossification. Expression of Runx2 increases progressively from immature to hypertrophic chondrocytes (HC). However, the role of Runx2 after chondrocyte hypertrophy is unknown. Runx2 gene was ablated in HC by using Col10-Cre mouse model. The Cre-expression was restricted to HC and absent in resting, proliferating and pre-hypertrophic zone. Surprisingly, HC-specific Runx2 null mice ($Runx2^{HC/HC}$) were born alive and survive to adulthood. $Runx2^{HC/HC}$ mice have a well-developed skeleton but extremities are poorly mineralized. Histological analysis of 3-day old limbs showed the length of HC zone was double in $Runx2^{HC/HC}$ mice. Decrease in length of the ossified region despite enhanced hypertrophy in the $Runx2^{HC/HC}$ mice was noted. Alcian-blue staining revealed the accumulation of cartilage matrix up to the mid-diaphysis region of the tibia. Consistent with the poor cartilage resorption, a significant decrease in the expression of matrix-degrading enzymes by HC was evident in mutant mice. Surprisingly, Von kossa staining showed increased mineralization under the growth-plate. Micro-CT analysis confirmed a 3-fold increase in the trabecular bone. Interestingly mutant bone showed no change in trabecular-thickness but a 55% increase in trabecular-number, resulting in a 25% decrease in trabecular-space. Cortical bones, however, were comparable among the littermates. To better understand increased trabecular bone, we evaluated osteoclasts activity. TRAP staining revealed a 35% decrease in the number of osteoclasts in $Runx2^{HC/HC}$ bones which is supported by the decreased Rankl/Opg ratio in $Runx2^{HC/HC}$ mice. Together our data demonstrate that Runx2 plays an essential role in hypertrophic chondrocytes for the resorption of transient cartilage matrix and development of trabecular bone.

Abstract #28

Undergraduate laboratory exercises on differential gene expression using Xenopus laevis

Emily Shifley
Northern Kentucky University, USA

In Developmental Biology classes, students are challenged with understanding how differential gene expression guides embryonic development. It can be difficult for students to realize that genes need to be turned on or off at the right time and place in order for development to proceed normally. In this series of lab exercises, students working in groups perform experiments with live embryos and visualize differential gene expression allowing them to become invested in their experiment and curious about the results. This lab also addresses the benefits of *Xenopus laevis* as a model organism and allows students to observe the changes *Xenopus* embryos undergo during early embryonic stages. After the students have chosen and fixed two stages of *Xenopus* embryos, they perform an *in situ* hybridization on the embryos to visualize gene expression at two different developmental stages. They then compare their results with those from other lab groups who analyzed their embryos for different genes. The students self-reported that they better understood the concept of differential gene expression during vertebrate development and enjoyed doing this series of lab experiments working with live materials.

Abstract #29

Transcriptional reprogramming of endothelial cells for arteriolar differentiation

Bin Ren
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Cellular transcriptional reprogramming may be essential for microvascular endothelial cell (MVEC) differentiation into arterioles and improvement of blood perfusion under ischemic conditions. Our previous studies suggest that lysophosphatidic acid (LPA), a lipid signaling mediator, initiates a genetic reprogramming of MVECs for arterial gene expression, accompanied by the transcriptional repression of CD36, an angiogenic regulator and fatty acid receptor, via signaling crosstalk between protein kinase D (PKD-1) and transcription factor FoxO1. We used cellular and molecular approaches, Zebrafish models, and the unique EC-specific *pkd-1* knockout mice with translating ribosome affinity purification (TRAP) system and *cd* 36 knockout TRAP mice. Our studies indicate an essential role of the LPA/PKD-1 signaling in promoting arteriolar differentiation via FoxO1-mediated transcriptional reprogramming (This study is supported by NIH and American Heart Association).

Abstract #30

The ciliary phosphatidylinositol phosphatase Inpp5e attenuates the Shh response in the mouse neural tube

Sandii Constable[1], Katharine Floyd[1], Chao Lin[1], Alyssa Long[1], Stephane Schurmans[2], Tamara Caspary[1]
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Sonic hedgehog (Shh) signal transduction specifies ventral cell fates in the neural tube and is mediated by the Gli transcription factors that play both activator (GliA) and repressor (GliR) roles. It is the ratio between GliA and GliR, observed as a gradient of Shh activity from ventral to dorsal in the neural tube, that determines cell fate. Cilia are essential for Shh signal transduction. The ciliary

phosphatidylinositol phosphatase, Inpp5e, modulates the phosphoinositide content of the ciliary membrane. When Inpp5e is inactive, phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) and its interacting protein Tulp3 accumulate in the cilium along with Gpr161, a negative regulator of the Shh pathway. Consistent with this, previous work using *Inpp5e*-null cell lines showed a diminished transcriptional response to Shh stimulation, suggesting Inpp5e normally plays an activating role in Shh signaling. In the course of a forward genetic screen for recessive mouse mutants, we identified a point mutation that creates a functional null allele of Inpp5e which we called *ridge top*, *Inpp5e*^{rdg}. At embryonic days (E) 9.5 and 10.5, *Inpp5e*^{rdg} mutants exhibit expanded ventral neural cell fates. This phenotype largely depends on the presence of cilia and on Smoothened, the obligate transducer of Shh signaling, indicating Inpp5e functions within the cilium to regulate the pathway. By E12.5, we observed normal neural patterning in *Inpp5e*^{rdg} embryos and determined that this correction over time required Gli3, the predominant repressor in the Shh signaling pathway. Our data indicate that Inpp5e plays a more complicated role in Shh signaling than previously appreciated. We propose that Inpp5e attenuates Shh signaling through temporal regulation of the GliA to GliR ratio: loss of Inpp5e allows some initial activator activity and a delayed formation of the repressor gradient. This finding contributes to our understanding of how Shh signal duration regulates ventral neural tube patterning.

Abstract #31

EMA6D Regulates Perinatal Cardiomyocyte Proliferation and Maturation in Mice

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Cardiomyocytes undergo dramatic changes during the fetal to neonatal transition stage to adapt to the new environment. The molecular and genetic mechanisms regulating these changes remain elusive. In this study, we showed *Sema6D* as a novel signaling molecule regulating perinatal cardiomyocyte proliferation and maturation. SEMA6D is a member of the Semaphorin family of signaling molecules. To reveal its function during cardiogenesis, we specifically inactivated *Sema6D* in embryonic cardiomyocytes using a conditional gene deletion approach. All mutant animals showed hypoplastic myocardial walls in neonatal hearts due to reduced cell proliferation. We further revealed that expression of MYCN and its downstream cell cycle regulators is impaired in late fetal hearts in which *Sema6D* is deleted, suggesting that SEMA6D acts through MYCN to regulate cardiomyocyte proliferation. In early postnatal mutant hearts, expression of adult forms of sarcomeric proteins is increased, while expression of embryonic forms is decreased. These data collectively suggest that SEMA6D is required to maintain late fetal/early neonatal cardiomyocytes at a proliferative and less mature status. Deletion of *Sema6D* in cardiomyocytes led to reduced proliferation and accelerated maturation. We further examined the consequence of these defects through echocardiographic analysis. Embryonic heart deletion of *Sema6D* significantly impaired the cardiac contraction of male adult hearts, while having a minor effect on female mutant hearts, suggesting that the effect of *Sema6D*-deletion in adult hearts is sex dependent.

Abstract #32

A steroid hormone promotes germ cell mitotic divisions and oocyte specification in Drosophila.

Danielle Finger, Taylor Hinnant
East Carolina University, USA

Reproductive function in many organisms, including *Drosophila melanogaster*, is controlled by steroid hormones. The main steroid hormone in *Drosophila* is ecdysone, which is similar to estrogen. Ecdysone is essential for many processes in oogenesis including germline stem cell function, encapsulation, and yolk uptake. Many studies have investigated the transcriptional targets of ecdysone during development, but, despite its diverse roles in oogenesis, much less is known about transcriptional targets in the ovary. As an initial approach to identify transcriptional targets of ecdysone signaling in the *Drosophila* ovary, we performed whole transcriptome sequencing (RNA-seq) comparing gene expression in ovaries from *Ecdysone Receptor* (*EcR*) loss-of-function mutants versus control ovaries. Intriguingly, the differentiation factor *bag of marbles* (*bam*) was significantly over-expressed in the absence of ecdysone, suggesting that ecdysone may play a previously unappreciated role in early cyst divisions and differentiation. We also observed increased expression of various APC/C targets, including *pimples* (the *Securin* homolog) and *Nek2*, a serine-threonine kinase, both required for mitosis. This suggests that ecdysone signaling may regulate the transition between mitotic divisions to meiosis in differentiated germ cells. Moreover, transcripts for the meiotic cohesion protein, orientation disruptor (ord) were reduced in the ecdysone mutants. Ord stabilizes the synaptonemal complex in presumptive oocytes and is essential for the earliest events of oocyte specification. Interestingly Orb, an RNA binding protein expressed in differentiated oocytes is also decreased in EcR^{ts} mutant ovaries. Taken together, these results suggest that ecdysone signaling may promote oocyte specification and differentiation. This data will give us further insight into cell type-specific interpretation of ecdysone signaling and increase our understanding of the functions of ecdysone signaling in oogenesis.

Abstract #33

TGFβ mediates fibrous tissue differentiation from mesenchymal stem cells during axial skeleton development

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Intervertebral Discs (IVDs) are an essential component of the spine that provide weight distribution. The structural integrity of IVDs is maintained by a fibrous tissue called the annulus fibrosus (AF), which degenerates with age causing chronic back pain. The development of the AF, but the signaling pathways required for its formation are unknown. By using mice in which TGFβ signaling is disrupted in collagen type II expressing cells, our lab previously showed that the AF of IVDs was missing. We've also shown that when sclerotomal cells are treated with TGFβ, there is an upregulation of fibrous tissue differentiation markers, most notably the transcription factor Scleraxis (Scx). We hypothesize that TGFβ regulates Scx expression to provide an instructive signal for fibrous tissue differentiation. To test this hypothesis, we sorted sclerotome cells from Scx-Mcherry transgenic mice into populations with high and low Scx expression and treated these cells with TGFβ ligand. We observed that TGFβ regulates Scx expression before any other fibrous markers, and the cell population highly expressing Scx is more primed to undergo fibrous tissue differentiation than Scx low expressing cells. To further elucidate the relationship between TGFβ signaling and Scx on fibrous tissue differentiation, we knocked down Scx using siRNA i*n vitro*. Scx expression was required for TGFβ mediated regulation of downstream fibrous tissue markers, ADAMTSL2 and Fibromodulin, in a temporal manner. These data suggest Scx is an important mediator of TGFβ signaling in regulating fibrous differentiation. Additional preliminary data suggested noncanonical TGFβ signaling through ERK regulated Scx expression, which in turn regulates fibrous tissue differentiation. By identifying the signaling mechanisms integral for fibrous tissue development in the spine, this study can be utilized for stem cell-based therapies to regenerate damaged fibrous tissues like AF, tendon or ligament in spinal degeneration.

Abstract #34

A Genetic Analysis of Functional Redundancy of the MAP Kinase Kinase (MKK) Gene Family in Arabidopsis

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Mitogen-activated protein (MAP) signals are found in all eukaryotes, and have a variety of functions ranging from cell growth/development to cell differentiation in zygotes. Plant zygotes elongate before dividing into a small apical daughter cell, the progenitor of the embryo, and the larger basal daughter cell, the progenitor of the suspensor. MAP signaling is required for this elongation and unequal first division of the zygote. Previously, we have shown that loss of the two Arabidopsis MAP kinase kinase genes, MKK4 and MKK5, results in slightly shorter zygotes and moderate defects in suspensor formation. These effects are similar but less severe when compared to the loss of the MAP kinase genes MPK3 and MPK6 or the MAPKK kinase YDA, which essentially blocks zygote elongation and results in suspensor-less embryos. Are other MKK genes involved in this cascade? By systematically expressing the other eight MKK genes in the family under the control of the MKK4 promoter in a double mutant background we aim to observe partial or full rescue of the MKK4/MKK5 mutant phenotype; implying functional similarity between MKK4/MKK5 and the transgene.

Abstract #35

The maternal epigenetic reprogramming function of the histone demethylase LSD1 is CoREST dependent and may contribute to inherited disease

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Somatic cell nuclear transfer has established that the oocyte contains maternal factors with epigenetic reprogramming capacity. However, the function of these maternal factors during the gamete-to-embryo transition remains poorly understood. In *C. elegans*, LSD1/KDM1A (lysine specific demethylase 1) acts with the CoREST repressor complex to enable this transition by removing H3K4me1/2 and preventing the transgenerational inheritance of transcriptional patterns. In mouse, the loss of maternal LSD1 results in embryonic arrest at the 1-2 cell stage, with arrested embryos similarly failing to undergo the maternal-to-zygotic transition. Moreover, partial loss of LSD1 protein levels maternally results in striking phenotypes weeks after fertilization, including perinatal lethality and abnormal behavior in surviving adults. To explore the mechanism underlying these heritable defects further, we developed a new maternally hypomorphic *Lsd1*allele that predominantly affects the binding of LSD1 to CoREST. This new allele phenocopies our mouse model with reduced LSD1, suggesting that the maternal reprogramming function of LSD1 is CoREST dependent. Additionally, preliminary data shows offspring from maternally hypomorphic LSD1 show a delay to reach physical milestones during development. We also find that the incidence of perinatal lethality is highest in early and advanced maternal age. This modulation of the phenotype by maternal age is reminiscent of the epidemiological data in autism, raising the possibility that defective maternal epigenetic reprogramming can contribute to neurodevelopmental disorders. We are currently using our new mouse model to investigate this hypothesis.

Abstract #36

The effect of age on epigenetic reprogramming in C. elegans

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Cellular fate is specified by differential gene expression, which is regulated by chromatin modifications. Gametes are highly differentiated cells, harboring distinctive histone marks. They require an epigenetic reprogramming event after fertilization to erase gamete fate and allow a totipotent zygote to develop. Dysregulation of this process leads to developmental problems and potentially human disease. In 1972 it was observed that parental age affects fertility of the following generation in wild type *C. elegans*. Offspring from the older hermaphrodites had a smaller brood size compared to their siblings from younger parents. To determine if this is due to defects in maternal epigenetic reprogramming, I performed analogous experiments in *spr-5* mutant worms. SPR-5 is a histone demethylase that removes the H3K4me2 from actively transcribed genes. Our lab has previously shown that *spr-5* mutant*s* have a transgenerational sterility phenotype, due to increasing H3K4me2. I found that progeny of *spr-5* mutant worms have further compromised fertility with advanced maternal age (AMA) compared to wild type. In addition, I found that progeny of *spr-5* mutant worms from early maternal age (EMA) also have reduced fertility. My results, coupled with original findings, suggest that the effect of paternal age on offspring fertility may be due to compromised H3K4me2 reprogramming at fertilization. In the future I will determine why the *spr-5* mutant line has an exacerbated decline in the fertility of progeny from EMA and AMA hermaphrodites,and determine genetically and molecularly whether this decline is due to trans-generationally increased H3K4me2. This study is important because the effect of age on fecundity is reminiscent of the maternal age effect on the rate of autism. In addition, mice and humans lacking the SPR-5 homolog have autism-like symptoms. Thus, we hope to provide a foundation for potential translational applications in the future.

Abstract #37

Investigating the role of MEP-1 in Caenorhabditis elegans embryogenesis

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Events during early embryogenesis are tightly regulated in order to develop an organism properly. These events can be carefully controlled via the epigenetic marks placed on chromatin, both before embryogenesis and after. Two widely characterized epigenetic marks in *Caenorhabditis elegans* are those that are removed and added by enzymes SPR-5 and MET-2, respectively. However, SPR-5 and MET-2 are surely not the only players involved in setting up proper embryogenesis. MEP-1 is a candidate for interacting with SPR-5 and MET-2 because of its role in suppressing germline genes in the soma (Unhavaithaya et al. 2002). In order to investigate the role of MEP-1 in embryogenesis of *C. elegans*, RNA interference was used to knock-down the transcription of MEP-1 in *spr-5, met-2,* and *spr-5;met-2* mutant animals. The mutant animals placed on *mep-1* RNAi were severely arrested in their development indicating that MEP-1 is yet another player in embryogenesis.

Abstract #38

The Ldb1 co-regulator is required for pancreatic endocrine progenitor appearance, differentiation, and survival.

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Pancreatic ß-cells are indispensable for glucose homeostasis and their dysfunction is central to diabetes mellitus. ß-cell development is regulated by transcription factor (TF) cascades, mediating differentiation of embryonic progenitors into mature ß-cells. TF roles have been highly characterized, yet the role of their interacting co-regulators is underappreciated. Our prior studies showed that the essential TF, Islet-1 (Isl1), interacts with a broadly-expressed transcriptional co-regulator, Ldb1, to regulate *late ß*-cell maturation. However, Ldb1 is also expressed *early* in development, before Isl1 is expressed, in embryonic multipotent progenitor cells (MPCs) and endocrine progenitors expressing the TF Neurogenin-3 (Ngn3). MPCs populate the endocrine *and* exocrine pancreas, while $Ngn3^{+}$endocrine progenitors ($Isl1^{-}$) delaminate from the ductal epithelium to form islets. We hypothesize that Ldb1 has Isl1-independent roles in maintaining islet progenitor identity and survival. To test this, we generated a pancreas-wide Ldb1 knockout (*Ldb1*$^{?panc}$) and observed severe developmental defects. At embryonic day 13.5 (E13.5) *Ldb1*$^{?panc}$ embryos exhibited disorganized progenitor pools, suggesting impacts on endocrine cell identity. At E15.5 *Ldb1*$^{?panc}$ mice had significant reductions of $Ngn3^{+}$progenitors and $Pdx1^{HI}$, a marker of new ß-cells. *Ldb1*$^{?panc}$ mice died by postnatal day 7 with severe hyperglycemia, hypoinsulinemia, and drastically reduced islet cell numbers. Considering that *Ldb1*$^{?panc}$ defects were endocrine specific, we generated a model of *Ldb1* deletion in $Ngn3^{+}$progenitors, termed *Ldb1*$^{?endo}$. We confirmed loss of Ldb1 in islets and observed hyperglycemia, with severe reduction of islet cells in *Ldb1*$^{?endo}$ neonates. We are assessing the developmental phenotype, specifically examining markers of apoptosis and delamination. Additionally, we have developed a new Ldb1$^{?endo;TOMATO}$ *Cre*-reporter mouse to be used for lineage tracing to assess endocrine cell fate in the absence of Ldb1.

Development in Cancer and Disease

Abstract #39

Optimizing Tol2 Transposition in Zebrafish

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Transposable elements are DNA segments that move within the genome when induced by transposase proteins. The *Tol2* transposable element from Medaka fish has successfully been adapted for integrating foreign DNA into a wide variety of vertebrates. In order to increase the usefulness of *Tol2* as a transgenic tool, we aim to optimize *Tol2* transposition in zebrafish. To achieve this, the project has two components. The first uses activation tagging, a form of transposon tagging, in order to induce overexpression of genes, allowing us to learn about the function of genes that may otherwise be hard to study because of lethality or redundancy. An activation tag is created when a strong enhancer is positioned within the transposable element. Our activation tag construct consists of the *Tol2* terminal inverted repeats flanking the enhancer region of the beta-actin promoter. This activation tag was cloned in front of the *mCherry* reporter gene to indicate when transposition occurs. A *Tol2* transposase construct controlled by a heat inducible promoter was engineered to induce transposition of the activation tag in zebrafish. The two constructs were coinjected into zebrafish embryos to create a population for measuring transposition rates. Upon heat shocking the embryos, a loss of *mCherry* expression within the zebrafish will indicate the transposition of the activation tag away from the reporter gene. As a result of the activation tag landing somewhere else within the genome, it is expected that a mutant phenotype can also be observed. The second study investigates

whether the removal of a Nuclear Export Signal (NES) from the *Tol2* transposase will increase the efficiency of *Tol2* transposition. We hypothesize that the NES is keeping *Tol2* transposase outside of the nucleus, thus lowering the rate of *Tol2* transposition. Comparison of rate of transgene integration for control and NES removed versions of transposase mRNA will indicate if the NES functions to suppress *Tol2* transposition.

Abstract #40

Deciphering the Triggering Mechanism of Ankylosing Spondylitis

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We study the molecular mechanisms controlling intervertebral disc remodeling and vertebral bone fusion during embryogenesis and animal development to understand disease processes in humans. Chicken is an ideal model to study both normal and pathogenic events as birds exhibit several axial skeletal regions that fuse, similar to human sacral and caudal fusion. We have discovered a role for complement and neutrophil cells in direct transdifferentiation of chondrocytes to osteoblasts in the intervertebral discs of the pygostyle in chicken - leading to remodeling and bone fusion. We hypothesize that Ankylosing Spondylitis, a major cause of back pain, is triggered when nuclei pulposi produce Complement C3 protein recruiting neutrophils to the intervertebral discs, which in turn triggers an inflammatory response, resulting in remodeling of intervertebral discs, inflammation, pain, and pathogenic bone fusion.

Abstract #41

The Transcription Factor Hypermethylated in Cancer 1 (Hic1) Interacts with Canonical and Non-canonical Wnt Signaling Pathways During Neural Crest Migration

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The transcription factor Hypermethylated in Cancer 1 (*HIC1*) resides within a genomic region associated both with cancer and the complex developmental disorder Miller-Dieker Syndrome. While several studies have characterized Hic1 as a tumor suppressor, Hic1 function in development is less understood. Loss-of-function mouse alleles show embryonic lethality accompanied with developmental defects, including craniofacial abnormalities that are reminiscent of human Miller-Dieker syndrome patients. In this study, we use the power of *Xenopus laevis* to further explore Hic1 function in craniofacial development. We find that *hic1* mRNA is expressed throughout early *Xenopus* development, including within the neural plate border and branchial arches, suggesting that Hic1 may be involved in cranial neural crest cell (CNCC) development. Targeted manipulation of *hic1* gene expression in the neural/CNCC domain by either overexpression (*hic1* mRNA) or knockdown (*hic1* morpholino) results in craniofacial defects including malformations of the craniofacial cartilages. *In situ* hybridization (ISH) indicates that while CNCC are specified correctly upon *hic1* manipulation, CNCC-specific gene expression patterns are altered during the stages of neural crest migration. Both CNCC transplant and explant experiments reveal that loss of *hic1* expression inhibits neural crest migration. Mechanistically, using ISH, immunohistochemistry, qRT-PCR and secondary axis formation assays, we find that *hic1* dysregulation impinges on the Wnt

signaling pathways required for neural crest delamination and migration via altered levels of both canonical and non-canonical Wnt signaling pathway components. These results identify Hic1 as a novel regulator of both canonical and non-canonical Wnt signaling pathways during neural crest development and shed new light on Hic1 function with implications for craniofacial development and cancer.

Abstract #42

Understanding the role of maternal hdac 4 in zebrafish craniofacial development

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In zebrafish, loss of maternal hdac4 results in premature ossification of the craniofacial skeleton, and in a subset of larvae, a loss of the skeletal elements of the maxillary domain and first pharyngeal arch. Affected larvae are missing the Meckel's cartilage, the anterior half of the palatoquadrate cartilage, and the anterior portion of the neurocranium. This study aims to investigate the role of maternal hdac4 on cranial neural crest cells patterning the craniofacial skeleton. We hypothesize that loss of maternal hdac4 in zebrafish causes loss of a specific population of cranial neural crest cells that form the anterior portion of the face. We predict that analysis of markers of cranial neural crest will indicate a loss of cells in maternal zygotic mutants and defects in expression of markers associated with formation or migration of cells. Previously, we identified carriers of the hdac4 mutant allele, including homozygous mutants, heterozygotes, and wildtype zebrafish using PCR genotyping. Female mutants were in-crossed with heterozygous males to generate maternal zygotic mutant and heterozygote larvae. At 7 days post-fertilization (dpf), the larvae were harvested and stained using Alizarin Red and Alcian Blue to score the anterior craniofacial defect. Embryos from stages 12 hours post-fertilization (hpf) up to 30 hpf will be used for mRNA in situ hybridization where we will use probes to label premigratory, migratory, and post-migratory neural crest cells (foxd3, sox10, snai1b, fscn1a). These larvae will be compared with wild-type larvae from a different cross. mRNA in situ analysis will tell us whether cranial neural crest cells are being specified, the migratory patterns of these cells, and their precise locations during development. We anticipate that this study will identify novel mechanisms for hdac4 in regulating cranial neural crest development in zebrafish.

Abstract #43

Identifying novel regulators of primary cilia function in C. elegans

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Primary cilia are microtubule-based structures that extend from nearly all mammalian cell types and play a role in cellular sensing and signal transduction. Ciliopathies are a spectrum of human disorders associated with defects in primary cilia formation and function. Mutations in the cilia-associated gene *MKS1* cause three different ciliopathies, Meckel-Gruber Syndrome, Joubert Syndrome, and Bardet-Biedl Syndrome with varying severity. It is not well understood why mutations in the same human gene, *MKS1*, can result in multiple disorders with varying clinical features. We hypothesize that this phenotypic variability may stem from the presence of modifier alleles causing different degrees of primary cilia dysfunction. Therefore, the **objective** of this work is to identify novel genetic mutations that can exacerbate the effects of loss of *MKS1* gene function. To identify these novel modifiers, we are using the tractable invertebrate model organism, *C. elegans*, which exhibits conservation of most aspects of primary cilia form and function when compared to mammals. We conducted an ethyl methanesulfonate (EMS) mutagenesis screen on *mks-1(yhw146)* mutants to identify secondary mutations that, when combined with an *mks-1* mutation, cause defects in primary cilia formation. These defects were identified by looking for loss of normal DiI dye filling of ciliated sensory neurons. We isolated 10 strains carrying homozygous recessive gene mutations that combine

with *mks-1(yhw146)* to result in defects in cilia function. Next generation sequencing is underway to identify the causal mutations. The isolation of multiple secondary mutations from our mutagenesis screen suggests that we will be able to identify and characterize novel genes influencing primary cilia formation and function in *C. elegans*. These discoveries could translate into new diagnostic and therapeutic possibilities for human ciliopathy patients.

Abstract #44

Investigating the Effects of Glucose on Choroid Plexus Development

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Brain barriers play an important role in maintaining homeostasis between the brain, blood, and cerebrospinal fluid. Proper brain barrier development is essential for sustaining neurological functions throughout the life of the organism. The blood-cerebrospinal fluid barrier consists of an epithelial-based structure called the choroid plexus (CP). Disruption of this barrier has been associated with several conditions including Alzheimer's disease and diabetes. Hyperglycemia has been shown to disturb normal brain barrier development, with long-term consequences affecting cognition and behavior. However, the effects of glucose specifically on the CP have not been fully determined. This study used zebrafish (*Danio rerio*) to investigate the effects of glucose on CP development. A transgenic zebrafish line was previously generated expressing the green fluorescent protein (GFP) in the CP which allows for its development to be visualized under a fluorescent microscope. Zebrafish were treated with various glucose concentrations and CP formation was observed by detecting GFP fluorescence. A glucose assay was also performed to determine internal glucose levels in zebrafish larvae. To determine if barrier integrity had been compromised after glucose exposure, zebrafish were injected intravenously with a fluorescent tracer and imaged to visualize tracer leakage into the brain ventricle. Our findings show that hyperglycemia negatively affects not only CP size, but also head size suggesting a delay in overall development. While the gluocse assay indicates that glucose levels do increase in exposed larvae, they may not be significant enough to cause a noticeable effect on CP function. Intravenous injection of a fluorescent tracer did not show a significant leakage into the brain ventricle. Further studies are needed to determine consistent methods for inducing hyperglycemia in zebrafish and observing CP function.

Abstract #45

Determining the role of ldlrap1a in cholesterol metabolism in zebrafish

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Low density lipoprotein receptor adaptor protein 1 (LDLRAP1) is a factor which interacts with low-density lipoprotein receptors (LDLR) in endothelial cells to endocytose lipids from the bloodstream. Humans with mutations in LDLRAP1 have familial hypercholesterolemia, an autosomal recessive inherited disorder, resulting in abnormally high levels of blood lipoproteins. We hypothesize, as in humans, Ldlrap1a functions in zebrafish to prevent the accumulation of blood lipoproteins. To study the role of *ldlrap1a* in zebrafish, a reverse genetics approach was taken by using CRISPR-Cas9 to generate a mutant zebrafish line for *ldlrap1a*. In-crosses with heterozygous F_2 *ldlrap1a* zebrafish were performed to generate F_3 embryos. Sequencing of F_3 mutant DNA and cDNA revealed a 7bp deletion in exon 3. This caused a frameshift resulting in a missense and a premature stop codon 37bp into exon 3. To study cholesterol clearance, we did a high cholesterol diet experiment. A heterozygous in-cross with the 7bp deletion line was performed, and the larvae were fed a high cholesterol or control diet from 4.5dpf until 9.5dpf, and then fish were stained with Oil Red O to label lipids. In fish fed the high cholesterol diet, homozygous mutants appeared to have more lipids in their blood stream compared to wild-type zebrafish, and in some cases, what appeared

to be lipid deposits were seen in the vasculature of the zebrafish. Next, we plan to quantify the cholesterol and perform qPCR on the genes *ldlr, srebp1*, *hmgcr,* and *pcsk9*, which are involved in lipid metabolism. We will also measure total cholesterol and trigyceride levels in tissues using quantifiable assays. If zebrafish have a lipid clearance defect, this line could be a useful model to study hypercholesterolemia in humans.

Abstract #46

Characterizing the role of Jagunal homolog 1 protein in neutrophil function

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The highly conserved endoplasmic reticulum protein Jagunal (JAGN1) was first identified as a requirement for Drosophila melanogaster oocyte development. Subsequently, JAGN1 mutations were correlated with Severe Congenital Neutropenia (SCN) in human patients. SCN is characterized by arrested granulocytic maturation in the promyelocyte stage. Neutrophils deficient in JAGN1 have altered granules, diminished fungal-killing capacity, and aberrant glycosylation of proteins - an important post-translational modification required for efficient cargo sorting and secretion. Overall, mutations in JAGN1 leave individuals susceptible to bacterial and fungal infections due to a decrease in circulating neutrophils and impaired release of normal granule content by the remaining cells. Despite its physiological importance, the cellular role of JAGN1 is poorly understood. Our goal is to use a zebrafish model, along with mammalian cell culture, to define the molecular functions of JAGN1. A human myeloid leukemia cell line capable of differentiation into neutrophil-like cells, HL-60 will be used to analyze neutrophil differentiation in the absence of JAGN1, define the step of secretion requiring JAGN1 function, and identify JAGN1 interactors. The zebrafish (Danio rerio) model offers a unique advantage for neutrophil studies as they are translucent, and neutrophil migration, degranulation and pathogen killing can be visualized in intact tissues in real time. Zebrafish have two homologs of human JAGN- JAGN1a and JAGN1b, an additional advantage since each may have a distinct function, providing additional information about the molecular mechanism of JAGN1 action. We have generated homozygous JAGN1a and JAGN1b-knockout fish, and are now poised to elucidate the role of these proteins by visualizing neutrophil migration and recruitment during wound response.

Abstract #47

Identifying a novel role for a canonically Golgi-associated guanine nucleotide exchange factor

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Cells must be able to transport a wide variety of proteins to and from the plasma membrane in order to ensure cellular growth, survival, and communication. Golgi Brefeldin-A resistant guanine nucleotide exchange factor 1 (GBF1) is a 206kDa guanine exchange factor that localizes to the *cis*-Golgi and is one of the earliest molecules in the COP-I secretory vesicle formation mechanism. GBF1 is ubiquitously expressed with higher expression in highly secretory tissues such as the pancreas, testes, and liver. Previously, it was observed that mutations within GBF1 are lethal in zebrafish before 96hpf [Chen et al, *Journal of Biological Chemistry* (2016)]. Similarly, in mice, rats, and *C. elegans*, null mutants in GBF1 are embryonic lethal. Though it is understood that GBF1 is absolutely essential for organismal viability, what remains unknown is the precise role of GBF1 in development. Here, we utilize biotin proximity labeling to investigate putative roles of GBF1 by identifying potential regulators of GBF1 activity and delve into one protein of interest from that list,

guanine nucleotide-binding protein subunit beta 1 (GNB1). GNB1 is part of the heterotrimeric guanine nucleotide-binding proteins and, when mutated, has been associated with global developmental delay in human patients [Lohmann et al, *Human Molecular Genetics* (2017)]. GBF1 is canonically thought to localize primarily to the cis-Golgi, but we have detected colocalization of GBF1 and GNB1 at the plasma membrane. If direct interaction is observed, a novel role for GBF1 at the plasma membrane may be revealed.

Abstract #48

Aligning the Aligners: Comparing RNA sequencing data and gene expression quantification tools for Formalin-Fixed, Parafin-Embedded (FFPB) breast cancer specimen and potential role of transposons in its early stages

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The rapid expansion of transcriptomics from increased affordability of next-generation sequencing (NGS) technologies generates rocketing amounts of gene expression data across biology and medicine, and notably in breast cancer research. Little is known as to why some patients diagnosed with atypia and DCIS remain cancer-free while others progresses to invasive ductal carcinoma (IDC). We tested the concordance of NGS RNA sequencing (RNA-seq) analysis outcomes between the two predominant programs for reads alignment, HISAT2 and STAR, and the two most popular programs for quantifying gene expression in NGS experiments, edgeR and DESeq2, using RNA-seq data from a series of breast cancer progression specimens microdissected from formalin fixed, paraffin embedded (FFPE) breast tissue biopsy blocks. Subsequently, to identify molecular signatures driving cell fate decisions at atypia and DCIS to transformation, we investigated expression of transcripts and TEs in atypia, DCIS and IDC. Methods: We created a Transcript and TE Enrichment Set Analysis (TESA) to identify transcripts and TEs in RNA sequencing datasets across four stages of breast cancer, normal atypia, DCIS, IDC (n= 8-23 samples per stage). Results: We identified significant differences in performance of the aligners: HISAT2 was prone to misalign RNA-seq reads to pseudogene genomic loci. TEs, compared to genes, exhibit substantially less variation in their expression and the majority of differentially expressed TEs were LTRs with the remaining split into DNA TEs, SINEs and unclassified at the atypia and DCIS stages. We used retroviral inhibitors to test the potential role of TEs, and the epigenetic mechanisms regulating their de-repression, in breast cancer cell lines, MCFDCIS.com and MCF10A (control) on proliferation, wound, and transformation assays. Conclusion: Our data provides support that TEs may play a role in early genomic changes as a potential mechanism underlying malignancy.

Abstract #49

Using zebrafish to define the mechanism of p53 tumor suppression

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p53 is critical for preventing tumors. Approximately 41% of human cancers have inactivating mutations in the TP53 tumor suppression gene and Li-Fraumeni patients, who inherited heterozygous loss-of-function mutations in TP53, develop various types of cancers before the age of 30. As a cellular stress sentinel, p53 protein levels are very low in unstressed, non-transformed cells, largely due to Mdm2-mediated degradation. In response to diverse cellular stresses, p53 induced transcription of a large subset of target genes resulting in activation of diverse cellular effector processes, including apoptosis, cell cycle arrest and others. However, which specific biological p53

target genes and following outcomes are critical to tumor suppression is unclear. We have used a cross species comparative analysis to define the critical regulators of p53 tumor suppression. We are using two approaches to characterize and validate which genes are true tumor suppressor genes. We hypothesize that loss of the key *p53*-induced tumor suppressive genes will mimic *p53*-null phenotypes; such that loss of these genes will rescue *mdm2*-null lethality like *p53* loss. First, we bred pro-apoptotic BH3 domain-only protein *puma*-null into *mdm2* heterozygotes and observed that loss of puma can rescue apoptosis but only partially rescue the morphological phenotype of *mdm2*-null lethality. pH3 staining indicates that there is lack of cell proliferation. To rescue the proliferative defect, we bred cyclin-dependent kinase inhibitor *p21*- null into the *mdm2*$^{-/-}$ *puma*$^{-/-}$ background and found *p21* fails to entirely rescue p53-dependent cell-cycle arrest. These data indicate that there is at least one undefined gene involved in *p53*- dependent growth arrest in zebrafish. We are pursuing other cell cycle regulators at this time. As an alternative approach, we are establishing cohort of Cas9 derived p53 target gene knockout G0 animals in a puma/noxa/p21 null background to identify genes required for tumor suppression.

Morphogenesis and Organogenesis

Abstract #50

Neural crest-derived neurons invade the ovary but not the testis during mouse gonad development

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Testes and ovaries undergo sex-specific morphogenetic changes and adopt strikingly different morphologies, despite the fact that both arise from a common precursor, the bipotential gonad. Previous studies showed that recruitment of vasculature is critical for testis patterning. However, vasculature is not recruited into the early ovary. Peripheral innervation is involved in patterning development of many organs but has been given little attention in gonad development. In this study, we show that while innervation in the male reproductive complex is restricted to the epididymis and vas deferens and never invades the interior of the testis, neural crest-derived innervation invades the interior of the ovary around E16.5. Individual neural crest cells colonize the ovary, differentiate into neurons and glia, and form a dense neural network within the ovarian medulla. Using a sex-reversing mutant mouse line, we show that innervation is specific to ovary development, is not dependent on the genetic sex of gonadal or neural crest cells and may be blocked by repressive guidance signals elevated in the male pathway. This study reveals a novel aspect of sexually dimorphic gonad development, establishes a precise timeline and structure of ovarian innervation and raises many novel questions for future research.

Abstract #51

The regulatory roles of Dpy30 and Ash2L in epigenetic control of vertebrate nervous system development

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The vertebrate nervous system comes from specific regions of the ectoderm that comprises of the neural plate and the neural crest. Although genetic mechanisms governing vertebrate neural development have been investigated in depth, there is a knowledge gap regarding the roles of epigenetic mechanisms in this process. As an epigenetic modulator, the COMPASS (also known as Set1/Mll) complex is responsible for deposition of activating histone H3K4 methylation marks at

promoters and enhancers. The critical structural subunits of COMPASS, Dpy30 and Ash2L, show high conservation from yeast to human and are involved in giving specificity to the complex by interacting with transcription factors (TFs) and regulatory proteins. Despite mounting *in vitro* and *in vivo* evidences indicating essential roles for Dpy30 and Ash2L in mesendodermal differentiation, their specific roles in neural development remain under-characterized mainly due to embryonic lethality of Ash2L and Dpy30 knockout mice. In this study, using *Xenopus laevis,*we demonstrate crucial roles of Dpy30 and Ash2L in development of the vertebrate nervous system. We show that targeted knockdown of Dpy30 or Ash2L within the developing neural tissues by antisense morpholino oligos (MOs) results in downregulation of the neural crest genes, such as Sox10, Snail2, FoxD3 and Twist, whereas the expression of the neural plate border specifier genes remain largely intact. We further show that higher doses of Ash2L MOs induce neural tube defects (NTD). Ash2L morphant embryos show a wider neural plate and fail in neural tube closure at tadpole stages. Collectively, our results indicate the importance of H3K4 methylation in regulating both the development of the neural crest and the morphogenesis of the neural tube. Funding source: National Science Foundation 1558067

Abstract #52

FGF targets such as Iroquois genes play important roles in pharyngeal development

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During early stages of development, embryonic cells differentiate to form adult tissues and organs. These cells use various genetic signals to help guide their development and differentiation. My study is focused on a genetic signaling pathway called the Fibroblast Growth Factor (FGF) pathway which is known to be important for the development of the pharynx, an embryonic structure that gives rise to craniofacial features, the thymus and parathyroid glands. We used *Xenopus laevis* as our model organism because the frog is an accessible model of vertebrate development. We performed a microarray to identify targets of the FGF signaling pathway in the foregut and pharynx of developing Xenopus embryos. We hypothesized that targets of the FGF signaling pathway may play important roles in the development of craniofacial structures. We analyzed the expression patterns of several FGF target genes with *in-situ* hybridization and RT-PCR to determine where and when genes are expressed. One group of FGF targets, the *Iroquois* genes, are expressed in the developing pharynx. We inhibited *Iroquois* translation in the developing pharynx by injecting Xenopus embryos with morpholinos. We analyzed these *Iroquois* loss-of-function embryos with *in situ* hybridization to visualize changes in gene expression in the developing pharynx. We also analyzed *Iroquois* loss-of-function tadpoles with skeletal preparations and found defects in their craniofacial features compared to sibling controls. Overall, we have identified and begun to characterize a number of FGF signaling pathway targets in developing Xenopus embryos. The more we learn about the genetic signals that help guide embryonic development, the more we will be able to understand why certain birth defects occur.

Abstract #53

Photoreceptor Packing in the Anolis sagrei Lizard

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In humans, the fovea is a specialized area of the retina that is associated with our ability to see fine detail and color. In primates, the development of the fovea is first characterized by the establishment of a pit followed by the packing of photoreceptor cells into the center of the retina. Determining how this process is governed is vital in understanding the development of visual systems. However, the

eyes of commonly used amniote models lack foveae. Therefore, very little is known concerning the genes and developmental mechanisms that contribute to the formation of this structure. In contrast, the brown anole lizard, *Anolis sagrei*, is ideal for studies of fovea development. This species develops two distinct foveae in each eye during embryogenesis. This reptile is also small, inexpensive to rear, and highly fecund. We aim to determine if the lizard eye exhibits similar photoreceptor packing patterns that have been recorded in primates. Towards this end, our study will explore retinal remodeling and fovea maturation in male and female juvenile brown anoles (2, 4 and 6 months of age) leading up to adulthood. We will do this by tracking changes in photoreceptor cell density in the fovea and assessing whether these packing trends are correlated with overall ocular growth. We predict that overall ocular growth will correlate with fovea development in terms of photoreceptor packing trends and fovea diameter.

Abstract #54

Exposure to the ubiquitous herbicide atrazine causes defects in left-right asymmetric organ development.

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Worldwide use of the herbicide atrazine (ATR) has raised serious concerns because it has been shown to have endocrine disrupting activities in larval animals, resulting in reproductive system defects. Moreover, in human epidemiology studies, ATR has also been associated with gastroschisis, omphalocele, and other structural birth defects, suggesting that it can affect development prior to the formation of the endocrine system. To begin to address this issue, *Xenopus laevis* embryos were continuously exposed to a range of concentrations of ATR during organogenesis and scored for developmental defects at tadpole stages. Interestingly, ATR-exposed embryos showed concentration-dependent left-right asymmetry defects of the digestive organs, including reversals of the gastroduodenal loop and malrotation of the intestine. To identify stages of embryogenesis sensitive to ATR exposure, embryos were then exposed to ATR starting at different times in digestive organogenesis. Embryos exposed prior to the onset of intestinal elongation had the highest frequency of reversed intestinal coiling, suggesting that the process of gut elongation is highly susceptible to ATR exposure and integral to asymmetric morphogenesis. These defects occurred at stages that precede gonadal development; thus, the underlying mechanism of teratogenicity is likely to be independent of the previously reported endocrine-disrupting effects of ATR. Intriguingly, another consistent effect was hemorrhaging throughout the embryo, indicating that ATR exposure disrupts hematopoiesis and/or angiogenesis. Our results suggest that similar cellular and/or molecular pathways may be involved in both left-right asymmetric morphogenesis and blood/vascular formation, and reveal a potentially novel, non-endocrine-mediated mechanism of action of ATR on left-right asymmetric organ development.

Abstract #55

Foxa2 is a possible downstream target of SHH pathway for the maintenance of Tbx1 expression and its role in patterning in the dorsal 3rd pharyngeal pouch.

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During mid-gestation mouse development (~E9-E10.5) the lateral aspects of the anterior endodermal gut tube evaginates to form a series of bilateral pharyngeal pouches. The thymus and parathyroid

develop from a common primordium derived from the 3rd pharyngeal pouches (3rd pp). How these two functionally and morphologically different organs derive from the same primordium is the focus of our research. T box 1 (*Tbx1*) is a key transcription factor regulating 3rd pp development. Initially important for pouch outgrowth, at E10.5 *Tbx1* is restricted to the dorsal region of the pouch and begins to regulate patterning in the pouch. *Tbx1* inhibits *Foxn1*, an important gene for thymus development, and may activate *Gcm2*, an important gene for parathyroid differentiation and survival. We know that *Tbx1* is activated by the SHH pathway. *Tbx1* is activated by SHH signaling during heart development; as SHH is also required for parathyroid fate specification, this suggests that SHH signaling could also regulate *Tbx1* in the 3rd pp. *Foxa2* has been identified as mediating SHH's effect on *Tbx1* in the pharyngeal endoderm during heart development, making it a strong candidate for regulating *Tbx1* in the 3rd pp. I have shown that FOXA2 is expressed in the 3rd pp and colocalizes with TBX1 in the dorsal region of the pouch. Using a hypomorphic mutant of Smoothened known as cabbie, which affects the highest levels of SHH response, I have shown that reduced SHH signaling reduces FOXA2 and TBX1 expression in tandem. These data suggest that *Tbx1* is a direct transcriptional target of FOXA2, filling a gap in our knowledge of how SHH signaling directs patterning in the 3rd pp. Funding provided to NRM from NIH/NIAID 1R01AI107096-01A1

Abstract #56

Ataxin10 as a novel ciliary associated gene involved in regulating early cardiac development in mice.

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Primary cilia are microtubule-based structures that are present on most mammalian cells. These structures play crucial roles throughout development; however, exact mechanisms through which cilia regulate developmental processes is still largely unknown. To elucidate ciliary functions during development we have incorporated an algorithm that compiles information from multiple databases to identify candidate genes involved in ciliary regulated developmental events, which also result in lethality in null animals. One of the candidate cilia related genes identified by the algorithm is *Ataxin10,* which encodes the largely uncharacterized protein ATXN10. Mutations in ATXN10are commonly associated with the penta-nucleotide expansion disorder spinocerebellar ataxia. Using an *Ataxin10* mutant mouse line generated by the International Mouse Phenotyping Consortium (IMPC), we have begun to test the hypothesis that ATXN10 is involved in ciliary mediated developmental processes in the mouse. We show strong expression of *Ataxin10* in the developing heart starting at E8.5. Our data indicates *Ataxin10* mutant embryos present with severe pericardial edema and developmental delay at E10.5 with lethality occurring by E11.5; suggesting that ATXN10 is necessary for proper cardiac development. Upon further histological analysis and contrast enhanced μCT we have observed reduced ventricular wall thickness and trabeculation abnormalities. Preliminary data suggests that the reduction in trabeculation is due to cell cycle irregularities. We have shown that primary cilia are present in *Ataxin10* mutants. Additionally, through the use of ATXN10 fluorescently labeled expression constructs and immunofluorescence microscopy we demonstrated that ATXN10 co-localizes with the basal bodies. These studies will show that a gene commonly associated with adult neurological disorders in humans has a critical role in cilia mediated, embryonic heart development in the mouse.

Abstract #57

Dact1 Function in the Planar Cell Polarity Pathway

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The planar cell polarity (PCP) pathway has been well characterized in Drosophila wing epithelial cells in which core PCP proteins localize in complexes on opposing cell cortexes, establishing polarity in the plane of the epithelium. In the fly, transmembrane protein Vangl2 and Prickle (Pk) localize to the proximal side of the plasma membrane, while on the opposing distal side, transmembrane protein Frizzled (Fz) and Dishevelled (Dvl) are localized to the plasma membrane. This intricate process effectively establishes sweeping polarity in the cells of the fly wing and eye, though it is not known how these proteins interact to regulate vertebrate development. The PCP pathway is thought to regulate convergent extension (CE), the collective cell movement wherein cells change shape and intercalate in an oriented fashion to cause the narrowing of one axis and the elongation of a perpendicular axis. We have shown that Vangl2 can regulate PCP in a bimodal fashion through recruiting and sequestering Dvl on the plasma membrane. A non-core PCP protein, Dact1, had previously been shown to genetically antagonize Vangl2 in the mouse. To understand mechanistically how Dact1 functions and how it may impact Vangl2-Dvl interaction, we are carrying out functional and biochemical studies in Xenopus. We confirmed the mouse study results, and found that Dact1 and Vangl2 antagonize each other in Xenopus. Consistent with our previous model, we have also shown that Dact1 and Dvl2 synergize to activate PCP signaling during CE. Our future imaging and biochemical studies will elucidate the molecular basis of these interactions to help us establish new models the PCP pathway during CE.

Abstract #58

Loss of KIAA0753 Impairs Ciliogenesis and Results in Severe Developmental Abnormalities Leading to Embryonic Lethality

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The primary cilium is a microtubule based cellular appendage that arises from the centrioles in quiescent cells. Primary cilia can be found on nearly every mammalian cell type and are present from embryonic implantation onward. The primary cilium facilitates many crucial signaling events, making it a required component for normal embryonic development. Mice that are unable to form primary cilia, such as Ift88 and Kif3a mutants, present with severe developmental defects, including neural tube patterning and closure abnormalities and heart tube looping defects, resulting in lethality by approximately E10.5. The aim of this project is to identify and characterize novel cilia-related genes that result in developmental defects leading to embryonic lethality. We have identified a novel ciliary candidate gene, *KIAA0753*, through the use of the bioinformatics algorithm, the INDICiliATOR, which compiles data from proteomes, transcriptomes, and published ciliary databases to score candidate cilia genes. This gene received a moderate INDICiliATOR score, and is reported to be homozygous embryonic lethal by the International Mouse Phenotyping Consortium (IMPC). *KIAA0753* encodes for the protein Moonraker (Moonr), a centriolar satellite component that is required for centriole duplication *in vitro*. Here, we show that complete loss of *KIAA0735* in mice results in a loss of primary cilia, and leads to neural tube patterning defects, body axis turning defects, pericardial edema and embryonic lethality by E11.5. Current experiments are focused on characterizing how loss of cilia in these mutants disrupts ciliary mediated signaling pathways, particularly the Sonic hedgehog (Shh) pathway, and defining how loss of Moonr impairs ciliogenesis.

Abstract #59

Identification of Akirin-interacting proteins that are critical for myogenesis

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The specification and differentiation of muscle precursor cells, or myoblasts, by the action of the Twist mesodermal regulator is a key event in the formation of the *Drosophila* larval musculature. Myoblast population dynamics are tightly controlled by gene expression moderated by Twist to determine somatic myoblast fates. Despite the primary importance of Twist for specifying and patterning the musculature, the identities of many molecular players involved in this process remain unknown. Akirin, a highly conserved nuclear transcriptional cofactor, regulates Twist-dependent gene expression via interactions with the Brahma chromatin remodeling complex during mesodermal specification and muscle development. Using a genetic interaction screen in *Drosophila*, we have begun to identify other Akirin interacting proteins that participate in the process of muscle specification, patterning, and development. Our screening method has determined that Akirin interacts with Mi-2, the catalytic subunit of the NuRD complex, to correctly pattern the skeletal musculature. Double heterozygous mutant embryos for *akirin* and *mi-2* demonstrate a host of deranged or misshapen muscle phenotypes. Further, genetic interactions between *akirin* and other subunits of the NuRD complex appear to display disruptions in muscle patterning, implicating the larger NuRD complex in this process. Finally, our screening process has identified other loci beyond the NuRD complex that genetically interact with *akirin* to facilitate myogenesis. Through the generation of an interactome of potential partners, we will gain crucial insight into mechanism of Akirin during myoblast specification and muscle patterning.

Abstract #60

Anomalous Incisor Morphology Suggests Tissue-Specific Roles for Tfap2A/B in Mouse Dental Development

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Mice have two types of differently-shaped teeth in their dentition, incisors with a single cusp and molars with multiple cusps. Mammalian teeth develop from two types of tissues, dental mesenchyme and epithelium. Reciprocal signaling between the epithelium and mesenchyme is required to form properly shaped teeth during development, and the molecular basis of these interactions has received considerable attention. The transcription factors AP-2 alpha and beta (*Tfap2a* and *Tfap2b*) are important for craniofacial and dental development in mice, but little is known about their roles in the regulation of tooth shape. To better understand the roles of *Tfap2a* and *Tfap2b* in development of incisor and molar morphology, we examined the shapes of incisors and molars in mice with conditional deletions of *Tfap2a* and *Tfap2b* in different tissue compartments. Tooth shape was analyzed using micro-CT at embryonic day 18.5, when the tooth crown shapes have developed. We examined two different tissue-specific genetic mutants: one with *Tfap2a* and *Tfap2b* deleted from the epithelium and one with *Tfap2a* and *Tfap2b* deleted from the mesenchyme. Dental tissue was digitally isolated from other oral tissues from micro-CT data and 3-D volumetric models were constructed for each tooth. Results from the 3-D reconstruction and histology revealed that in mice with the epithelial-specific deletion of *Tfap2a* and *Tfap2b*, the upper and lower incisors are longer and are more curved than those of the control mice. Furthermore, some individuals have duplicated lower incisors. By contrast, the dental phenotype of mice with the mesenchyme-specific deletion of *Tfap2a* and *Tfap2b* did not differ from the control embryos. Overall, these results demonstrate that epithelial expression of *Tfap2a* and *Tfap2b* is important for controlling tooth number, length, and shape, whereas mesenchymal expression of *Tfap2a* and *Tfap2b* is not required for tooth development.
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Abstract #61

Inhibition of class I and II Hdac function affects tbx5 expression in zebrafish

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Zebrafish embryos exposed to high doses of valproic acid (VPA, 0.5-1.0 mM), a class I and II Hdac inhibitor, show a dose-dependent pattern of cardiac malformations, reduced pectoral fin growth, ocular coloboma, and pharyngeal skeleton malformations. Previous studies with *tbx5a* and *tbx5b* knock-out zebrafish show similar phenotypes to VPA-exposed fish, and mimic Holt-Oram syndrome (*Tbx5* loss-of-function) in humans, indicating that Hdacs and *Tbx5* may function in the same genetic pathways. In zebrafish, *tbx5a* and *tbx5b* are co-expressed in the heart, eye, and pectoral fins, although subtle differences in expression and mutant phenotypes indicate that paralogs may have sub-functions in development, and therefore different mechanisms of regulation. Our hypothesis is that the *tbx5* genes in zebrafish are regulated by Hdacs. Using RT-qPCR, we observed in VPA-treated embryos a significant dose-dependent downregulation of *tbx5b* and upregulation of *tbx5a.* mRNA *in situ* hybridization of *tbx5a* and *tbx5b* expression in VPA-treated fish shows a similar trend to the RT-qPCR data, with elevated levels of *tbx5a* expression in the eye and normal levels in the pectoral fin, and diffuse and minimal expression of *tbx5b* in the heart and pectoral fin. Our findings suggest that *tbx5b* may be a target of Hdac function, but *tbx5a* is not. Further study of expression of downstream targets of *tbx5a* and *tbx5b* in the heart (*vcana, nppa* and, *hand2*) will be examined using mRNA *in situ* hybridization. Rescue of VPA-treated fish using *tbx5a* and *tbx5b* mRNA is also planned.

Abstract #62

Determining how phf21aa affects craniofacial development in zebrafish

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In humans, mutations in the transcriptional repressor *PHF21A* (PHD finger protein 21A)causes Potocki-Shaffer syndrome which is associated with craniofacial defects. Previously, it was observed that knockdown of *phf21aa* in zebrafish caused defects to larval craniofacial cartilage. It is therefore hypothesized that *phf21aa* functions in zebrafish similarly to how it functions in humans, and thus we can use zebrafish to understand the physiology of Potocki-Shaffer syndrome. In our lab, we generated lines of zebrafish with insertions and deletions in *phf21aa* using CRISPR-Cas9. An F_0 line was outcrossed to create an F_1 generation. The F_1 generation was genotyped to identify heterozygotes. Heterozygotes were outcrossed to wild-type fish to create an F_2 generation. F_1 and F_2 heterozygotes were identified using PCR and T7 endonuclease digest. For *phf21aa* heterozygotes, thePCR product is 995 base pairs and the T7 endonuclease digest products are 720 and 275 base pairs. Recently we developed PCR primers that can identify heterozygote, mutant, and wild-type fish based on shifts in PCR band size. In this case, the wild-type PCR product is 641bp and the heterozygote product contains a 641bp band and a lower band representing a deletion. The F_3 generation was in-crossed and resulted in roughly 25% homozygous mutants. Sequencing analysis revealed a 7bp deletion in exon 6, producing a frameshift which is predicted to generate extensive missense and a premature stop codon. Currently, skeletal preparations of heterozygous in-crosses are being screened for evidence of patterning defects. Studying how disruptions to *phf21aa* affect skeletal development in zebrafish can help us to understand the normal functions of this gene in craniofacial development, and how mutations cause Potocki-Shaffer syndrome defects in humans.

Abstract #63

Epigenetic Regulation of Sex Determination in The Mouse Fetal Gonad

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XX and XY fetal gonads are initially bipotential, poised between the ovary and testis fate. Multiple lines of evidence suggest that commitment to testis fate requires the repression of genes associated with ovary fate. It was previously shown that loss of CBX2, the subunit of the Polycomb Repressive Complex 1 (PRC1) that binds H3K27me3 and mediates silencing, leads to ovary development in XY mice and humans. While it had been proposed that CBX2 is an activator of the testis-determining gene *Sry,* we investigated the alternative possibility that CBX2 has a direct role as a repressor of the antagonistic ovary-promoting pathway. To investigate this possibility, we developed a quantitative genome-wide profile of the repressive histone mark H3K27me3 and its active counterpart H3K4me3, in isolated XY and XX gonadal supporting cells before and after sex determination. We show that testis and ovary sex-determining (SD) genes are bivalent before sex determination, providing insight into how the bipotential state of the gonad is established at the epigenetic level. After sex determination, many SD genes of the alternate pathway remain bivalent, possibly contributing to the ability of these cells to transdifferentiate even in adults. The finding that many genes in the Wnt signaling pathway were targeted for H3K27me3-mediated repression in Sertoli cells led us to test whether deletion of *Wnt4* could rescue testis development in *Cbx2* mutants. We show that *Sry* expression and testis development were rescued in XY *Cbx2*$^{-/-}$*;Wnt4*$^{-/-}$ mice. Furthermore, we show that CBX2 directly binds the downstream Wnt signaler *Lef1*, an ovary-promoting gene that remains bivalent in Sertoli cells. Our results suggest that stabilization of the testis fate requires CBX2-mediated repression of bivalent ovary-determining genes, which would otherwise block testis development.

Abstract #64

Identifying targets of TBX5 in developing forelimbs and genitalia

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Tbx5 encodes a transcription factor that is necessary to regulate the growth and development of the forelimb, but not the hindlimb. A recent study has identified thousands of putative enhancers targeted by TBX5 in mouse embryonic forelimbs. Our lab has previously shown that many enhancers active in the developing forelimb are also active in the genital tubercle (GT). Although *Tbx5* has been found to also be expressed in the developing genitalia, its role in this tissue is almost completely unexplored. We have performed TBX5 ChIP-seq in mouse embryonic forelimbs and GT and find that a significant subset of TBX5 binding sites are shared between these appendage types. In both the forelimb and GT, TBX5 binding sites are significantly enriched near genes involved in limb development. In order to identify *Tbx5*-dependent genes and to determine the functional importance of *Tbx5* during GT development, we will conditionally knockout *Tbx5* in developing forelimbs and

genitalia and perform RNA-seq. The impact of ablating *Tbx5* function on GT morphology will be assessed, and we will intersect our ChIP-seq and RNA-seq datasets to further investigate the enhancer regions and target genes directly regulated by TBX5 in developing appendages.

Abstract #65

Determining the function of phf21ab in craniofacial development in zebrafish

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PHF21A is an element of the BRAF-HDAC complex that causes target gene transcription to be repressed. In humans, mutations in PHF21A can result in a disorder known as Potocki-Shaffer syndrome, which causes craniofacial malformation and neurological defects. Previously, we generated potential mutant lines in zebrafish targeting phf21ab using CRISPR-Cas9 mutagenesis. Recently, we genotyped F_1 offspring of an F_0 line with potential mutations in phf21ab. We identified a female F_1 heterozygote that was crossed with a wild-type male to generate an F_2 line composed of heterozygotes and wild-types. The identification was made using PCR and T7 endonuclease assay. Results were analyzed to look for differences in band sizes to indicate genotypes. The original PCR product is 613bp. If a sample is from a heterozygote, two lower bands (447bp and 166bp digestion products) are created through T7 endonuclease digest. Fish that display all three bands are heterozygous while the fish with just a 613bp band are wild-type. We plan to create two additional lines of fish (using other F_1 parents) and rear them into adulthood to generate additional F_2 generations. Once F_2 fish are adults, heterozygotes will be genotyped and in-crossed to create 25% homozygous mutants. The fish will then be screened for craniofacial phenotypes. This will be done through a staining process to label bone and cartilage two separate colors to make them identifiable. Potential mutant offspring will be sequenced to characterize lesions produced using CRISPR-Cas9 mutagenesis. Through this project and other projects in the lab, we hope to identify the function of the paralogs of phf21a (phf21aa and phf21ab) in zebrafish craniofacial development, and explore hypotheses for how mutants in these genes may reproduce aspects of PHF21A loss of function in humans. Potentially, this study could result in a zebrafish model for Potocki-Shaffer syndrome.

Abstract #66

Bimodal regulation of Dishevelled function by Vangl2 during morphogenesis

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Convergent extension (CE) is a fundamental morphogenetic mechanism that underlies numerous processes in vertebrate development, and its disruption can lead to human congenital disorders such as neural tube closure defects. The dynamic, oriented cell intercalation during CE is regulated by a group of core proteins identified originally in flies to coordinate epithelial planar cell polarity (PCP). The existing model explains how core PCP proteins, including Van Gogh (Vang) and Dishevelled (Dvl), segregate into distinct complexes on opposing cell cortex to coordinate polarity among static epithelial cells. The action of core PCP proteins in the dynamic process of CE, however, remains an enigma. In this report, we show that Vangl2 (Vang-like 2) exerts dual positive and negative regulation on Dvl during CE in both the mouse and Xenopus. We find that Vangl2 binds to Dvl to cell-autonomously promote efficient Dvl plasma membrane recruitment, a pre-requisite for PCP activation. At the same time, Vangl2 inhibits Dvl from interacting with its downstream effector Daam1 (Dishevelled associated activator of morphogenesis 1), and functionally suppresses

DvlàDaam1 cascade during CE. Our finding uncovers Vangl2-Dvl interaction as a key bi-functional switch that underlies the central logic of PCP signaling during morphogenesis, and provides new insight into PCP-related disorders in humans.

Abstract #67

Investigating the mechanism by which the platelet-derived growth factor receptor alpha (Pdgfra) regulates heart tube assembly in zebrafish.

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Paracrine signaling is essential to organ formation. During cardiac development, tissue-tissue communication is critical for the proper formation of the heart tube. Previous work in our laboratory analyzing an ENU-induced mutation in platelet-derived growth factor receptor alpha (*pdgfra)* has identified a role for the PDGF signaling pathway in directing the bilateral populations of myocardial precursors towards the embryonic midline, where they merge and form a heart tube, in a process known as cardiac fusion. Based on this work we have hypothesized that the PDGF signaling pathway acts in a paracrine manner, in which signals from the endoderm are received by the myocardial precursors to direct their movement toward the midline. To examine this hypothesis, we are examining the tissue-specificity of PDGF signaling and the cellular behaviors regulated by PDGF signaling. To determine the tissue specificity of PDGF signaling, we will create a conditional *pdgfra* mutation. Using CRISPR-Cpf1 gene editing approach, we are inserting LoxP sites in two introns (introns 16-17 and 28-29) flanking the catalytic kinase domain of *pdgfra*. Following generation of the floxed alleles, we will induce a myocardial specific deletion of *pdgfra* using *Tg(myl7:Cre)*. Based on our hypothesis, we anticipate failure of myocardial cells to fuse in the midline at 21 hpf in these conditional mutants. To examine the cellular behaviors regulated by PDGF signaling, we will analyze myocardial cells for migratory protrusions, such as filopodia and/or pseudopodia. We have hypothesized that PDGF signaling regulates the formation of medially-directed migratory protrusions in the myocardium. Since cardiac disorders are often a result of defects in cardiac morphogenesis, in the long-term we expect these studies to contribute to our understanding of the etiology of congenital heart defects. Research funding provided by an AHA career development award (18CDA34080195)

Abstract #68

Investigations into cellular forces regulating cardiac fusion.

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During organ morphogenesis, cells often function as a community to coordinate changes in movement and shape. The process of heart tube formation involves collective movement of bilateral populations of myocardial cells to the midline, where they meet and merge, a process called cardiac fusion. During cardiac fusion, myocardial cells undergo a mesenchymal-to-epithelial (MET) transition forming strong cell-cell contacts between the myocardial cells. Previous studies in our laboratory have found that PDGF signals are required to direct the myocardial cells to the midline. However, the mechanisms by which PDGF signalling rearranges the cellular forces within the myocardial populations to facilitate medial movement remains unclear. To analyse whether PDGF signalling affects MET during cardiac fusion we are using immunofluorescence analysis of the tight junction protein ZO-1 and live imagining of adherens junctions using the N-cadherin reporter *Tg(cdh2:cdh2-EGFP*) in wildtype and *pdgfra* mutant embryos. This analysis will also allow us to visualize the changes in cell morphology that occur during cardiac fusion. To directly measure

differences in tension within myocardial cells and between wildtype and *pdgfra* mutant cells, we will use laser ablation techniques. Altogether we aim to elucidate how forces within and between myocardial cells are regulated during collective movements occurring during heart tube formation. Research funding provided by an AHA career development award (18CDA34080195).

Cell-Cell Communication and Signaling

Abstract #69

Local ecdysone signals in Drosophila larval wing regeneration

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Regeneration has long fascinated scientists, perhaps due to the contrast between the restorative regenerative responses seen in certain animals, such as salamander limbs, and scar tissue formation that occurs in most mammals, such as humans. Variation in regenerative capacity is not only observed between species, but can also occur between different developmental ages within a single tissue and organism. For example, neonatal mouse cardiac tissue demonstrates a strong regenerative response, but injuries later in life produce scar tissue and impaired cardiac function. This pattern of a progressive loss of regenerative potential with age suggests that a better understanding of molecular mechanisms that guide regeneration might allow us to reactivate regenerative responses in aged tissue or confer *de novo* regenerative ability. *Drosophila* larval imaginal wing discs (precursors of adult fly wings) have been used to study the mechanisms of regenerative growth since the mid-1900s. Many of the pathways involved in formation of a blastema, or localized zone of proliferating cells around the wound, and subsequent tissue regrowth have been elucidated. Intriguingly, these are primarily well-conserved pathways also involved in developmental growth (e.g. Wnt, JAK/STAT, Hippo). In 2015, our lab discovered that a physical interaction between the Yorkie and Taiman proteins, respectively coactivators for the Hippo and Ecdysone pathways, is required for transcription of genes involved in cell proliferation, survival, and stemness. My PhD work has focused on the question: “Is there a role for this signaling axis in regenerative growth?” I will review data suggesting that the Ecdysone pathway is specifically induced in the blastema and that local synthesis of the Ecdysone steroid hormone is a required element of the imaginal disc regenerative program in *Drosophila* that drives regeneration through the Taiman-Yorkie axis.

Abstract #70

Elucidating novel roles of Gdi2 in ciliary mediated signaling during embryonic development

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The primary cilium is a microtubule-based structure critical for signaling throughout embryonic development. Failure of cilia to form causes lethality, and mutations that cause disruptions to cilia structure or function produce a spectrum of disorders. Ciliogenesis and cilia mediated signaling is a complex process that involves regulation of vesicular transport mechanisms, many of which are not well understood. Through a bioinformatics algorithm, a Rab associated GDP dissociation inhibitor (Gdi) 2 was identified as a potential gene involved in cilia signaling. Although many Rabs have been identified as regulators of cilia formation, maintenance, and function, the role of regulatory factors such as GDIs remain unclear. A Gdi2 knockout mouse is being used to address this. To determine if ubiquitously expressed Gdi2 is required for cilia formation, we generated MEFs. Gdi2 mutant MEFs appear to have cilia frequency and length similar to that observed in control MEFs. Whether there are ciliogenesis defects in Gdi2 mutant embryos is currently being analyzed. Thus far we have recovered one live mutant that subsequently died within one day of birth. The cause of death is unknown but preliminary data suggest it is related to respiratory insufficiency. Additionally, we have

observed one mutant at E18.5 that exhibited impaired orofacial development. Based on our initial data, we are testing the hypothesis that Gdi2 is involved in regulating vesicular trafficking to the primary cilia that is not necessary for ciliogenesis, but rather for regulation of ciliary sensory and signaling events. To address this, we are analyzing the localization of Gdi2, cilia-associated Rabs, and cilia localized receptors, in both MEFs and embryonic tissue as well as assessing potential differences in cilia number, length, and morphology by IF, TEM, and SEM. Future directions include generating conditional lines to explore the role of Gdi2 in post-embryonic development and in specific cell types.

Abstract #71

Understanding the Secretion Mechanism of VAPB MSP

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Most eukaryotic, secreted proteins travel through the Golgi-ER secretory pathway. However, certain secreted proteins travel via an alternative mechanism independent of the Golgi-ER. VAPB (VPR-1 in *C. elegans*) is a type-II ER transmembrane protein whose N-terminal Major Sperm Protein (vMSP) domain is cleaved and secreted. This vMSP domain extends into the cytosol and does not harbor a conventional signal peptide. Our goal is to understand how vMSP is proteolytically processed, secreted, and regulated. We hypothesize that vMSP is secreted in a regulated fashion via an unconventional mechanism. To test our hypothesis, we developed an RNAi screen using *C. elegans* to identify genes required for vMSP secretion. Then, we used genome editing techniques to tag the termini of endogenous *vpr-1*. These techniques will allow for a direct read-out of vMSP secretion in *C. elegans*. Our results show that *vpr-1* null worms are sterile and have striated muscle mitochondrial abnormalities. We predicted that RNAi of genes essential for vMSP secretion would cause sterility and mimic the muscle mitochondrial defect. Candidate genes resulting from the 420 genes screened will be targeted to further determine their role in vMSP secretion. Western blots of epitope tagged *vpr-1* endogenous termini revealed two stable N-terminus cleavage products. Immunofluorescence revealed a polar localization of *vpr-1* termini in intestinal cells. Further work is in progress to determine whether vMSP cleavage and secretion is developmentally regulated.

Abstract #72

Wnt1/8-Fzl5/8-JNK-ATF2 signaling and the gene regulatory network essential for anterior-posterior specification and patterning in the sea urchin embryo

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A posterior-to-anterior gradient of Wnt signaling plays a fundamental role in anterior-posterior (AP) axis formation in most metazoan species. We have recently discovered that canonical Wnt/β-catenin as well as non-canonical Wnt signaling pathways (Wnt16-Frizzled1/2/7 and Wnt1/8-Frizzled5/8-JNK) work as an interconnected Wnt signaling network responsible for specifying and patterning the early AP axis in the sea urchin embryo. We previously showed that Wnt1/Wnt8-Fzl5/8-JNKsignaling plays a critical role in down-regulating the initially broadly expressed anterior neuroectoderm (ANE) gene regulatory network (GRN) in the equatorial ectodermal in the anterior half of the embryo. In this study, we performed high-throughput whole-transcriptome differential screens to identify Wnt signaling components and transcription factors (TFs) regulated by Fzl1/2/7-PKC signaling and Wnt1/Wnt8-Fzl5/8-JNK signaling. We observed that Fzl1/2/7-PKC signaling, which antagonizes Wnt1/Wnt8-Fzl5/8-JNK signaling during

ANE restriction, represses the expression the TF ATF2, a known c-Jun N-terminal Kinase (JNK) target. ATF2 knockdown embryos showed an expansion of ANE GRN expression while the gain of function of ATF2 eliminated it. In addition, We have identified several TFs activated by the Wnt1/8-Fzl5/8-JNK pathway and focused our initial analysis on the GRN regulated by Wnt1/Wnt8-Fzl5/8-JNK signaling on Sp5, a TF that is activated by Wnt8 and is essential for early AP neuroectoderm patterning in vertebrates. Our results indicate that similar to vertebrates Sp5 is also necessary to down-regulate ANE GRN factors from posterior ectoderm cells. Together, our results show that we identified an important transcriptional level interaction between the Fzl1/2/7-PKC and Fzl5/8-JNK signaling pathways through the regulation of *atf2* expression. In addition, our data strongly suggest that Sp5 is a conserved TF essential for restricting ANE GRNs around the anterior pole in deuterostomes.

Abstract #73

Rgp1 is required for normal craniofacial development in zebrafish

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Cells produce and secrete proteins to the extracellular space that assemble to form an extracellular matrix (ECM). The ECM is essential for growth, wound healing, and structural development. Defects in the ECM proteins themselves or their associated secretory machinery often manifest in clinical phenotypes. Much of the secretory machinery was discovered in yeast. Yeast, as single-cell organisms, do not secrete ECM proteins. Thus, the role of secretory machinery in ECM secretion has remained largely unexplored. A protein known as Rgp1 had been shown in yeast and mammalian cells to bind Ric1 and function as a guanine nucleotide exchange factor (GEF) complex for Rab6. However, the role of Rgp1 has not been examined *in situ* in multicellular organisms, and there is currently no vertebrate model to study its function. To understand the role of Rgp1 in a vertebrate, I used CRISPR/Cas9 genome editing to generate a stable *rgp1*-null (*rgp1*$^{-/-}$) zebrafish line.My preliminary studies have shown that in the absence of Rgp1, zebrafish embryos manifest with malformed craniofacial cartilage, a tissue primarily composed of chondrocytes. The *rgp1*-deficient chondrocytes accumulate collagen intracellularly and adopt a biconcave discoid morphology, suggesting a more complex function of Rgp1 in multicellular organisms. Zebrafish and human sequences are highly conserved; therefore, I predict that findings in zebrafish will inform us of Rgp1 biology in humans and other vertebrates. I present my progress on the characterization of the two CRISPR-induced mutant lines and their phenotypic description.

Abstract #74

Genetic analysis of the ciliary transition zone in *Danio rerio*

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Cilia are microtubule-based organelles that project from the surface of cells and play important roles in protein trafficking, signaling cascade regulation, mechanical movement of fluids, and cell motility. Mutations that affect ciliary components are associated with a multitude of human diseases collectively called ciliopathies. A subset of these disease-associated proteins localizes to a specialized domain located proximally to the base of the cilium known as the transition zone (TZ). The localization of these proteins to the TZ and protein-protein interaction analysis indicates that

these proteins form a multi-protein complex to control the entry and exit of molecules within the cilia. While these TZ proteins are viewed to function as a single unit which gates the same molecules, phenotypic variability between diseases suggests they may have specific functions or gate specific molecules. Our goal was to provide a comprehensive analysis of null zebrafish mutants in ciliopathy genes associated with the TZ, such as *cc2d2a* (*mks6*), *rpgrip1l* (*mks5*), *tmem216* (*mks2*), *mks1, b9d2*, *nphp4*, and *nphp1* in hopes of identifying common or unique phenotypes associated with a mutation in specific TZ genes. We see that *mks6, mks5,* and *mks2* display embryonic lethality and a ventrally curved body axis and tail; while, *mks1*, *b9d2*, *nphp1*, and *nphp4* null mutants display grossly normal body morphology and embryonic survival. Across our TZ null mutants, we see variations in kidney cyst formation, motile cilia within the pronephric duct, primary cilia of the mechanosensory hair cells, and embryonic viability. Our data indicate the presence of these strong variations in phenotype suggesting specific roles for each of these TZ genes. Moreover, our phenotypic data suggest that certain subsets of these proteins act together while others act independently of one another.

Evolution and Development

Abstract #75

Investigating the transposition of the Harbinger3n_Dr transposable element in yeast

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DNA transposable elements are mobile sequences of DNA that use a cut-and-paste mechanism to "jump" from one site in the genome to another. They are found in all kingdoms of life and are sorted by homology into groups called superfamilies. The *Harbinger3n_Dr* transposable element, from zebrafish belongs to the *PIF/Harbinge*r superfamily. We are interested in studying this element because it has previously been shown to transpose in human cells, where it can be used as a tool for transgenesis or mutagenesis. Our goal is to learn more about its transposition characteristics, as well as develop hyperactive versions that transpose at higher rates. Previous experiments have shown that the *Harbinger* ORF1 and *Harbinger* Transposase (TPase) proteins must be present for *Harbinger3n_Dr* to jump. I have developed *Harbinger* ORF1 and *Harbinger* TPase expression constructs and transformed them into yeast together with a *Harbinger3n_Dr* reporter construct. Yeast transposition assays showed that *Harbinger3n_Dr* transposes at a very low rate. Sequence analysis revealed that *Harbinger3n_Dr* transposition can result in imprecise repair of excision sites. These results suggest that either the yeast cells are not a very compatible host of this element or that our assay is not effectively measuring transposition. Because we observed some imprecise repair of *Harbinger3n_Dr* excisions sites, we are testing if providing a homologous template in a diploid yeast will allow for precise repair of the excision sites. If imprecise repair was the limiting factor, this strategy should allow us to observe the true number of transposition events.

Abstract #76

TGFb in C. elegans regulates a novel mechanism of prostaglandin synthesis

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In internally fertilizing animals, sperm must nagivate the convoluted female reproductive tract to fertilize the oocyte. Our lab has developed *C. elegans* as an in vivo model to study sperm guidance. The clear epidermis of *C. elegans* allows for direct visualization of labeled sperm in an intact oviduct. Using this model, we have identified a specific class of F-series prostaglandins (PGFs) that are important for guiding sperm toward the fertilization site. Prostaglandins are classically synthesized from polyunsaturated fatty acids (PUFAS) via the cyclooxygenase (COX) enzymes, but these genes are not encoded by the *C. elegans* genome. Recent data from *C. elegans* show that PGF

levels are regulated by the DAF-7/TGFbsignaling pathway. DAF-7, the homolog of TGFb, is a neuroendocrine factor secreted by the *C. elegans*ASI sensory neurons in response to food and pheromone cues. It signals through the DAF-1 Type I and DAF-4 Type II TGFbreceptors, which act through downstream R-SMADs DAF-8 and DAF-14 to inhibit DAF-3 Co-SMAD. In this study, we show that the sperm guidance defect seen in *daf-1*mutant is suppressed in the *daf-1;daf-3*double mutant. Further studies using mass spectrometry showed the sperm guidance phenotype correlated with the levels of PGF detected in these mutants. To further understand the mechanism by which DAF-3 affects sperm guidance and PGF metabolism, we created *daf-3* mosaic animals to identify the tissues where DAF-3 function was necessary to promote sperm guidance. We found that expression of *daf-3* in the intestine and germline is important to promote sperm guidance. Furthermore, using an in vitro biochemical reaction of worm lysates and the PGF precursor, arachidonic acid, we found that TGFbpathway mutants can synthesize similar levels of PGFs. Together, these data suggest that the DAF-7/TGFbpathway may be regulating PGF levels by modulating the transport of PGF precursors from the intestine to the oocytes, where they are converted to PGFs.

Abstract #77

Interaction of Casein Kinase 1 delta/epsilon and Dishevelled within puncta in a novel cortical domain at the vegetal pole specifies egg and embryo polarity in sea urchins

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The Dishevelled (Dvl) protein plays a critical role in regulating Wnt signaling but how Dvl is "activated" during Wnt signaling remains an enduring gap in knowledge. Dvl is commonly seen in cytoplasmic "puncta" that have been shown to be key sites for Dvl regulation, and in sea urchin eggs Dvl puncta are embedded in a novel vegetal cortical domain (VCD). Ultrastructurally, endogenous Dvl puncta have a flat, petal-like appearance and Dvl localized to puncta become differentially post-translationally modified. During early cleavage, VCD Dvl puncta are inherited by vegetal blastomeres where Wnt/β-catenin (cWnt) signaling is initially activated. Functional studies have indicated that localized Dvl activity mediates cWnt signaling in vegetal blastomeres, but the mechanism for its activation during Wnt signal transduction is not known. To identify Dvl interacting proteins (DIP) that may regulate its activity before and after Wnt signaling is initiated we carried out Dvl Co-Immunoprecipitation (Co-IP) from lysates of isolated unfertilized egg cortices and 16-cell stage micromere cells where cWnt is first activated and identified putative DIPs using mass spectrometry (MS). One DIP identified from cortex and micromere lysates was Casein Kinase 1 δ/ε (CK1δ/ε). A CK1δ/ε::GFP fusion protein co-localized with Dvl in VCD puncta and overexpressing a kinase-dead CK1δ/ε resulted in a lack of endomesoderm gene expression and an anteriorization of embryos. Downregulation of CK1δ/ε activity also resulted in an expansion of the vegetal domain of Dvl puncta suggesting that these structures may repress Wnt signaling. Overexpression of CK1δ/ε or Dvl alone did not affect endomesoderm gene expression, but CK1δ/ε and Dvl co-overexpression led to a dramatic upregulation of these genes and posteriorization of embryos. Our results indicate that CK1δ/ε and Dvl function together in VCD puncta to specify egg polarity and to positively regulate Wnt signaling during early sea urchin embryogenesis.

Abstract #78

The role of sex and hormones on craniofacial development

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Differences between males and females, or sexual dimorphisms, can be seen across animals. For example, the sex of a human can be determined simply by shape of facial bones. While these differences can be affected by hormones during puberty, differences between sexes in human skulls are apparent as early as 1-3 years old. However, we don't fully understand the role of hormones in

initial patterning of facial bones and the patterns of sexual dimorphism between species. To answer this, we used cichlid fishes, which have a large and continuous spectrum of craniofacial variation. In a group with such large differences in facial shapes between species, we first asked if there is variation between males and females. Shape analysis of 400 F2 hybrids of *Aulonocara koningsi* and *Maylandia mbenji* show significant divergence in skeletal structures between sexes. Specifically, females have a decreased dorsal-ventral head depth ($p<2e-16$), smaller pre-orbital distance ($p=4.01e-10$), and shorter mandible length ($p=1.75e-15$) compared to males, despite corrections for overall body size. We also assessed species-specific patterns of sexual dimorphism, which revealed distinct patterns of sexual dimorphisms among species. We hypothesize that differences in estrogen levels can result in both differences between sexes and potentially between species. While estrogen has known major roles in bone development during puberty and bone remodeling, this hormone could also influence bone patterning earlier. To investigate this, we altered estrogen levels in *Danio rerio* from 8 hours to 5 days post fertilization and are currently analyzing the effect on the craniofacial skeleton. Overall, this work will clarify the effect of sex during early bone patterning, the specific role of estrogen in this process, and how differences in hormone levels may influence species-specific differences in facial shape.

Abstract #79

Trichostatin A Effects on Craniofacial Development in Danio rerio using Geometric Morphometrics

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There are several aspects that impact head shape during development including, environmental, genetic, and epigenetic factors. Epigenetic modifications alter DNA packaging and influence gene expression without changing nucleotide base pairs by impacting chromatin (histone acetylation/deacetylation) and DNA (methylation/demethylation) landscapes. Previous research has shown distinct changes in craniofacial morphology in *Danio rerio* (zebrafish) when treated with chromatin disrupting compounds. However, critical intervals of craniofacial development were not explicitly examined and changes in face shape were not quantified. Therefore, we examined the effects of Trichostatin A (TSA), a global histone deacetylase 1 inhibitor, on early head shape development. We treated zebrafish during neural crest cell migration from 12-24hr post fertilization with 0.05- 0.4 uM TSA. At 5 days post fertilization, fish were euthanized, cleared and stained, and photographed. To quantify craniofacial variation, shape analysis was performed on each specimen using geometric morphometrics. TSA concentrations over 0.2 uM significantly altered facial shape. Specifically, increases in histone acetylation due to TSA treatment resulted in a wider Meckel's cartilage, wider branchial structures, and overall shorter head ($p=6e-7$ to $p=0.0048$ for 0.2-0.5 uM versus vehicle controls using ANOVA and Tukey's HSD in R). This work in zebrafish reinforces that epigenetic factors play a significant role in craniofacial development. Future work will assess the effects of altering chromatin structure during cartilage and bone development, the role of DNA methylation during the same time intervals, and how these factors influence species-specific facial structures. Investigating the epigenetic effects on craniofacial development in *Danio rerio* may elucidate unknown epigenetic mechanisms that implicate head shape diversification across vertebrates.

Abstract #80

Isl1 Targets Conserved Limb Enhancers During Development of The Amniote Phallus

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The development of the genital tubercle (GT) – the embryonic precursor to the penis and clitoris – involves expression of many genes that play a role in limb development. Similarities in early genital budding across amniotes suggests homologous mechanisms from their last common ancestor over 300 million years ago. ChIP-seq experiments show that many limb enhancers are active during phallus development, raising the possibility that limb regulatory elements were coopted during the evolution of the phallus. The *Isl1* gene encodes a transcription factor required for initiation of hindlimb outgrowth in mice. Conditional knockouts also demonstrate a crucial requirement of *Isl1* for GT outgrowth. Using a combination of RNA-seq and ChIP-seq, we reveal putative direct targets of ISL1 during GT development. Limb genes are overrepresented among these targets, and many appear to be activated via known limb enhancers. ChIP-seq in chick, lizard, and turtle genitalia also reveals conserved binding at some of these enhancers, possibly highlighting targets of functional importance maintained from their common ancestor. A notable example of a conserved target is *Tbx4* and its enhancer HLEB, which is also an ISL1 target during the initiation of hindlimb budding.

Abstract #81

Expression of *Kolobok* DNA transposons in *Nematostella vectensis* development

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Previously we have reported upregulation of transposable elements (TEs) during oogenesis and early development of phylogenetically distant species across many *Metazoa*. Here, we report detailed analysis of TE expression in starlet sea anemone (*Nematostella vectensis*), an established model organism in evo-devo studies. Repetitive sequences are abundant and comprise approximately 30% of *N. vectensis* genome. Among likely active TEs, most prominent are DNA transposons of *Kolobok* family. Kolobok transcripts are highly abundant throughout development, with steady increase from the fertilized egg to young polyp stage. Spatial distribution of Kolobok mRNA, revealed by *in situ* hybridization, suggests its expression is confined to undifferentiated cells. DNA TEs of *Harbinger* and *piggyBac* families, two Polinton DNA transposons, and several retrotransposons of *Penelope* family, are also expressed in early *N. vectensis* development, with notable peaks at the gastrula and early planula stages. Our findings underscore the notion that eukaryotic species' genome variability depends upon unrestricted expression of recently "co-opted" TEs in early development and undifferentiated cells.

Abstract #82

Developmental Basis of Pesticide Sensitivity: Searching for PON Genes in Manatees

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Manatees are known to have a mutation in their Paraoxonase 1 (PON1) gene. The purpose of this research is to discover if manatees have normal copies of two genes that may help them survive in environments polluted with pesticides. PON1 helps organisms to repair oxidative damage and break down organophosphate pesticides into non-toxic chemicals. Humans use a lot of organophosphate

pesticides, mostly for farming and mosquito control, and the majority of these run off after rain into lakes, springs, and the ocean. Because manatees lost the PON1 gene, they cannot defend themselves against the organophosphate pesticides. There are two more genes in the PON family in mammals, PON2 and PON3. These two other genes are similar to PON1 and may also help to defend against damages by organophosphates. It is unknown if manatees and other marine mammals or other aquatic animals, such as sea birds and sea turtles, contain PON2 and PON3. Our hypothesis is that manatees have a normal functional PON2 and PON3 proteins and genes because they contain a mutation in their PON1 gene, the PON2 and PON3 might not have been affected. In addition, our second hypothesis is that aquatic birds, but not terrestrial birds, lack the PON1 gene because water fowl have been reported dying in Floridian lakes associated with the presence of organophosphates. Used bioinformatics, we conducted homology searches and phylogenetic analysis in 11 animals: 3 mammals, 4 birds, 3 turtles, 1 cephalochordate (Florida lancelet). There are two mutations in PON1 of the manatee and orca but have PON2 and PON3. Aquatic birds and turtles have only one PON gene, which is most similar to PON2 of mammals and terrapins have no PON gene. In contrast, the phylogenetically oldest organism, Florida lancelet, contained 8 PON genes. This may explain why birds quickly die from organophosphate pesticides, as they do not have a gene similar to PON1.

Abstract #83

Discovery of putative gene duplications of ANAPC1 in the molecular evolution of APC/CCDC20 pathway and potential impacts on health

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Trisomies caused by chromosomal nondisjunction during meiotic divisions in eggs, or during mitotic cell divisions of a developing embryo, are among the most prevalent birth defects in humans. While age, genetic and environmental factors, have great predictive value about the risks of such abnormalities, unfortunately, the underlying causes of these abnormal cell divisions are not well understood. The process of chromosome separation during mitotic and meiotic divisions is triggered by activation of anaphase-promoting complex, also known as cyclosome (APC/C). In mammals, APC/C is a large protein complex containing 12 known structural and enzymatic subunits. The largest component of the APC/C complex, encoded by *ANAPC1* gene, unexpectedly has multiple segmental duplications restricted to the human genome. At least two of these human-specific gene duplications are expressed producing severely truncated protein products compared to wild-type APC1 sequence. To understand the molecular evolution of and origin of the ANAPC1 gene duplications in the APC/C^{CDC20} pathway, our team conducted deep phylogenetic of each gene encoding APC/C subunits, and its regulators CDC20 and CDH1, across 56 species representing major eukaryotic phyla. Based on transcriptomics data in human tissues, at least two of these gene duplications are expressed. The rates of molecular evolution of APC/C^{CDC20} pathway in cell division calculated using bioinformatic and mathematical models for each gene encoding APC/C subunits, and its regulators CDC20 and CDH1, across 56 species representing major eukaryotic phyla. The next steps are to use RNAi and overexpression constructs and RNAi for ANAPC1 gene duplications and homologs in *C. elegans* and subsequently detecting disruptions in cell divisions by assessing fertility, embryo viability and growth using our established integrated health protocol.

Stem and Progenitor Cell Biology

Abstract #84

Expansion and maintenance of gonad progenitors in 3D culture

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Gonadal sex determination in mice is regulated by a network of antagonistic interactions between the male and female pathway. The complex nature of these interactions is challenging to study in vivo, and traditional cell culture methods fail to capture many aspects of organ development. In response to the limitations of these systems, we sought to develop an experimental model of the fetal gonad. Here we show that embryonic gonad progenitors can be isolated and expanded in 3D culture. The resulting structures self-organize into a sphere and mirror endogenous expression patterns for gonadal markers. Proliferation and subsequent maintenance of expression is only observed from a starting colony of progenitors, highlighting a requirement for cell-cell interaction in this system. A unique aspect of this model is that it can potentially be used to study two distinct fates, the testis, or the ovary, dependent on supplementation with growth factors. Overall, the generation of these bipotential structures enables the study of gonadal sex determination within a physiologically relevant and experimentally accessible model.

Abstract #85

Orphan nuclear hormone receptor ftz-f1 is necessary to promote essential developmental checkpoints during oogenesis

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Gamete production is dependent on the balance between cell fate decisions and maintaining tissue viability. Nuclear receptors (NRs) link physiological status to a cellular transcriptional response and are important mediators of reproduction, physiology, and tissue homeostasis. The distinct roles of NRs in gamete production remain unclear due to extensive cross-talk between receptors and duplication of NR coding genes in many eukaryotic genomes. Mammalian NR5A family members *SF-1* and L*RH-1* are essential for sex determination, gonad development, and sex steroid production. We hypothesize that NR5A ortholog *fushi tarazu factor 1* (*ftz-f1*) fills a conserved role in Drosophila oogenesis. *Ftz-f1* is expressed throughout the ovary. Germline-specific knockdown of *ftz-f1* resulted in fewer germline stem cells as female flies aged. Germ cell mitotic divisions were also impaired in the absence of *ftz-f1*. Moreover, reduced *ftz-f1* in ovarian escort cells blocked follicle formation and survival. Taken together, these observations highlight multiple essential roles for *ftz-f1* at essential checkpoints during oocyte development. Our future experiments seek to elucidate whether and how *ftz-f1* interacts with other NR family members, including the Ecdysone Receptor and/or impairs steroid hormone production in late oogenesis. Our data add to a growing body of literature underscoring the importance of nuclear receptors in the control of reproduction.

Abstract #86

Single-cell sequencing for all: making the most of the cost

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For all its appeal, single-cell RNA-seq (scRNA-seq) remains inaccessible to most researchers. The main reason is sequencing depth: many argue that sampling thousands of single-cell barcodes across independent replicates inherently requires billions of sequenced reads, which few investigators can afford. Instead, we ask: does deeper sequencing improve data sparsity? Here, we show the answer is no. First, we sequenced scRNA-seq libraries in various sequencing platforms (Illumina, IonTorrent), from three separate biological models (human PBMCs, mouse embryonic testes, mouse embryonic kidney stroma; N≥3 independent specimens each, >10K cells/specimen) and with three different sc-RNAseq modalities (10X Genomics, DropSeq, sci-RNA-seq). Then, we implemented the SALSA workflow (doi: 10.1101/551762) to compare gene detection rates, barcode re-incidence, and clustering reproducibility for each specimen at different sequencing run outputs. We found that barcodes added only in high-depth sequencing runs (>3x more barcodes than low-depth in all scRNA-seq modalities) represented RNA/gDNA debris and had to be discarded from expression analyses; in contrast, barcodes scoring as single cells at low-depth were independently rescored as such in all sequencing outputs. Newly captured UMIs at high-depths aligned predominantly to either constitutive genes in all barcodes or rare transcripts in discarded ones. Finally, compared to low-depth output, single-cell clusters inferred from high-depth runs lost statistical support. In sum, statistical insight from scRNA-seq libraries tracks with their underlying UMI diversity, and sequencing beyond those limits only increases the likelihood of “false” single-cell barcodes and transcripts becoming readout. Our findings posit a new paradigm to extracting reproducible biological insight from scRNA-seq experiments, in which minimal (and inexpensive) sequencing depths, with as many cells supplied per assay as possible, are always best.

Notes

Notes

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