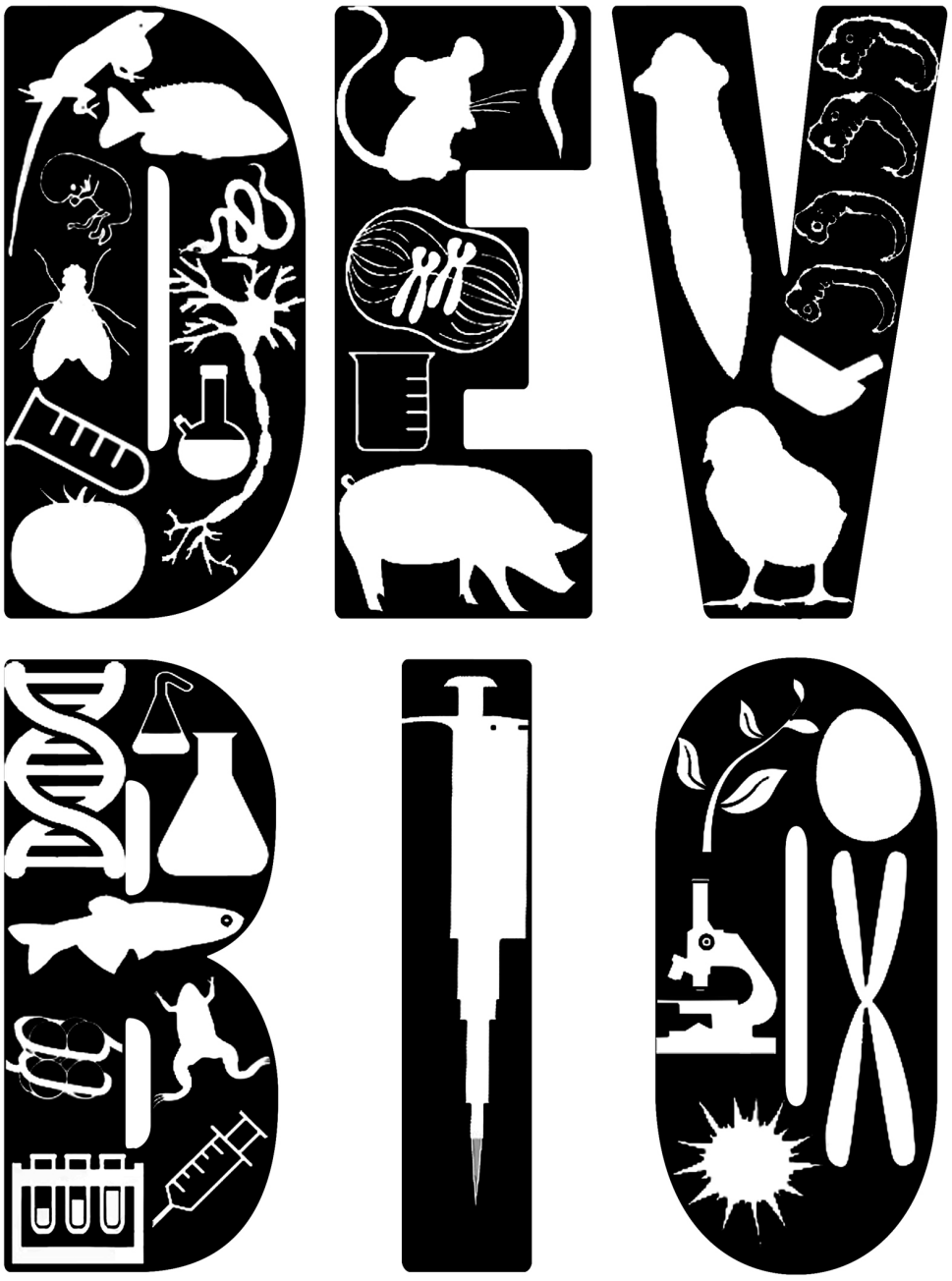
Southeast Regional Society for Developmental Biology 2018 Annual Meeting



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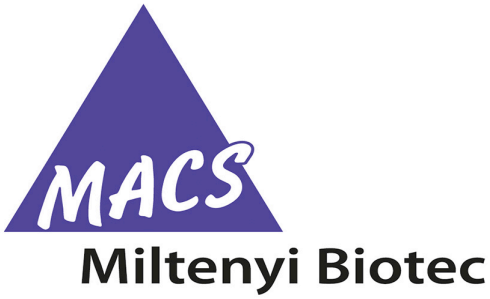

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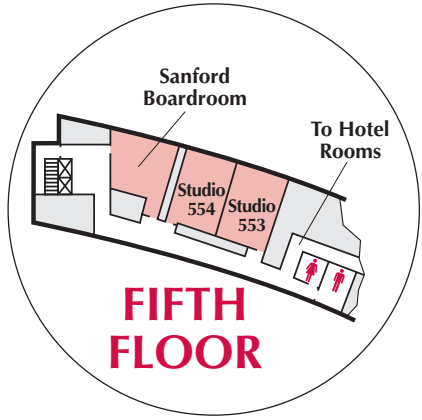

Elevators to Conference Rooms, Hotel Rooms, Sanford Boardroom, and Studios 553 & 554

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PDR

Magnolia Ballroom

Conference Registration

Georgia Java

Pecan Tree Galleria

Concierge

Oak Room

Masters Hall

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Lumpkin Plaza

Hotel Front Desk

Lumpkin Street Entrance

Hill Atrium

Guest Services

Mahler Auditorium

Carlton Street Main Entrance

EDUCATION SESSION & WORKSHOP

"Communicating Science to the Public"

Wednesday, May 23rd from 3:30 – 5:00 PM

Location: Masters Hall

We will hold an education workshop: "Communicating Science to the Public". The workshop will cover ways that individual scientists at all career levels can effectively engage with the public to communicate their science, and why this is important. The planned structure is a panel discussion format to encourage direct participation and interaction with the panel. The panel will include academic scientists at different career stages who have experience engaging with the public as well as individuals who work with scientists to communicate their research to the public.

Moderator:

Barbara Del Castello, Graduate Student, Genetics, and former White House Science Policy Intern
Email: bjdelcas@uga.edu

Panelists:

James Hataway, Public Relations Specialist, Office of Research, University of Georgia
Email: jhataway@uga.edu

Suzanne Pilaar Birch, Assistant Professor, Anthropology, and Co-Founder of TrowelBlazers
Email: sepbirch@uga.edu

Amanda Shaver, Graduate Student, Genetics, and Editor-in-Chief of Athens Science Observer
Email: amanda.shaver@uga.edu

Sara Beresford, Communications & Outreach Coordinator of ECOGIG
Email: sarab@uga.edu

Stephanie Halmo, Graduate Student, Biochemistry & Molecular Biology, and Director of Athens Science Cafe
Email: shalmo27@uga.edu

PROGRAM OF EVENTS

Day 1: Wednesday, May 23, 2018

2:00 – 5:00 PM **REGISTRATION & POSTER SET-UP**
Registration: Pecan Tree Gallery
Posters: Pecan Tree Gallery/Hill Atrium

Note – All posters will remain up from Wednesday afternoon through Thursday night. Odd-numbered posters will be presented on Day 1 and even-numbered posters on Day 2.

3:30 – 5:00 PM **EDUCATION SESSION & WORKSHOP**
Location: Masters Hall

Panel Discussion: Communicating Science to the Public

Moderator: ***Barbara Del Castello****, Graduate Student, Genetics, and former White House Science Policy Intern*

Panelists:
James Hataway, Public Relations Specialist, Office of Research, University of Georgia
Suzanne Pilaar Birch, Assistant Professor, Anthropology and Co-Founder of TrowelBlazers
Amanda Shaver, Graduate Student, Genetics, and Editor-in-Chief of Athens Science Observer
Sara Beresford, Communications & Outreach Coordinator of ECOGIG
Stephanie Halmo, Graduate Student, Biochemistry & Molecular Biology, and Director of Athens Science Cafe

5:30 – 8:05 PM **DINNER & KEYNOTE ADDRESS**
Location: Magnolia Ballroom (dinner) & Masters Hall (keynote)

7:00 – 7:05 PM **WELCOME ADDRESS** – in Masters Hall
Doug Menke
Associate Professor and Director of the Developmental Biology Alliance, Department of Genetics, University of Georgia

7:05 – 8:05 PM **KEYNOTE ADDRESS I** – in Masters Hall
Scott Poethig – ID # 4444
John H. and Margaret B. Fassitt Professor, Department of Biology, University of Pennsylvania

From weeds to trees: the molecular mechanism and evolution of vegetative phase change in plants

8:05 – 9:30 PM **POSTER SESSION I & RECEPTION**
Odd-numbered posters will be presented
Location: Pecan Tree Galleria/Hill Atrium

Day 2: Thursday, May 24, 2018

7:30 –9:00 AM **REGISTRATION & BREAKFAST**
Registration Desk & Magnolia Ballroom

9:00 – 10:30 AM **SESSION 1: Transcriptional Regulation and Epigenetics**
Location: Masters Hall

9:00 – 9:30 AM **Mary Goll, University of Georgia (Session Chair) – ID # 4435**
Developmental regulation of heterochromatin establishment at the maternal to zygotic transition

9:30 – 9:50 AM Brandon Carpenter, Emory University – ID # 4033
SPR-5; MET-2 maternal reprogramming antagonizes H3K36me3 in the transgenerational control of germline versus soma

9:50 – 10:10 AM Saeid Parast, University of Alabama at Birmingham – ID # 4479
The regulatory role of Dpy30/Ash2L in epigenetic control of neural development

10:10 – 10:30 AM Marina Martinez-Bartolome, Mississippi State University – ID # 4483
A non-canonical role for Wnt16 in the Wnt signaling network involved in specifying and patterning the early anterior-posterior axis of sea urchin embryos

10:30 – 10:45 AM **BREAK – AM Refreshment Break**
Location: Pecan Tree Galleria/Hill Atrium

10:45 – 12:15 PM **SESSION 2: Organogenesis and Morphogenesis**
Location: Masters Hall

10:45 – 11:15 AM **Scott Nowak, Kennesaw State University (Session Chair) – ID # 4427**
Akirin is critical for early tinman induction and subsequent formation of the heart in Drosophila melanogaster

11:15 – 11:35 AM Sarah Suciu, Emory University – ID# 4430
The ciliary protein arl13b regulates axon guidance in the developing mouse hindbrain

11:35 – 11:55 AM Alexandra Carroll, University of Florida – ID # 4484
Hedgehog signaling initiates genital tubercle development

11:55 – 12:15 PM Camerron Crowder, University of Alabama at Birmingham – ID # 4482
Nuclear androgen receptor regulates testes organization and oocyte maturation in zebrafish

12:15 - 1:30 PM **LUNCH**
Location: Magnolia Ballroom

1:30 – 3:00 PM **SESSION 3: Neural Development**
Location: Masters Hall

1:30 – 2:00 PM **Daichi Kamiyama, University of Georgia (Session Chair) – ID # 4449**
Imaging contact-dependent signaling in neural circuit assembly

2:00 – 2:20 PM Edwin Corgiat, Emory University – ID # 4471
Defining links between an intellectual disability-associated RNA-binding protein and planar cell polarity in dendritic arborization

2:20 – 2:40 PM Mary Morton, Clemson University – ID # 4039
Extracellular Vesicles Released from Subventricular Zone Neural Stem Cells Alter Microglia Morphology in the Perinatal Brain.

2:40 – 3:00 PM Heather Ray, University of Alabama at Birmingham – ID # 4415
The Tumor Suppressor Hypermethylated in Cancer 1 (Hic1) Interacts with Wnt Signaling Pathways During Neural Crest Migration

3:00 – 4:30 PM **AFTERNOON BREAK & POSTER SESSION II**
Even-numbered posters will be presented
Location: Pecan Tree Galleria/Hill Atrium

4:30 – 5:30 PM **KEYNOTE ADDRESS II**
Location: Masters Hall

Greg Wray – ID # 4424
Professor of Biology and Director of the Duke Center for Genomic and Computational Biology, Duke University

Tinkering with networks: evolution of developmental mechanisms and life history in sea urchins

5:30 – 8:00 PM **BANQUET DINNER & ENTERTAINMENT**
Featuring: Indiana Heights
from 5:45 – 7:45 PM
Location: Magnolia Ballroom

7:30 – 8:00 PM **Business Meeting (Concurrent with Entertainment)**
Planning for SESDB 2019
Location: Pecan Tree Courtyard

Entertainment from 5:45 – 7:45 PM

in the Magnolia Ballroom:

Indiana Heights

Indiana Heights is a rock-fusion band out of Boston, Massachusetts. Their style is reminiscent of the 70's and 80's, and they are influenced by artists such as Billy Joel, Chicago, and Steely Dan.

The band formed in 2016 at Berklee College of Music, and since then, they have performed at venues such as the Red Room at Berklee and the Hard Rock Café in Boston. The members are from places all around the country including Georgia, Michigan, New Jersey, Tennessee, and Indiana.

After running an Indiegogo campaign to produce their debut album, Indiana Heights was able to surpass the goal they had set and has released their single "Smooth Operator" on all platforms. They are currently in the process of recording that album with Plaid Dog Recording in Boston.

Day 3: Friday, May 25, 2018

7:00 – 8:30 AM **BREAKFAST**
Location: Magnolia Ballroom

8:30 – 10:30 AM **SESSION 4: Growth and Differentiation**
Location: Masters Hall

8:30 – 9:00 AM **Dongfang Wang, Spelman College (Session Chair) – ID # 4419**
The effect of micropylar endosperm on early embryo development

9:00 – 9:20 AM Manashree Malpe, University of Georgia – ID # 4461
G-Protein signaling accelerates Drosophila germline stem cell divisions upon repeated mating

9:20 – 9:40 AM Phil Byun, Emory University – ID # 4457
Genetic analysis of invasive pathways engaged by the EcR-coactivator protein Taiman

9:40 – 10:00 AM Sade William, University of Alabama at Birmingham – ID # 4422
TGFß mediates fibrous tissue differentiation from mesenchymal stem cells during axial skeleton development

10:00 – 10:15 AM **BREAK – AM Refreshment Break**
Location: Pecan Tree Galleria/Hill Atrium

10:15 – 11:45 PM **SESSION 5: Evolution and Development**
Location: Masters Hall

10:15 – 10:45 AM **Kara Powder, Clemson University (Session Chair) – ID # 4413**
Cis-regulatory enhancers in cichlid craniofacial evolution

10:45 – 11:05 AM Sergio Minchey, University of Georgia – ID # 4474
Conserved targets of ISL1 in genital and hindlimb development

11:05 – 11:25 AM Melissa Bentley, University of Alabama at Birmingham – ID # 4448
Genetic interactions between ciliary proteins Nphp4 and Bbs5 and their role in development

11:25 – 11:45 PM Elijah Lowe, Georgia Institute of Technology – ID # 4458
A massively parallel screen for morphogenesis genes by RNAseq of interspecific hybrid embryos

11:45 – 11:55 AM Award Committee Meeting

11:55 – 12:00 PM **Travel and Regional Awards Announced**
Location: Masters Hall

12:00 PM **DEPARTURE**

KEYNOTE ADDRESS I

Wednesday, May 23rd from 7:05 – 8:05 PM

Location: Masters Hall

Dr. Scott Poethig

John H. and Margaret B. Fassitt Professor,
Department of Biology, University of Pennsylvania

"*Temporal regulation of shoot development in plants*"

Plant shoots progress through several distinct phases during their development. The first transition occurs shortly after germination and is known as "vegetative phase change". This transition regulated by a decline in the abundance of the miRNAs, miR156 and miR157, and the resulting increase in the expression of their targets, SPL transcription factors. In *Arabidopsis*, the decline in miR156 is mediated by the epigenetic silencing of two of the 8 genes that encode this miRNA, *MIR156A* and *MIR156C*. miR156 initially decreases very rapidly, and then decreases slowly and more uniformly throughout shoot development. This expression pattern explains the changes in leaf morphology that occur as the shoot develops, and also accounts for variation in the developmental stability of leaves produced at different times in shoot development; juvenile leaves produced early in shoot development are less plastic than juvenile leaves produce later in shoot development because they contain significantly more miR156/miR157. To determine if changes in the timing of miR156/miR157 expression have played a role in plant evolution, we examined the expression patterns of these miRNAs in species in the genus, *Acacia*. Most of the >1,000 *Acacia* species native to Australia produce compound leaves early in shoot development and then switch to producing a simple leaf known as a phyllode. This transition is associated with a dramatic decline in the level of miR156/miR157. Species that never undergo this transition, or display delayed phase change, evolved independently at least 7 times. In the largest of these neotenous clades—which includes approximately 40 species—this phenotype is associated with the prolonged high expression of miR156. This expression pattern is the result of a regulatory mutation in a single *MIR156* gene.

KEYNOTE ADDRESS II

Thursday, May 24th from 4:30 – 5:30 PM

Location: Masters Hall

Dr. Gregory A. Wray

Professor of Biology and Director of the Duke Center for Genomic and Computational Biology, Duke University

"Tinkering with networks: evolution of developmental mechanisms and life history in sea urchins"

The developmental gene regulatory network (GRN) of the sea urchin is well-studied, primarily by knock-downs of transcription factors and so the urchin *Lytechinus variegatus* is well suited to study the functional consequences of direct manipulation of gene expression in cis.

Previous work has identified the knockdown phenotype and both cis- and trans- acting regulators of the endodermal marker *Endo16*. Module "A" of the *Lv-Endo16* promotor is an evolutionary conserved module required for its expression.

We used the CRISPR-Cas9 system to modify the *Lv-Endo16* promoter module A, resulting in a morphologically similar but more severe phenotype than the previously published *Endo16* translation-blocking morpholino. This manipulation will serve as a positive control for silencing the enhancer. We will next validate the molecular phenotype with qPCR and in situ hybridization.

Using these validated guides, we will next attempt to silence *Lv-Endo16* expression with CRISPR-interference by using a deactivated Cas9 fused with a repressor protein called KRAB which condenses the chromatin, making the locus inaccessible to transcription factors. To our knowledge, this technique has not yet been used in invertebrates. When fully developed, CRISPR-i can be used to regulate genes temporally and to explore cis-regulatory architecture and evolution.

ID #3913

Modeling Peripheral Nervous System Disease with Human Pluripotent Stem Cells

Nadja Zeltner[1], Faranak Fattahi[4], Shuibing Chen[3], Lorenz Studer[2]

[1]University of Georgia, USA; [2]Memorial Sloan Kettering Cancer Center, USA; [3]Cornell University, USA; [4]University of California San Fransisco, USA

Functional and molecular aspects of human genetic disease can be recapitulated *in vitro* using patient-specific, human induced pluripotent stem cells (iPSCs). Familial Dysautonomia (FD) is a debilitating developmental and degenerative disorder with onset at birth that primarily affects derivatives of the neural crest (NC), particularly the peripheral nervous system (PNS). Symptoms are defective sensation of pain, spine deformations and the inability to regulate the the sympathetic nervous system. For unknown reasons, FD patients present with mild or severe disease symptoms despite carrying the identical, homozygous point mutation in *IKBKAP*. We used iPSCs derived from FD patients and differentiated into various PNS cell types to present phenotypes at various stages of development that capture severe and mild FD symptoms accurately. Via genetic rescue of the FD mutation and whole-exome sequencing (WES), we identified candidate mutations that were only found in severe but not mild FD patients, providing evidence that FD may constitute two genetic sub-diseases. Our study demonstrates that human iPSC-based disease modeling is sensitive in recapitulating disease severity. This paves the road for applications in personalized medicine and raises the prospect that individual patient's disease could be studied *in vitro*. Screening a library of small molecules, we further identified a novel compound that rescued severe FD defects. This compound is the active chemical in a Traditional Chinese Medicine, making it an interesting possible treatment option for preventing neurodegeneration in FD and possibly more common peripheral neuropathies.

ID #4033 – Session 1: Transcriptional Regulation and Epigenetics

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

Brandon Carpenter, David Katz

Emory University, USA

Epigenetic information is acquired within each generation in order to regulate proper gene expression. In *C. elegans,* the H3K4me2 demethylase, SPR-5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte and cooperate to reestablish the epigenetic ground state of the zygote by reprogramming histone methylation. Progeny of worms lacking *spr-5* and *met-2* exhibit a maternal effect sterile phenotype due to the rapid accumulation of H3K4me2 and the resulting ectopic expression of spermatogenesis genes in the soma. Here, we show that the progeny *spr-5; met-2* of mutants display additional developmental defects including defects in gut granule accumulation, and a severe L2 developmental delay in both the germline and soma. Strikingly, these somatic defects are rescued by *mes-4* RNAi. Previously, the Strome and Kelly Labs demonstrated that maternally deposited MES-4 transgenerationally maintains H3K36 at germline genes in a transcription-independent manner, and this is required to reactivate germline genes in the subsequent generation. Thus, we hypothesized that the removal of H3K4me2 by SPR-5 followed by the addition of H3K9me2 by MET-2 at fertilization may be required to prevent MES-4 from ectopically licensing germline gene expression in somatic tissues. To test this model, we performed an RNAseq on L1 progeny from *spr-5; met-2* mutants, and found that transcription-independent MES-4 targets are ectopically expressed in the soma. Thus, we propose that SPR-5; MET-2 maternal reprogramming antagonizes H3K36me3 to enable the proper transgenerational control of germline versus somatic cell fates. We are currently testing this by performing H3K36me3 ChIP-seq, and will present data from this analysis at the meeting.

ID #4039 – Session 3: Neural Development
Extracellular Vesicles Released from Subventricular Zone Neural Stem Cells Alter Microglia Morphology in the Perinatal Brain.
Mary Morton, Victoria Neckles, Caitlin Seluzicki, Jennie Holmberg, David Feliciano
Clemson University, United States
Subventricular Zone (SVZ) Neural Stem Cells (NCSs) persist in the perinatal neurogenic niche, and give rise to neurons early and late into adulthood. Microglia are the immune cells of the central nervous system that help form the intricate neural circuitry of the mammalian brain. Extracellular vesicles (EVs) are cellular-derived, nano-sized vesicles that encapsulate miRNA and proteins. It is thought that EVs transfer molecular information from donor to recipient cells which may play a role in normal development or could potentially contribute to the pathogenesis of neurodegenerative diseases. In this study, we tested the communicative potential of SVZ NSC EVs and microglia in the perinatal brain. Using a fluorescent fusion EV protein, CD9-GFP, to study EV release, it was found that SVZ NSCs generated EVs. The fusion protein was expressed in Nestin positive NSCs in the ventro-lateral SVZ, and could be detected outside of labeled cells. Scavenging microglia selectively took up tagged NSC EVs. Small RNA sequencing identified miRNAs within NSC EVs that regulate microglia physiology and morphology. In support of this finding, NSC EVs induced a transition to a CD11b/IBA1 non-stellate, rounded morphology. This morphological shift was accompanied by an altered microglial transcriptional state and cytokine profile which contributed to a negative feedback loop that controlled NSC proliferation. These data suggest that SVZ NSCs generate EVs that are targeted to and modify microglia within the perinatal brain. These findings implicate a NSC-EV-microglia axis that provides insight to normal and pathophysiological brain development.

ID #4056
Longevity and its transgenerational inheritance is enabled by repressive chromatin
Teresa Lee, Amanda K. Engstrom, Heidi David, David J. Katz
Emory University, USA
Longevity is a complex trait influenced by a mix of environmental, genetic, and epigenetic factors. WDR-5, a member of the COMPASS complex, methylates histone 3 at lysine 4 (H3K4), a modification associated with active chromatin. Previously, the Brunet lab has shown that *wdr-5* mutants are long-lived, and this longevity is inherited by wild-type descendants. We demonstrate that longevity in thisbackgroundis a transgenerational phenotype that takes at least 18 generations to manifest after the loss of WDR-5. Consistent with the gradual appearance of longevity in *wdr-5* mutant populations, we see that lifespan correlates with levels of dimethylation of histone 3 at lysine 9 (H3K9me2), a mark associated with repressive chromatin. Using ChIP-seq to examine global levels of H3K9me2 in *wdr-5* mutants, we find that late-generation, long-lived *wdr-5* mutants have higher levels of genomic H3K9me2 both globally and at locus-specific regions. To examine whether H3K9me2 may confer longevity in *wdr-5* mutants through its inheritance and generational accumulation, we mutated *met-2*, the methyltransferase required for all germline H3K9me2, in *wdr-5* mutants. The extended lifespan of *wdr-5* mutants is dependent on *met-2*, indicating that H3K9me2 promotes longevity in late-generation *wdr-5* mutants. Additionally, when we mutated *met-2* in otherwise wild-type descendants of *wdr-5* mutants, we find that its loss can also abolish the inheritance of longevity. Taken together, these data support a model in which germline H3K9me2 is transgenerationally inherited, thereby facilitating longevity and its epigenetic inheritance.

ID #4090

The effect of age on epigenetic reprogramming in C.elegans

Onur Birol
Emory University, USA

Cellular fate is specified by differential gene expression, which is regulated by chromatin modifications. Gametes are highly differentiated cells, harboring distinctive histone modifications. 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 is 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. Remarkably, I found that progeny of *spr-5* mutant worms have a 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.

For future directions 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, and the fact that mice and human lacking the SPR-5 homolog manifest autism-like symptoms. We hope to provide a foundation for potential translational applications in the future.

ID #4115

The transcription factor ztf-29 is required for C. elegans nervous system development

Ciara Hosea, Wendy Aquino Nunez, Dr. Martin L. Hudson
Kennesaw State University, USA

In humans, Kallmann syndrome (KS) is classified by lack of sense of smell in addition to delayed or absent puberty. KS is a polygenic disorder, with twenty-one genes being associated with the disease to date. These KS-related genes display X-linked recessive, autosomal recessive and autosomal modes of inheritance, making investigation of the causes of KS difficult. In addition, half of KS cases have no known specific genetic cause, leading to the conclusion that there are multiple factors at play and not just one gene, protein, or transcription factor. We are using the nematode C. elegans to investigate the underlying genetic causes of KS. In particular, C. elegans has an ortholog gene of the human gene kal-1, which is mutated in X-linked KS. We hypothesize that transcription factors that regulate kal-1 expression may be KS genes in their own right. To investigate kal-1 gene regulation, we are using the kal-1 promoter to drive GFP expression. We then cross in candidate transcription factor mutations to create a double mutant, then assess the effect of the mutation on kal-1-GFP expression. Our data indicates that mutations in the transcription factor ztf-29 has a striking effect on kal-1-GFP expression, displaying a wider nerve ring than normal. In addition, we consistently see additional kal-1-GFP positive cells in ztf-29 mutants, suggesting that ztf-29 normally represses the fate of one or more kal-1-positive cells in the head. The aim of this project is to determine how ztf-29 plays a role in the cell adhesion or axon guidance of kal-1 expressing neurons, as well as understand how ztf-29 controls cell fate.

ID #4121

srw-85 expression is controlled by the basic-helix-loop-helix transcription factor cnd-1/NeuroD1

Derrica McCalla, Wendy Aquino-Nunez
Kennesaw State University, USA

To drive cell fate specification, differentiation and the overall development of the nervous system, it is essential to have the right pro-neural transcription factors and cell signaling pathways to mediate these processes. What is of particular interest are the downstream targets of these pro-neural factors and the lack of research being done in understanding their relevance in the nervous system development. Unpublished data from our lab has identified a small number of genes controlled by the pro-neural transcription factor *cnd-1*. This protein is a member of the basic-helix-loop-helix family of transcription factors and is orthologous to human NeuroD1. *cnd-1* mutants were previously shown to have defects in axon morphology as well as in cell fate specification, so by studying this transcription factor and its possible targets, we can assess its role in neuronal cell fate specification. The question then is how can we find genes that are controlled by *cnd-1* and the role they play in the development of the nervous system? We performed RNAseq assays and bioinformatics, comparing RNA transcripts from WT and *cnd-1* mutant embryos. We identified a set of *cnd-1* downstream targets that show significant p-values when comparing transcripts isolated from WT and *cnd-1* mutants. We are validating these targets using GFP reporter genes and genetic approaches. Preliminary data indicates that the G protein-coupled receptor gene *srw-85* is strongly regulated by *cnd-1*. In addition, we find that *srw-85* is exclusively expressed in a single pair of sensory neurons. Our data adds to previously published work, indicating that *cnd-1* controls a panel of genes required not just for fate specification and axon outgrowth, but also for neural function.

ID #4413 – Session 5: Evolution and Development (Session Chair)

Cis-regulatory enhancers in cichlid craniofacial evolution

Kara Powder
Clemson University, USA

Alteration of gene expression can generate both clinical and natural phenotypic variation. Most pathogeneic mutations in human disease affect non-coding DNA and evolution of *cis*-regulatory enhancers, which regulate the spatiotemporal pattern of gene expression, has been proposed as a primary mechanism for morphological evolution. However, identifying relevant enhancers and characterizing their *in vivo* function remains a challenge. Work in model organisms has experimentally identified >33000 enhancers active during facial, cartilage, and/or bone development. To identify which of these enhancers may mediate the unparalleled craniofacial variation that is a hallmark of the adaptive radiation of cichlid fishes, we utilized a bioinformatic approach using multi-species genetic data sets. Specifically, we mapped enhancers experimentally identified in mammals to the tilapia genome, and cross reference these to genomic regions that (1) exhibit high levels of genotypic divergence (i.e. high FST) among phenotypically divergent cichlid species and (2) are associated to craniofacial phenotypes via quantitative trait loci (QTL) mapping. This approach efficiently prioritizes enhancers in an unbiased way from ten of thousands to dozens for functional assays. Using this method, we prioritized a putative *sox9b* enhancer that lies within QTLs for lower jaw width and length and has fixed mutations in species that vary in jaw phenotypes. Using CRISPR in zebrafish, we show that genetic variation in the *sox9b* enhancer results in the specific loss of ceratobranchial cartilages as well as phenotypic variation in the lower jaw that mimics natural variation between cichlid species. Overall, we suggest that bioinformatic integration of data from evolutionary models like cichlids and model organisms offers a powerful approach to both prioritize and functionally evaluate enhancers, advancing our understanding of how non-coding genetic variation impacts development, disease, and evolution.

ID #4415 – Session 3: Neural Development

The Tumor Suppressor Hypermethylated in Cancer 1 (Hic1) Interacts with Wnt Signaling Pathways During Neural Crest Migration

Heather Ray, Chenbei Chang
University of Alabama Birmingham, USA

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

ID #4418

Pigmentation: straddling between development and evolution of chordates

Ugo Coppola[1,2]
[1]*Stazione Zoologica Anton Dohrn, Napoli (SZN), IT;* [2]*School and Biological Sciences, Georgia Institute of Technology, Atlanta, US*

Pigmentation is one of the most fascinating phenomena in nature, with several types of pigment cells among animals carrying out various biological functions. Coupling developmental biology and comparative genomics, we have found new players in pigmentation.

We studied the evolutionary history of the Rab32/38 subfamily, a fundamental set of proteins responsible for intracellular trafficking related to melanogenesis. Using amphioxus and zebrafish, we found conservation of *Rab32/38* function in pigment cells but also functional diversification of duplicates. Since many Rabs are involved in the transport of molecules associated with pigmentation, we employed eleven species inside metazoans (from cnidarians to human) generating an updated reconstruction of whole Rab complement in metazoans, which showed great variablity in chordates.

To find new genes implicated in pigmentation, we surveyed a Kelch-like gene family member in sea squirt *Ciona robusta* (*Cr-Klhl21*). It is localized exclusively in pigment cells, with a dynamic expression profile during embryogenesis: from mid- tailbud stage onward, it becomes restricted to the otolith, a melanin-containing cell critical for gravity sensing. We identified the minimal promoter region for this gene, hinting at *Cr-Mitf* as a major regulator of a key pigmentation gene. Moreover, we found that *Cr-msxb* and *Cr-Dmrt* work as co-regulatory transcription factors for *Cr-Klhl21* expression. Thus, we have elucidated a gene regulatory network for *Cr-Klhl21* based on combinatorial regulation by two distinct *cis*-regulatory modules, one bound by the "major" regulator (*Cr-Mitf*) and one bound by the "minor" co-regulators (*Cr-msxb*, *Cr-Dmrt*). We have begun to probe *Cr-Klhl21* function, using CRISPR/Cas9to knock this gene out in the pigment cell lineage of *C. robusta.* Taken together, our data serve as a template for studying novel players in pigment cell specification and differentiation in *C. robusta*.

ID #4419 – Session 4: Growth and Differentiation (Session Chair)
The effect of micropylar endosperm on early embryo development
Dongfang Wang
Spelman College, USA
Proper seed development requires coordinated growth among the three genetically distinct components, the embryo, the endosperm, and the seed coat. This growth coordination is partly achieved through the interaction between the embryo and the surrounding endosperm. Like many angiosperms, Arabidopsis has a nuclear type endosperm. During the initial syncytial stage, the endosperm differentiates into three functional domains: micropylar, peripheral, and chalazal domains. The subsequent endosperm cellularization requires the Fertilization-Independent Seed (FIS)-Polycomb Repressive Complex 2 (PRC2). After endosperm cellularization, the endosperm ceases to grow and is eventually absorbed by the embryo. We discovered two putative invertase inhibitors (InvINH1 and InvINH2) that are involved in the acceleration of embryo growth after endosperm cellularization. InvINH1 and InvINH2 were preferentially expressed in the micropylar endosperm that surrounds the embryo. After endosperm cellularization, InvINH1 and InvINH2 were down regulated in a FIS-PRC2-dependent manner. We hypothesized that FIS-PRC2 complex either directly or indirectly represses InvINH1 and InvINH2 to increase invertase activity around the embryo, making more hexose available to support the accelerated embryo growth after endosperm cellularization. In support of our hypothesis, embryo growth was delayed in transgenic lines that ectopically expressed InvINH1 in the cellularized endosperm. We also discovered the nature of the regulatory connection between the FIS-PRC2 complex and InvINH1. Our data suggested a novel mechanism for the FIS2-PRC2 complex to control embryo growth rate via the regulation of invertase activity in the micropylar endosperm.

ID #4421
A miRNA-Mediated Pathway Regulates Age-dependent disease resistance During Plant Maturation
LANXI HU, LI YANG
Department of Plant Pathology, University of Georgia, Athens, GA, USA
Plants are constantly exposed to pathogen during growth in natural conditions. Plants express different level of resistance to pathogen depending on their maturation stage, known as Aged-Related Resistance (ARR). The onset of ARR is associated with the juvenile-to-adult vegetative transition in some plants. It remains unclear how the specificity and potency of defense is regulated during plant maturation. We show that the ARR onset againsts multiple microbial pathogens, *in Arabidopsis,* is mediated by the miR156/SPLs pathway. MicroR156s suppress members of the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)* family. Temporal decrease of miR156 accumulation is concomitant with the increase of SPL expression during the vegetative phase change. Reducing miR156 level in juvenile phase enhances resistance to both pathogenic oomycete *Hyaloperonopora arabidopsidis(Hpa)* and bacteria *Pseudomonas syringae (Psy)*, suggesting a board spectrum resistance may be mediated by miR156. Gain-of-function studies of SPL family members indicates that they have distinct and overlapping function in activating immune response. By defining the roles of miR156/SPLs pathway in defense-development crosstalk, we are hoping to provide molecular mechanisms that shed light on resistance engineering in field.

ID #4422 – Session 4: Growth and Differentiation
TGFß mediates fibrous tissue differentiation from mesenchymal stem cells during axial skeleton development
Sade Williams, Rosa Serra
University of Alabama at Birmingham, USA
Intervertebral Discs (IVDs) are an essential component of the spine that provide shock absorption and weight distribution. The structural integrity of IVDs is maintained by a fibrous tissue called the annulus fibrosus (AF), which degenerates with age or trauma causing severe, chronic back pain. The proper development and maintenance of fibrous tissue is essential for spinal function, 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 severely under-formed. We also previously showed that when sclerotomal cells (embryonic mesenchymal progenitors) are treated with TGFβ, there is an upregulation of fibrous markers, most notably the transcription factor Scleraxis (Scx). We hypothesize that TGFβ signaling regulates Scx expression to provide an instructive signal during fibrous tissue differentiation. To test this hypothesis, we sorted sclerotome cells from Scx-Mcherry transgenic mice into populations with high and low Scx expression. With TGFβ treatment, we observed that TGFβ directly regulates Scx expression before any other fibrous markers, and TGFβ promotes fibrous tissue differentiation specifically in the cell population highly expressing Scx. These data suggest Scx is an important mediator of TGFβ signaling in these cells. To further elucidate the relationship between TGFβ and Scx on fibrous tissue differentiation, we will knockdown and overexpress Scx using the chick model and in primary sclerotomal cells to determine how TGFβ signaling uses Scx to regulate fibrous marker expression. 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 in spinal disorders.

ID #4423
The Role of nou-darake in Planarian Brain Regeneration
Jennifer Jenkins
University of Georgia, United States of America
Humans cannot repair central nervous system (CNS) injuries, which leads to devastating consequences when areas of the brain are damaged. While humans do not have robust mechanisms for regenerating CNS tissue, other organisms can achieve full, functional regeneration. Planarians—flatworms with astounding, whole-body regenerative abilities—have emerged as a model system for studying regeneration of the CNS. Our laboratory focuses on these animals, with the idea that learning about the processes that allow planarians to regenerate could help develop future therapies for human neural repair. We have begun tackling this problem by focusing on the gene *nou-darake (ndk)*, which encodes an FGFR-like molecule. *ndk* plays a critical role in proper scaling of the brain during regeneration and is expressed in the anterior and brain of the worm. Nou darake (Ndk) protein is proposed to act as an FGF signal sink, but the ligand and other related downstream effectors of Ndk have yet to be characterized. In order to better understand the function of Ndk, we used RNA-Seq to identify a group of genes with mRNA abundance that is significantly altered after reduction of *nou-darake* by RNA interference (RNAi). We are determining the expression patterns of each gene through whole mount in situ hybridization. We are also using RNAi to test whether these genes function in CNS regeneration or maintenance.

ID #4424 – Keynote Address II on Thursday, May 24
Tinkering with networks: evolution of developmental mechanisms and life history in sea urchins
Gregory Wray
Duke University, USA
The well-characterized gene regulatory network (GRN) of sea urchins is a valuable framework for understanding how developmental mechanisms can evolve to alter ecologically significant traits. We study the genus *Heliocidaris*, which contains two species with dramatically different embryonic development and larval morphology, but which converge on a similar morphology during metamorphosis. Prior studies identified changes in the timing of axis formation and cell fate specification in *H.erythrogramma*. By experimentally manipulating gene expression, we find that some key components of the GRN retain their ancestral roles in cell fate specification despite changes in timing of expression. Other GRN components, however, appear to have lost their ancestral roles, likely contributing to altered developmental timing and larval morphology. Analyses of embryonic transcriptomes from hybrids and both parental species indicate that many evolutionary changes in gene expression result from mutations in *cis*-regulatory elements, arguing against a model where a single master regulator imposes widespread changes in late gene expression in *trans*. Together, these findings suggest that the highly derived larval and life history traits of *H. erythrogramma* result from broadly distributed *cis*-regulatory mutations, some of which altered core interactions within the GRN.

ID #4425
Identifying targets of TBX5 in developing forelimbs and genitalia
Aaron Alcala, Sungdae Park, Doug Menke
University of Georgia, USA
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.

ID #4427 – Session 2: Organogenesis and Morphogenesis (Session Chair)
Akirin is critical for early tinman induction and subsequent formation of the heart in Drosophila melanogaster
Scott Nowak
Kennesaw State University, USA
Among the metazoans the heart is one of the earliest discrete organ structures to form during embryogenesis, in a process highly conserved across the phyla. In *Drosophila melanogaster* the specification of cardiac progenitors from mesoderm, differentiation and patterning of 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. Previous work in the Nowak lab has 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 a significant initial decrease in tinman expression levels compared with wild-type embryos. *akirin* mutants have reduced numbers of cardiomyoblasts, along with disrupted patterning and organization of the heart, including ectopic lumen formation, collapse of the aortic outflow tract, and hypertrophic cardiomyoblasts. Finally, while hearts do indeed form in *akirin* mutants, these mutant hearts display highly disorganized, irregular, and asymmetric contractions. Taken together, these results indicate that Akirin is crucial for the first induction of *tinman* by the Twist transcription factor, and that the success of the cardiac patterning program is highly dependent upon establishing the proper level of Tinman at the earliest steps of the cardiac developmental pathway.

ID #4428
Cis-enhancing regions that influence craniofacial development in cichlids
Emily Hawkins, Emily Furno, Kara E. Powder
Clemson University, USA
Up to 1/3 of birth defects are associated with abnormalities in craniofacial development, and faces show a great deal of natural variation. Cichlid fishes from East Africa have an extraordinary range of facial shapes that correlate to their feeding mechanism, making them an ideal model system to study the genetic basis of craniofacial variation. Changes in regulatory DNA, including enhancers, can alter gene expression which manifest in varying morphologies. We have identified three putative enhancers that lie in immediate proximity to genes involved in craniofacial, cartilage, and bone development. Hs844 and hs1007 is genetically associated with bone strength and shape of the upper jaw in cichlids. These are both thought to regulate the gene *Sp8*, which controls bone production. Hs1602 is thought to regulate *Six1*or *Six4*, both of which have been previously associated with facial development. Deletion of *Six1* or *Six4* in mice results in truncation of rounding of the face which mimics natural variation in cichilds. We sequenced these regions in cichlids that are representative of this phenotypic range, namely *Metriaclima zebra* and *Labeotropheus fuelleborni*. Using alignment software, these regions were compared to identify single nucleotide variants (SNVs) that were distinct between the two species. Hs1602 shows marked genetic variation between species, which may result in altered enhancer activity. Future work will (1) assess where and when in an embryo that enhancer is active using cloning and reporter assays and (2) determine the phenotypic impact of these enhancers using CRISPR/cas9 genetic editing. Given the conservation of facial development, understanding the regulatory basis of evolved variation in cichlids can bring new understanding to how craniofacial development is regulated normally and in human diseases.

ID #4430 – Session 2: Organogenesis and Morphogenesis
THE CILIARY PROTEIN ARL13B REGULATES AXON GUIDANCE IN THE DEVELOPING MOUSE HINDBRAIN
Sarah Suciu[1], Julien Ferent[2], Jiami Guo[3], Laura Mariani[1], Eva Anton[3], Frederic Charron[2], Tamara Caspary[1]
[1]Emory University, USA; [2]IRCM, Canada; [3]University of North Carolina, USA
The ciliopathy Joubert Syndrome (JS) presents with physical anomalies, intellectual disability, and is diagnosed by the hindbrain malformation "molar tooth sign" (MTS). The MTS results from cerebellar hypoplasia and axon guidance defects in the superior cerebellar peduncles (SCPs) white matter tract. Mutations in 35 cilia-associated genes including *ARL13B* cause JS but the underlying mechanism(s) remains unknown. ARL13B regulates transcription-dependent Shh signaling, which requires cilia to regulate cell-fate specification and cerebellar precursor proliferation. *Arl13b^{hnn}* null mouse mutants display constitutive, low-level transcription-dependent Shh signaling, consistent with MTS cerebellar hypoplasia. Additionally, transcription-independent Shh signaling regulates commissural axon guidance in the embryonic neural tube and so we hypothesized that Arl13b regulates transcription-independent Shh signaling to provide a common mechanism for MTS formation. To test Arl13b's role in transcription-independent Shh signaling, we examined commissural axon guidance *in vitro* and *in vivo*. We tested the ability of cultured commissural neurons to turn towards Shh and found a significant loss of response upon Arl13b knock down. Furthermore, we observed a loss of commissural axon midline crossing in *Arl13b^{hnn}* null embryos. These results indicate Arl13b regulates transcription-independent Shh signaling. We extended our work to directly examine the SCPs and test whether they are guided by a Shh cue and whether Arl13b regulates this guidance. We examined SCP guidance in mouse brains lacking either Smoothened, the obligate transducer of Shh, or Arl13b and found significant guidance defects in both. These data indicate Arl13b regulates axon guidance in projection neurons that use Shh as a guidance cue, implicating a cilia-associated gene in axon guidance. Taken together, our data suggest that disruption of Shh signaling may be a unifying mechanism underlying the MTS phenotype seen in JS.

ID #4435 – Session 1: Transcriptional Regulation and Epigenetics (Session Chair)
Developmental regulation of heterochromatin establishment at the maternal to zygotic transition
Kathrin Laue[1], Shrivarsha Rajshekar[2], Abigail Courtney[1], Zackary Lewis[1], Mary Goll[1]
[1]University of Georgia, USA; [2]Weill Cornell Medical College, USA
Heterochromatin has important functions in the organization of nuclear structure, the maintenance of genome integrity, and the regulation of gene expression programs[1-3]. Here, we demonstrate that the genome of the early zebrafish embryo is packaged in an atypical chromatin state that lacks features characteristic of heterochromatin. The heterochromatic histone modification, histone H3 lysine 9 trimethyl (H3K9me3), is undetectable in embryonic chromatin and embryonic nuclei are devoid of condensed chromatin prior to activation of the zygotic genome. The BAF complex catalytic subunit Smarca2 is a guardian of this unique chromatin state, and miR-430 mediated degradation of maternal *smarca2* RNA is required for heterochromatin establishment at the maternal to zygotic transition. Our results reveal a period of profound chromatin reorganization in the early zebrafish embryo and identify Smarca2 as an essential regulator of heterochromatin establishment during early development

ID #4436

An Inducible Fluorescent Extracellular Vesicle Transgenic Mouse for Spatial and Temporal Examination of In Vivo Intercellular Communication

Victoria Neckles, Mary Morton, Jennie Holmberg, David Feliciano
Clemson University, United States of America

Extracellular Vesicles (EVs) are membranous particles released from cells. EVs facilitate intercellular communication by transferring miRNA and proteins from donor to recipient cells. However, the *in vivo* sources, contents, targets and functions of EVs remain unknown. To this end, we generated a novel transgenic mouse to study EVs. This model utilizes the Cre-LoxP system to induce the expression of a fluorescent EV marker protein, CD9-GFP. CD9 was fused to copepod turbo GFP that contains a His tag, allowing for biochemical purification and analysis. Turbo GFP results in a brighter fluorescence that matures quickly, making it perfect for small, fast appearing particles, such as EVs. CD9-GFP was inserted within the Rosa26 locus. A CAG promoter was placed upstream of a stop sequence flanked with LoxP sites and CD9-GFP. The CAG promoter sequence is used to drive ubiquitous and persistent gene expression. Mice were electroporated with DNA plasmids that encode for CAG-CRE recombinase or mated with mice that have a Tamoxifen (TAM) inducible form of Cre-ERT2 driven by nestin or CAG promoters. Electroporated mice have focal recombination in target cells in close proximity to the lateral ventricles of the brain. CAG-Cre-ERT2 mice have Cre-ERT2 expression in all cell types, whereas nestin-Cre-ERT2 expression is selective for neural stem cells. TAM acts as an estrogen receptor modulator that upon binding to Cre-ERT2, causes translocation into the nucleus. The Cre recombinase excises the flanked stop sequence, resulting in subsequent CD9-GFP expression. This allows for spatial and temporal control of CD9-GFP. Following the injection of TAM, different tissues including the stomach, liver, kidney, heart, lung and brain were analyzed. Future studies will isolate fluorescent EVs from this model system that will be subjected to small RNA sequencing. Furthermore, this new tool will allow for the assembly of a cell type and spatially resolved map of *in vivo* intercellular communication.

ID #4440

Anolis sagrei lizard—a novel model system for studying fovea development

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The fovea—a pit-like depression in the retina with a high photoreceptor cell density, is specialized area that is important to the vision of primates and many non-mammalian amniotes. However, very little is known concerning the genes and developmental mechanisms that contribute to the formation of the fovea as the eyes of commonly used model systems—like the mouse, chick, frog, and zebrafish lack a fovea. Therefore, we introduce the bifoveated lizard*, Anolis sagrei,* as a novel model organism for eye-related research. We report here a morphological and histological analysis of foveae development in the lizard as well as highlight functional approaches for gene manipulation in the developing lizard embryo eye.

ID #4444 – Keynote Address I on Wednesday, May 23rd
Temporal regulation of shoot development in plants
Scott Poethig
University of Pennsylvania, USA
Plant shoots progress through several distinct phases during their development. The first transition occurs shortly after germination and is known as "vegetative phase change". This transition regulated by a decline in the abundance of the miRNAs, miR156 and miR157, and the resulting increase in the expression of their targets, SPL transcription factors. In *Arabidopsis*, the decline in miR156 is mediated by the epigenetic silencing of two of the 8 genes that encode this miRNA, *MIR156A* and *MIR156C*. miR156 initially decreases very rapidly, and then decreases slowly and more uniformly throughout shoot development. This expression pattern explains the changes in leaf morphology that occur as the shoot develops, and also accounts for variation in the developmental stability of leaves produced at different times in shoot development; juvenile leaves produced early in shoot development are less plastic than juvenile leaves produce later in shoot development because they contain significantly more miR156/miR157. To determine if changes in the timing of miR156/miR157 expression have played a role in plant evolution, we examined the expression patterns of these miRNAs in species in the genus, *Acacia*. Most of the >1,000 *Acacia* species native to Australia produce compound leaves early in shoot development and then switch to producing a simple leaf known as a phyllode. This transition is associated with a dramatic decline in the level of miR156/miR157. Species that never undergo this transition, or display delayed phase change, evolved independently at least 7 times. In the largest of these neotenous clades—which includes approximately 40 species—this phenotype is associated with the prolonged high expression of miR156. This expression pattern is the result of a regulatory mutation in a single *MIR156* gene.

ID #4445
Ciliary protein Arl13b is required for the full activation of G-protein coupled receptor Smoothened.
Eduardo Gigante, Tamara Caspary
Emory University, USA
The primary cilium, once thought to be vestigial, is now an extensively studied organelle due to its connection with several signaling pathways, including vertebrate Sonic Hedgehog (Shh) signaling. Several Shh components are dynamically shuttled through cilia. Shh ligand binds its receptor, Patched, at the cilium, removing Patched mediated inhibition of Smoothened (Smo). Smo is a G-protein coupled receptor (GPCR) whose Shh-dependent enrichment in cilia is thought to be critical for its activation, although the exact mechanism remains mysterious. Smo is aberrantly enriched in cilia of cells lacking the ciliary GTPase Arl13b. *Arl13b*^{hnn} null mouse mutants display a low-level Shh response. Thus, the Smo enriched in *Arl13b* mutant cilia is interpreted to not be fully activated. Arl13b is ancient, duplicated from *Arl13* in urochordates around the same time that Hedgehog signaling co-opted the cilium. We identified a hypomorphic *Smo* allele, *cabbie* (*cbb*), which survives until birth and misspecifies cell fates requiring the highest level of Shh activity. In *Smo*^{cbb} mutants, Smo is not fully activated and does not enrich in cilia. Based on the constitutive Smo enrichment in *Arl13b*^{hnn}, we predicted that Smo protein in a *Smo*^{cbb} mutant would localize to cilia, allowing Smo to be fully activated. Thus, we generated *Arl13b*^{hnn} *Smo*^{cbb} double mutants and were surprised to find the phenotype was more severe than either single mutant, displaying embryonic lethality at E10.5 and a head shape reminiscent of mutants lacking all Hh signaling. We interpret this to be a synergistic interaction and hypothesize Arl13b is required for full Smo activation. Through use of a new *in vitro* assay for Smo activation that uses its intrinsic GPCR function we can directly test the role of ciliary Arl13b in full Smo activation. Through this approach we will dissect the relationship among Arl13b, Smo, and cilia, and corroborate our findings *in vivo* in the mutant embryos.

ID #4447

Mutant Inpp5e disrupts Sonic Hedgehog and Wnt response in mouse neural tube patterning

Katharine Floyd, Sandii Constable, Alyssa Long, Chao Lin, Tamara Caspary
Emory University, USA

The simple model of vertebrate neural patterning posits a morphogen gradient of Sonic Hedgehog (Shh) specifies the 5 ventral neural cell fates in a concentration- and time- dependent manner. Here we describe a novel mouse allele of *Inositol polyphosphate-5-phosphatase-E*, called *Inpp5e*^{M2} caused by a D511G mutation. Inpp5e localizes to the primary cilium, the cell's microtubule-based projection required for Shh signal transduction. We found *Inpp5e*^{M2} embryos display expanded ventral cell fates at E9.5 and E10.5 suggesting increased Shh activity. This was unexpected as previous work showed Inpp5e loss inhibited Shh response by increasing PIP2 on the ciliary membrane, which bound Shh negative regulators. To investigate the relationship of *Inpp5e*^{M2} to cilia, we tested whether the *Inpp5e*^{M2} phenotype was cilia-dependent by generating *Inpp5e*^{M2} lacking cilia (*Inpp5e*^{M2} *IFT172*^{wim}) embryos. We found no ventral fates were specified, as is true in embryos lacking cilia, indicatingInpp5e function requires cilia. Smoothened (Smo) is the obligate transducer of Shh signaling. To understand the relationship of *Inpp5e*^{M2} to Shh signal transduction, we tested whether the *Inpp5e*^{M2} phenotype was Smo-dependent by generating *Inpp5e*^{M2} lacking Smo (*Inpp5e*^{M2} *Smo*^{bnb}) embryos. We found no floor plate or adjacent p3 cells were specified, indicating that Inpp5e requires Smo to specify cell fates requiring the highest level of Shh activity. However, *Inpp5e*^{M2} *Smo*^{bnb} double mutants specified Nkx6.1 and Olig2 expressing cells, which are absent in *Smo*^{bnb} single mutants. This result indicates that Inpp5e doesn't require Smo to specify cell fates requiring moderate levels of Shh activity suggesting Inpp5e may derepress the Shh response. Previous work showed alterations in Wnt signaling can alter ventral cell fates. We used the Wnt reporter *BAT-gal* allele and observed expanded Wnt response in *Inpp5e*^{M2} embryos. Taken together, our data indicate Inpp5e functions in both Shh and Wnt signal transduction.

ID #4448 – Session 5: Evolution and Development

Genetic interactions between ciliary proteins Nphp4 and Bbs5 and their role in development

Melissa Bentley, Scott Henke, Dawn Landis, Corey Williams, Svetlana Masyukova, Mandy Croyle, Bradley Yoder
University of Alabama at Birmingham, USA

The primary cilium is a microtubule-based structure that protrudes from the surface of most mammalian cell types and is crucial for developmental processes and cell signaling events. When ciliary proteins or structures are disrupted it often results in disorders collectively termed *ciliopathies.*The low genotype to phenotype correlation in ciliopathy patients makes it apparent that critical genetic modifiers of ciliopathy-associated genes still need to be identified. Through a screen performed in *C. elegans,* we have discovered that evolutionarily conserved genetic interactions between transition zone protein NPHP4 and BBSome protein BBS5 are necessary for proper ciliary function. We hypothesize that *Nphp4* and *Bbs5* mediate the primary cilium dependent Hedgehog (Hh) signaling pathway. In the zebrafish model *Nphp4; Bbs5* mutants are viable into adulthood but present with severe scoliosis. However, in mice we have shown that, while *Nphp4* or *Bbs5* single mutants are viable, *Nphp4;Bbs5* doublemutants are embryonic lethal between E11.0 and E12.5. Using μCT we have generated three-dimensional renderings of double mutant embryos up to E11.0. These embryos show irregularities in the formation of the atrioventricular canal of the heart. To study the potential postnatal effects of viable mutations in the patient population we have employed the use of a conditional *Bbs5* mouse model. When deletion of *Bbs5* is induced in *Nphp4* mutant pups at postnatal day 7 most animals do not survive past 7-10 days following induction. Lethality in these animals is often preceded by uncoordinated behaviors characteristic of cerebellar defects. We are currently working to generate CRISPR cell lines that will allow us to study how Nphp4 and Bbs5 affect cilia regulated signaling pathways such as Hh. The heterogeneity seen in these models is typical of Hh signaling defects and potentially provides insight into cardiac, neurological, and skeletal phenotypes commonly seen in ciliopathy patients.

ID #4449 – Session 3: Neural Development (Session Chair)
Imaging contact-dependent signaling in neural circuit assembly
Daichi Kamiyama, Ryo Tamura, Rie Kamiyama
University of Georgia, United States
Our brain consists of billions of neurons assembled into circuits. One of the primary goals of cellular neuroscience is to understand the principles behind assembly of neural circuits. We have been studying the molecular bases underlying neural morphogenesis in establishing the *Drosophila* central nervous system. Using single-neuron genetics and various microscopy techniques, we revealed the mechanism by which inter-neural contact of Down syndrome cell adhesion molecules positions a dendrite outgrowth site by guiding local cytoskeletal rearrangement. To elucidate how signaling molecules work together in a tiny volume of neural morphological features, we've also developed fluorescent labeling methods. we have recently developed small epitope tags based on self-complementing split fluorescent proteins (FP11-tags). The small size of the tags enables their cloning-free insertion into endogenous genomic loci by a CRISPR-mediated genetic knockin approach, potentially applicable for a large-scale knockin collection in many model organisms. By further developing unique quantitative imaging toolkits, our long-term objective is to decipher contact-signaling pathways of neural morphogenesis in neural circuit assembly.

ID #4450
The effect of HDAC inhibition on expression of tbx5 paralogs in zebrafish
Alec Jones
University of South Carolina Aiken, United States
Valproic acid (VPA) is known to inhibit class I and II HDAC activity, and exposure of zebrafish to high doses of VPA results in a striking phenotype, which includes cardiac defects, reduced forelimb growth, and ocular coloboma. These symptoms are identical to phenotypes observed in Tbx5 loss-of-function studies in zebrafish, and reflect phenotypes seen in Holt-Oram syndrome in humans. *tbx5a* and *tbx5b*, paralogs of *tbx5*, are responsible for the development of fin buds, for establishing cardiac asymmetry, and formation of the retinal axis. Current research suggests that these paralogs may be sub-functionalized in zebrafish and expression may be regulated differentially during development. Our initial observations support a hypothesis that Tbx5 is a target of HDAC repression during development, and that loss of Tbx5 produces the phenotypes associated with VPA exposure. However, semi-quantitative RT-PCR, along with preliminary RT-qPCR data, of *tbx5a* and *tbx5b* in VPA-treated zebrafish, reveals an increase in *tbx5a* expression, and a decrease in *tbx5b* expression. The reduced expression of *tbx5b* suggests that it is positively regulated by HDACs, while the increase in *tbx5a* expression implies that HDACs normally repress *tbx5a* transcription. Additionally, *in situ* hybridization of both *tbx5a* and *tbx5b* reveals increased *tbx5a* expression, and less localized expression of *tbx5b* in response to VPA exposure. Additional RT-qPCR analysis of *tbx5a* (along with its downstream targets *tbx2b, bmp4*, *fgf24*, *hey2*, and *nppa*)and *tbx5b* in VPA-treated fish is in progress. We will also be analyzing tissue-specific mRNA expression of *tbx5a*, *tbx5b*, *fgf24*, and *vcana* in embryos and larvae using mRNA *in situ* hybridization to establish if expression localization changes with VPA exposure. By examining downstream target expression we will be able to determine how divergent functions of *tbx5* paralogs may underlie differential expression of targets important for development.

ID #4451

Tbx1 Parathyroid Domain Expression Time Point

Todd Mudd, Barbara Del Castello
University of Georgia, United States

The thymus and parathyroid each originate from the third pharyngeal pouch in mouse and human embryos. Tbx1, a transcription factor from the T-box family of genes, is found within the nucleus of the regulatory network that dictates the cell fate decision for cells of the third pharyngeal pouch. Knockout experiments have implicated loss of Tbx1 with the DiGeorge Syndrome phenotype in which hypoplasia of the third pharyngeal pouch occurs, while upregulation of Tbx1 expression results in suppression of thymic epithelial cell differentiation. While Tbx1 expression is initially exhibited throughout the third pharyngeal pouch early in embryonic development, it later localizes to the parathyroid domain upon differentiation of the third pharyngeal pouch. There have been experiments to examine the phenotypic results of various Tbx1 expression levels within the third pharyngeal pouch, but the timeframe for Tbx1 expression within the pouch has yet to be elucidated. Through the use of immunofluorescence staining, we have explored the expression of Tbx1 in the parathyroid domain of embryonic mice around the age of E.13 in an attempt to identify the time point when Tbx1 expression is halted in the parathyroid of the developing mouse embryo. Identification of the expression timeframe for Tbx1 can provide a framework for further study of the role that it plays in the regulatory network that determines parathyroid/thymus cell fate.

ID #4452

Understanding the secretion mechanism of VAPB MSP

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Most eukaryotic secreted proteins travel through the conventional Golgi-ER secretory pathway. Other proteins are secreted unconventionally, independent of the Golgi-ER. VAPB/VPR-1 is a ubiquitously expressed type II membrane protein found anchored into the endoplasmic reticulum with the N-terminal Major Sperm Protein (vMSP) domain extending into the cytosol.This vMSP domain is cleaved and secreted, but does not harbor a signal peptide characteristic of conventionally secreted proteins. The goal is to understand how vMSP is proteolytically processed, secreted, and regulated. Interestingly, in humans, vMSP secretion has been linked to Amyotrophic Lateral Sclerosis (ALS). Based on published studies, we hypothesize that vMSP is secreted in a regulated fashion via an unconventional mechanism. In order to test our hypothesis, we developed an RNAi screening method using transgenic *C. elegans* to identify genes required for vMSP secretion. Then, we used genome-editing techniques to tag the termini of endogenous *vpr-1* and, are currently developing a direct read-out of vMSP secretion in *C. elegans*. Our results show that *C. elegans* mutants null for *vpr-1* 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. *ykt-6*, a v-SNARE, was a candidate gene resulting from the 420 genes screened. Further genetic studies are being used to determine the relationship between *ykt-6* and vMSP. Western blots of epitope tagged *vpr-1* endogenous termini revealed various stable N-terminus cleavage products. Immunofluorescence revealed a polar localization of *vpr-1* termini in intestinal epithelial cells. Further work is in progress to determine whether vMSP cleavage and secretion is developmentally regulated. The results from the screen and transgenic work will further our understanding of vMSP secretion and may pave way to understanding ALS pathology.

ID #4453

Regulation of embryonic cell fate decision by histone methylation

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Genetic and epigenetic information are transmitted from one generation to the next through the germline. Although the heritability of genetic information is stable from one generation to the next, epigenetic information is highly modified within each generation to regulate proper gene expression. After fertilization, each embryo must reprogram their epigenome and reestablish an epigenetic ground state to allow normal development to proceed. In *C. elegans*, two epigenetic enzymes, the H3K4me2 demethylase, SPR5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte and cooperate to reestablish the epigenetic ground state by modifying histone methylation. Progeny of worms lacking *spr-5* and *met-2* accumulate high levels of H3K4me2 within two generations, resulting in complete sterility and improper somatic expression of spermatogenesis genes. However, the precise developmental consequences of misregulating germline/soma identity in embryos is unclear. To interrogate how reprogramming defects may affect early embryonic development, we are using confocal imaging to perform automated lineage tracing experiments. The C. elegans embryonic lineage is normally invariant. I will investigate how inappropriate inheritance of histone methylation affects cell fate specification in the early embryo. By identifying defects in the embryonic lineage, we hope to gain mechanistic insight into the consequences of improper germline/soma identity. We hypothesize that an abnormal accumulation of histone methylation could affect cell fate, cause abnormal cell divisions, and perhaps lead to inappropriate cell death.

ID #4454

Improving the Transposition Efficiency of the Harbinger3n_Dr Transposable Element

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DNA transposable elements, or transposons, are mobile sequences of DNA that jump from one site in the genome to another in a cut-and-paste manner. They are found in all kingdoms of life and are sorted by homology into groups called superfamilies. The transposable elements from the *PIF/Harbinge*r superfamily are one of the more recently discovered superfamilies. We are interested in studying these elements in particular because the *Harbinger3N_DR* transposable element from zebrafish has been shown to be able to transpose in human cells, where it can be used for transgenesis or mutagenesis. We hope to learn more about its transposition characteristics, as well as develop hyperactive versions that transpose at higher rates. In order for *Harbinger3N_DR* to "jump" the proteins *Harbinger* ORF1 and *Harbinger* Transposase (TPase) must be present. I have developed Harbinger ORF1, Harbinger TPase expression constructs and transformed them into yeast together with a *Harbinger3N_DR* reporter construct. We are performing yeast transposition assays to determine the transposition rate and analyze the excision sites. These results will be compared to *mPing*, another *PIF/Harbinger* superfamily transposable element from rice. We anticipate that these elements will show similarities in their transposition strategies. We can then use strategies that worked for increasing *mPing* transposition rates to make a more efficient *Harbinger3N_DR* element. This includes removal of nuclear export sequences and mutation of internal sequences.

ID #4455

USING CRISPR/CAS9 TO STUDY THE ROLE OF ZMYM2 AND ZMYM3 IN ZEBRAFISH CRANIOFACIAL DEVELOPMENT

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Potocki-Shaffer syndrome (PSS) is a rare contiguous gene-deletion caused by heterozygous interstitial microdeletions of chromosome region 11p11-p12 and is characterized by developmental defects including intellectual disability and craniofacial anomalies. PSS is associated with mutations in genes encoding factors in the PHF21A protein complex, including ZMYM2, and ZMYM3 proteins. It is hypothesized these protein complexes affect craniofacial development of zebrafish in a way that reflects their function in humans. F_0 founder fish carrying mutations in *zmym2* and *zmym3* were generated by microinjection of CRISPR constructs including a gRNA and Cas9 mRNA at the 1-cell stage. Founders were screened by PCR and T7 endonuclease digest to identify mutations. F_1 lines were generated and screened by using tail fin DNA in PCR and T7 digest. Zebrafish with potential frameshifts were out-crossed to produce an F_2 generation. To confirm the nature of the mutations as frameshifts or early stop codons, *E. coli* cells were transformed with *PminiT* plasmids containing PCR amplicons from individual confirmed heterozygote fish. Ampicillin-agar plates were used for positive selection of transformation. Four colonies from each plate were sequenced.In principle, half of clones will contain mutant sequence and half of clones will contain wild-type sequence. If frameshift lesions are detected in DNA samples, these lines will be used to generate homozygous mutants. Lines will be screened using PCR and T7 digest, or if there is a large enough indel, PCR products run on a 2% gel will be used to resolve genotypes. The F_3 generation will be studied at 7 days post fertilization for anatomical abnormalities in craniofacial development by using Alcian Blue and Alizarin Red histological stains for cartilage and bone. The goal of this project will be to identify the roles of *zmym2* and *zmym3* in zebrafish development, and how loss of function of these factors may underlie the defects seen in PSS.

ID #4456

Tactile Versus Electrical Sensory Input to Stimulate the Zebrafish (Danio rerio) Escape Response

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The escape response, or c-start response in zebrafish, provides a quantifiable assay for measuring changes in the communication of the neural circuit components that govern survival behaviors. Initiation of the escape response relies on the detection of mechanostimuli that trigger Mauthner neurons or M-cells, a pair of commissural neurons that extend the full length of the spinal cord to integrate stimuli through interneurons. Mauthner cells integrate directional information from stimuli, and convey bilateral, excitatory responses to motor neurons innervating trunk muscles. Communication between Mauthner neurons by commissural interneurons induces reciprocal inhibition necessary for coordinated movement. Even from an early embryonic period (16 hours post fertilization), zebrafish receive and respond to direct touch stimuli with escape behaviors. Previous studies comparing head touch-induced responses with responses elicited by an auditory cue indicate that fish do not develop auditory/ vestibular induced responses until 70 hours post fertilization. From this developmental milestone, instead of needing direct touch stimuli, the fish employs the octavolateralis system to detect disturbances in the water. The escape response has been extensively characterized in larval zebrafish and is often used for testing innate and genetically altered reactions to stimuli. As a prelude to my thesis research regarding treatment of spasticity through reestablishment of balanced excitatory and inhibitory inter-neuronal signaling in the escape circuit, we investigate the relationship between c-start responses evoked through electrical and tactile stimuli. The importance of standard repeatable methods to elicit escape behaviors removes variables that may influence the integrity of the measured escape response and our ability to elicit consistent behaviors.

ID #4457 – Session 4: Growth and Differentiation

Genetic analysis of invasive pathways engaged by the EcR-coactivator protein Taiman

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The transformation of non-motile epithelial cells to a migratory state plays a significant role in normal development and diseases such as cancer. During *Drosophila* oogenesis, a specialized group of cells termed border cells (BCs) acquire the ability to detach from their host epithelium and migrate through surrounding cells to the posterior end of the oocyte. The steroid receptor transcriptional co-activator *taiman* (*tai*) plays an important role in promoting this motility process, but its downstream transcriptional targets remain poorly defined. Here we introduce a novel, pathogenic model of Tai-driven tissue invasion that allows for rapid genetic screening for elements of the Tai-induced transcriptional program. Overexpression of *tai* in non-motile pupal wing cells causes these cells to invade through adjacent thoracic cuticle and into internal tissues, leading to a high-penetrance adult phenotype of wing tips embedded into the thorax. RNA-seq analysis of wing tissue overexpressing Tai led to identifying upregulation of components of the Toll pathway and innate immunity pathway including an extracellular serine protease *gastrulation defective (gd)* and *spätzle (spz), spätzle4 (spz4), and spätzle6 (spz6)*. Published work shows excessive Toll signaling can lead to cell death via pro-apoptotic factor *hid*. We have investigated the genetic interaction between Toll pathway components and Tai-driven wing invasion as well as transcriptional induction of these components by Tai and where *hid* is expressed in this context.

ID #4458 – Session 5: Evolution and Development

A massively parallel screen for morphogenesis genes by RNAseq of interspecific hybrid embryos

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

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ID #4459

The ciliary protein Inpp5e regulates axon guidance in mice

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Joubert Syndrome (JS) is a ciliopathy defined by recessive mutations in 35 cilia-associated genes, including *INPP5E.* Patients display symptoms consistent with ciliopathies as well as a failure of axons to cross the midline at the corticospinal tract, the optic chiasm, and the superior cerebellar peduncle (SCP) of the hindbrain. Neurons guide their axons by sensing external guidance cues with the growth cone found at the tip of the axon. The primary cilium, a specialized microtubule-based organelle protruding from the cell body, is distant from the growth cone so how cilia proteins, such as INPP5E, regulate axon guidance remains elusive. One possibility is through regulation of Sonic Hedgehog signaling (Shh). Shh signals through two pathways; a Gli-transcription dependent process for neural cell identity and proliferation that is dependent on the cilium, and a transcription-independent process for axon guidance. INPP5E can be linked to Gli-dependent Shh signaling. INPP5E is a phopsphatidylinositol phosphatase highly enriched in cilia which hydrolyzes PIP_2 to PI(4)P. PI(4)P is required for activation of Smoothened, the obligate transducer of Shh signaling. To test whether Inpp5e directs SCP axon guidance, I conditionally deleted *Inpp5e* in developing projection neurons and examined the SCP tract. Axons crossed the midline but did not project properly to the thalamus indicating a defect in SCP axon guidance. Next, I examined projections of commissural neurons, known to respond to Shh using transcription-independent signaling. I examined a D511G missense allele of Inpp5e, $Inpp5e^{M2}$. Commissural axons in $Inpp5e^{M2}$ mice lacked a ventral commissure indicating Inpp5e regulates commissural axon guidance. Together these data argue that Inpp5e is important for axon guidance of multiple tracts in the mouse nervous system and implicate Shh in the mechanism.

ID #4460

Dynein independent role for dynein light chain in meiotic progression

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

ID #4461 – Session 4: Growth and Differentiation

G-Protein signaling accelerates Drosophila germline stem cell divisions upon repeated mating

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Stem cells play critical roles in development and in adult animals due to their ability to create functional tissues in the body. Stem cells self-renew and create a pool of precursors for the differentiation into specialized cells that are lost due to cell death or usage. Most adult stem cells are associated with a specialized microenvironment, which is essential in the regulation and maintenance of stem cell fate decisions. However, little is known about the regulation of stem cell mitotic activity in response to a demand for specialized cells.Our lab discovered that *Drosophila* wild-type males display a significant and reproducible increase in their germline stem cell (GSC) division frequency after repeated mating with virgin females. Signaling via the highly conserved G-proteins is required for this acceleration of GSC divisions. Expression of a dominant negative version of Gγ1 or repression of Gα-subunits via RNA-Interference (RNA*i*) in the germline eliminated the GSC response to mating.To corroborate the role of G-protein signaling in this process, we reduced the expression of 34 G-protein coupled receptors (GPCRs) from the germline cells via RNA*i*. Surprisingly, RNA*i* against any of seven distinct GPCRs reproducibly prevented the acceleration of GSC divisions in mated males, while RNA*i* against the other GPCRs did not. The GPCRs required for the increase in GSC division frequency were the Serotonin Receptors 5HT-1A, 5HT-1B and 5HT-7, Metuselah (Mth), Metuselah-like5 (Mth-l5), Octopamineβ 2R (Octβ 2R), and CG12290.Conversely, feeding non-mated males serotonin at micro molar levels was sufficient to elicit a heightened rate of GSC divisions that was similar to the GSC division frequency in mated males. We conclude that the increase in GSC division frequency is regulated by G-protein signaling. We hypothesize that GSC mitotic activity is regulated via GPCR oligomerization and cross-talk among downstream signaling pathways.

ID #4462

ANALYZING A TOL2-BASED ACTIVATION TAG CONSTRUCT IN ZEBRAFISH

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Transposable elements are segments of DNA that can move from one region to another within the genome when induced. The *Tol2* transposon from Medaka fish has successfully been adapted for transgenesis, integrating foreign DNA, in a wide variety of vertebrates. Our goal is to develop the *Tol2* element into a mutagenesis tool for gene discovery. Activation tagging, a form of transposon tagging, is when a strong enhancer is positioned within the element. Activation tags can 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. Zebrafish are a model for vertebrate development, therefore activation tagging within zebrafish will allow for the discovery of developmental-related genes. A *Tol2*-based activation tag construct was engineered by PCR, digestion, and sequence analysis. The construct consists of the *Tol2* terminal inverted repeats (TIRs) flanking the enhancer region of the Beta-actin promoter. This activation tag was cloned next to the remainder of the Beta-actin promoter, and a *mCherry* reporter gene to indicate if transposition has occurred. A *Tol2* transposase construct was previously engineered to provide transposase, which is necessary to induce transposition of the activation tag in zebrafish. These constructs were coinjected into zebrafish embryos to create a population for measuring transposition rates and look for altered phenotypes.

ID #4463

Regulation of Foxn1 expression during embryonic development and in post-natal thymic maintenance –

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The thymus is a primary lymphoid organ. It provides a functional microenvironment for the development of a mature, functional and self tolerant T cells repertoire. Thymic epithelial cells (TECs) are crucial component of the functional thymic microenvironment. Transcription factor *Foxn*1 is necessary for fetal TEC differentiation and proliferation. No functional thymus is developed in absence of *Foxn*1. We have limited information about how *Foxn*1 is regulated during embryonic development. Literature suggests that, the regulatory elements sufficient to drive *Foxn*1 expression in fetal TECs, are present in intron1 of the gene itself. We want to map these regulatory elements. We identified putative enhancer elements based on evolutionarily conserved sequences. The functionality of these elements was validated *in vivo* by transient transgenic mouse assay. We identified ~1500bp sequence in the intron1 of *Foxn*1. This sequence is sufficient to drive expression of *Foxn*1 in fetal TECs.*Foxn*1 is also required in a dosage dependent manner for post-natal thymic maintenance (Chen L *et al*, 2009). Limited information is available about regulation of *Foxn*1 in post-natal TECs. Current literature proposes a model depicting regulation of *Foxn*1expression in post-natal TECs. According to the model, in postnatal stages, *Foxn*1 expression is under direct control of Rb/E2F pathway. This in turn regulates TEC proliferation and thymus size. Two binding sites for E2F factors were identified in *Foxn*1 promoter by computational analysis (Garfin P *et al*, 2013). We mutated these binding sites via CRISPR genome editing to test their functionality *in vivo*. We screened the CRISPR mutants and established 2 mouse lines with mutations in one of the binding sites. Phenotypic analysis of the thymus size and histology in the homozygous mutants is currently under study.

ID #4464

Gene expression analysis of migrating neural crest cells in craniofacial evolution in cichlid fishes

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Neural crest cells (NCCs) play a significant role in development and are the primary source of all bones in the face. Before they differentiate into facial bone and cartilage, NCCs migrate from the hindbrain into the pharyngeal arches. Variation in the pattern and amount of NCC migration can lead to congenital birth defects in humans ("neurocristopathies") or natural variation in facial skeleton. Cichlid fishes of Lake Malawi are an extreme example of natural variation in craniofacial morphologies. Adaptations related to feeding mechanisms have resulted in the evolution of hundreds of species in just one million years. We hypothesize that differential expression of candidate genes involved in NCC migration and development create early developmental differences. These changes in craniofacial morphologies would then lead to large phenotypic differences in adult cichlids. In fact, the earliest variation in morphology in cichlids is evident at NCC migration. We assessed changes in gene expression using *in situ*hybridization in two species of cichlids that either develop long or short faces (*Maylandia zebra* and *Labeotropheus fuelleborni*, respectively). Embryosranged from 2-4 days post fertilization, during which time NCCs are migrating. We focused on genes involved in NCC specification and delamination (*fox3d, snai2,*and*twist1a*) and migration and patterning (*dlx2a*, *efnb2a*, *epha4b,* and *tfap2a*). Overall, genes are expressed similarly in species, and there are suggestive differences between species in distribution of NCCs migrating to the anterior and posterior head. Future work will quantify the differences found in each species by measuring the volume and area in which NCCs are found. By better understanding how early craniofacial development leads to differences in phenotype, these results can lead to a better understanding of the developmental processes behind human diseases related to craniofacial structure such as cleft palate.

ID #4465

Reprogramming MEFs into Thymic epithelial cells

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Thymus is one of the primary lymphoid organs---the major source of self-restricted, self-tolerant naïve T cells required for robust adaptive immunity. 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.

The transcription factor FOXN1 plays a crucial role in thymus development, postnatal function, and involution. Our collaborator lab and our lab both successfully reprogramed Mouse Embryonic fibroblast (MEF) into induced functional thymus epithelial cells (iTECs) by only using enforced exogenous Foxn1 expression. In this study, we further analyze the gene expression profile of the iTECs as well as the reprogramming pathway. Our 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 data shows that the reprogramming pathway acts in a step-by-step manner, in which several Foxn1-pathway gene and non-Foxn1-pathway gene is involved.

In this study, we are developing a non-transgenic method of iTEC generation for future clinical application. Our data shows by using dCAS9 plasmid, we can successfully activate Endogenous Foxn1 gene expression both from Foxn1 promoter region and potential enhancer region. ICC staining shows that several cells shows morphology change after activating endogenous Foxn1. By using Foxn1-EGFP MEFs, we can successfully separate MEFs based on their different endogenous foxn1 expression. Further experiments still need to done to further analyze the reprogramming completeness and efficiency by using this non-transgenic methods.

ID #4466

Structural changes in the adult brain of PAX6-deficient mammals

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Aniridia is a congenital and progressive disorder affecting approximately 1 in 83,000 live births. Although the disorder is most well known for its ocular phenotypes, the condition has several other neural abnormalities, which are only recently emerging as prominent features of the disorder. Development of aniridia in humans is predominately caused by heterozygous loss-of-function mutations in the *PAX6* gene, a highly conserved transcription factor critical for normal eye and brain development. Our lab has utilized 3T MRI to show structural changes in the brains of aniridia patients as compared to their *PAX6*-normal comparisons. We found reductions to major fiber tracts such as the anterior commissure, posterior commissure, and optic chiasm, in addition to lack of or reduction to the pineal gland. The cellular basis for these changes are not well understood. To better understand the role of *PAX6* in brain development and adult brain function we have turned to the rodent model of aniridia, *Small eye*, in which we can utilize a variety of tools to assess *Pax6* expression and the neural consequences of mutations in the brain. The current study employed 7T MRI to acquire structural brain images and volumetric data and histological examination of the adult brain to examine the consequences of loss of one functional copy of the *Pax6* gene. Our results indicate that *Small Eye* have some structural brain changes similar to our human population; however, there are structures in our mouse model that do not seem to be changed to the same extent as our human population, namely the anterior commissure and posterior commissure. These data suggest that the structural changes seen in the aniridia patient population could be more severe, as compared to our mouse model, due to modifier effects or species-specific roles for PAX6. We are currently using the tissue-clearing method, iDISCO, in our mouse model, *Small eye,* to better understand the role Pax6 plays during adult brain maintenance.

ID #4467

Genetic Variation in the Cranial Shape of Malawi Cichlids

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Changes in development produces phenotypic differences. This can be normal variation, birth defects, or evolved phenotypes. Cichlids are an extreme example of an adaptive radiation, having an unparalleled range of craniofacial morphologies that relate to their diverse feeding mechanisms. Most of this variation falls on a continuous spectrum. Cichlids that eat by suction feeding have narrow faces, longer mandibles, and a more shallow craniofacial profile to enable fast jaw closure. Alternatively, cichlids that eat by biting or scraping attached algae from rocks have wider faces, shorter mandibles, and a steeper craniofacial profile to enable more power with each bite. We examined phenotypic variation in facial structures for two species of Lake Malawi cichlids, *Aulonacara koningsi*and *Maylandia mbenji*, that represent a different sides of this spectrum. Parentals of both species and ~400 of their F2 hybrids were x-ray imaged, and craniofacial variation was quantified using linear measurements and geometric morphometrics to assess overall shape changes. All measurements were size corrected and statistics were calculated in R. *Aulonocara*, which eats by suction feeding, has a decreased dorsal-ventral height of the head (p=0.0061), longer mandible (p=5.2e-6), and a more downturned mouth (p=1.2e-5) compared the *Maylandia*generalist; all p-values are the species interaction in ANOVA. Geometric morphometrics agrees with linear measurements. *Aulonocara* has a PC1 score associated with a longer anterior-posterior length of the head, decreased dorsal-ventral height, and a shallow craniofacial profile (PC1=43% of total shape variation, p=0.0097 by t-test between species). Future work will identify the genetic basis of these phenotypic differences via quantitative trait loci mapping. By understanding the genetic basis of natural variation in cichlids, we hope to identify novel genes and mechanisms that underlie craniofacial development in general, as well as human birth defects.

ID #4468

Determining how PHF21a affects craniofacial development in zebrafish

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In humans, mutations in the transcriptional repressor *PHF21a* (PHD finger protein 21A) results in Potocki-Shaffer Syndrome which causes craniofacial and neurological defects. Previously, it was observed that knockdown of *phf21a* in zebrafish resulted in defects in embryonic cartilage. It is therefore hypothesized that Phf21a 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 the co-orthologs of *phf21aa* and *phf21ab* using CRISPR-Cas9. An F_0 line was outcrossed to create an F_1 generation, and F_1 zebrafish were screened to identify heterozygotes using PCR and T7 endonuclease digest. PCR produces a product which is gel extracted. If fish are heterozygotes,T7 endonuclease digests mismatched PCR products which results in two DNA fragments that equal the PCR product. For *phf21aa* heterozygotes, the PCR product is 995 base pairs and the T7 endonuclease digest products are 720 and 275 base pairs. For *phf21ab* heterozygotes,the PCR product is 613 base pairs and the T7 endonuclease digest products are 447 and 166 base pairs. The fish that were confirmed as heterozygotes were outcrossed to AB wild type fish to generate an F_2 line and these fish are currently being genotyped. The F_2 generation will later beincrossed, resulting in 25% homozygous mutants which will be sequenced to confirm the nature of mutations. Mutants will also be stained with dyes to label bone and cartilage. Studying how disruptions to *phf21a* affect skeletal development in zebrafish can help us understand the normal functions of these genes in craniofacial development and can lead to a better understanding for how loss of PHF21A in humans causes the Potocki-Shaffer syndrome phenotype.

ID #4470

Maternally provided LSD1 enables the maternal-to-zygotic transition and prevents defects that manifest postnatally

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Autism spectrum disorders (ASDs) are characterized by deficits in social interactions, repetitive behavior patterns, and restricted interests. Although these disorders affect 1-2% of the world's population, the underlying mechanisms that contribute to ASDs are not fully understood. Recent data from our lab suggests that maternal inheritance of reduced levels of the enzyme LSD1 (lysine specific demethylase 1), may be a contributing factor to autistic-like behavior in mice. LSD1 is an epigenetic reprogramming enzyme that removes H3K4me1/2 (histone H3 lysine 4 mono- and di-methylation), which are typically associated with actively transcribed genes. These 'active marks' around transcribing oocyte genes need to be erased during reprogramming in the early embryo in order for the oogenesis transcriptional program to be repressed and normal development to continue. A complete loss of maternal LSD1 in mice results in embryonic arrest at the 1-2 cell stage, indicating the importance of this enzyme during reprogramming of the early embryo. On rare occasions when there is only partial loss of LSD1 maternally, the surviving offspring exhibit autistic-like behaviors such as high anxiety and repetitive behaviors. Furthermore, there is a decrease in the amount of LSD1 in late stage oocytes in mice with increasing maternal age. This correlates with epidemiological data showing that the risk of ASDs increase significantly with each 10-year increase in parental age. We hypothesize that reduced amounts of maternally-inherited LSD1 due to advanced maternal age contributes to the risk of developing ASDs. To test this hypothesis, we've generated a hypomorphic *Lsd1* allele to mimic the partial loss maternal phenotype. Our goal is to discover whether subtle defects in LSD1-mediated epigenetic reprogramming at fertilization can lead to the development of autistic-like behaviors via inappropriately inherited histone methylation, a novel mechanism potentially underlying ASDs.

ID #4471 – Session 3: Neural Development

Defining links between an intellectual disability-associated RNA-binding protein and planar cell polarity in dendritic arborization

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The human *ZC3H14* gene encodes a ubiquitously expressed zinc-finger polyadenosine RNA-binding protein. Mutations in *ZC3H14* that impair function of it encoded proteinhave been linked to an inherited form of non-syndromic intellectual disability (NS-ID). We developed a *Drosophila melanogaster* model of ZC3H14 NS-ID by deletion of *dNab2*, the fly ortholog of *ZC3H14*. These *dNab2*-deficient animals display defects in survival, locomotion, and memory which correlate at a cellular level with neurodevelopmental defects. Importantly, pan-neuronal expression of human ZC3H14 in *Drosophila* neurons can rescue the overt locomotor and survival phenotypes of *dNab2*-deficient flies, suggesting that dNab2 and ZC3H14 serve conserved roles in neurons. To probe this role, we used a dominant-modifier approach to identify genes that interact with *dNab2*. This approach has uncovered genetic interactions between *dNab2* and multiple components of the planar cell polarity (PCP) pathway, such as *Disheveled*, that can rescue dNab2-deficient neurodevelopmental defects. Furthermore, loss of function alleles of PCP components can rescue a portion of dNab2 null neuro-morphology defects observed in the mushroom bodies, twin neuropil structures analogous to the mammalian hippocampus. Importantly, mutations that inactivate either PCP factors or the *Drosophila* Fragile-X protein homolog, dFMRP, which physically interacts with dNab2, both result in increased dendritic arborization. These correlations suggest that dNab2 may regulate dendrite morphology via the PCP pathway. To begin to test this, we have examined the effect of dNab2 loss on dendritic structure, particularly among L3 larval class IV dorsal dendritic arborization C (ddaC) neurons. Here, we show that knockdown of dNab2 within ddaC neurons leads to an increase in dendritic complexity and follow this up with genetic analysis of the role of various PCP components and known dNab2 interactors (dFMRP, Atx2) in this dendritic phenotype.

ID #4472

Rheb-Ulk1 Regulation of Cortical Neuron Morphology

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Anabolic and catabolic processes are required for the proper growth and maintenance of dendrites within cerebrocortical neurons. mTORC1 is a protein kinase that activates anabolic protein translation and inhibits autophagy. mTORC1 activity is controlled by the GTPase Ras homology enriched in brain (Rheb). mTOR hyperactivation causes a constellation of neurodevelopmental disorders known as "mTOR-opathies". mTOR-opathies, including Rheb mutations, are frequently accompanied by abnormal neuron morphology and dendrite arborization. Abnormal morphology may contribute to neurological manifestations including intellectual delay and epilepsy. While Rheb and mTORC1 are implicated in the formation of cortical lesions, seizure activity, and defects in neuronal migration, the contribution of Rheb to changes in neuron soma size and dendrite morphology is not well established. In this study *in utero* electroporation (IUE) of radial glia, cortical pyramidal neuron stem cells, was used to label and study cortical neurons. Soma size and dendrite growth were assessed in layer II/III neurons within the anterior cingulate cortex (ACC) and somatosensory cortex (SCC). Between P0 and P21, neuronal soma size increased by 50 and 122 percent in the ACC and SSC, respectively. The increased size was accompanied by an increase in the number of basal dendrites and enhanced dendrite complexity. Phosphorylation of mTOR substrates, including the pro-autophagic kinase Ulk1, was detected within developing neurons. Notably, Rheb IUE caused cortical malformations comprised of ectopically positioned cytomegalic neurons with dendrite hypertrophy. To assess whether Ulk1 regulates neuron morphology, Ulk1 was electroporated into radial glia. Ulk1 IUE decreased neuron growth. Based on these findings, we propose that Rheb and Ulk1 can regulate cortical neuron morphology which may contribute to the neurological manifestations seen in mTOR-opathies.

ID #4473

Thymic Epithelial Cells Accumulate Lipid During Age-Associated Thymic Involution

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One of the phenotypic hallmarks of age-associated thymic involution is an increased presence of adipocytes in the thymus. Most studies to date have shown that a decrease in the functional tissue of the thymus occurs closely parallel to the accumulation of adipogenic tissue. The result is a lower capacity of the thymus to generate functional T-cells required for adaptive immunity. However, the cellular origins of thymic adipocytes remain obscure, with some investigators suggesting that adipocytes infiltrate the thymus during involution and others indicating that they differentiate directly from thymic stromal cells. Our lab has identified a potential regulator of adipogenesis in the thymus, the Activating Transcription Factor 3 (ATF3), in which deletion of the *Atf3* gene results in an increased presence of lipid-laden thymic stromal cells at 2-months of age in mice. We have also conducted a lineage trace study using $Atf3^{null/null}$; $Foxn1^{Cre/+}$; $Rosa26^{tdTom/+}$ mice to identify if a subset of thymic epithelial cells (TECs) undergo adipogenesis by using imaging flow cytometry and histological analysis. Preliminary results show that cells with markers for cortical TECs (cTECs) and medullary TECs (mTECs) accumulate lipid with age. These data suggest that both cTECs and mTECs give rise to a subpopulation of thymic adipocytes and that ATF3 is a likely repressor of adipogenesis during thymic involution.

ID #4474 – Session 5: Evolution and Development
Conserved targets of ISL1 in genital and hindlimb development
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Early development of the genital tubercle – the embryonic precursor to the penis and clitoris – involves expression of many genes that play a role in limb development. Additionally, ChIP-seq experiments in mice have suggested that many enhancers are active in both tissues. The *Isl1* gene encodes a transcription factor that is required for initiation of hindlimb outgrowth in mice. Conditional knockouts also demonstrate a crucial requirement of *Isl1* in genital development. Similarities in early external genital development across amniotes suggests derivation from their last common ancestor over 300 million years ago. Using a combination of RNA-seq and ChIP-seq, we reveal putative direct targets of ISL1 in the mouse genital tubercle. We find that limb genes are overrepresented among these targets, and that many appear to be regulated via known limb enhancers. Furthermore, we use ChIP-seq in the chick genital tubercle to identify many conserved enhancers bound by ISL1 in both species, possibly highlighting targets of functional importance across amniotes. Finally, our analysis of ISL1 targets in the hindlimb field reveals genes that appear to be regulated via shared limb-genital enhancers.

ID #4475
Characterizing a role for CoREST in regulating the function of LSD1
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Histone modifying enzymes are vital for transmitting epigenetic information across generations. In C. elegans, the H3K4me2 demethylase, *spr-5*, and H3K9me2 methyltransferase, *met-2*, synergize to reprogram the chromatin landscape and prevent the inappropriate expression of germline genes at fertilization. If either is lost, developmental defects occur; both *spr-5* and *met-2* mutants display a progressive sterility phenotype. This is more apparent in *spr-5; met-2* double mutants, which exhibit maternal effect sterility and developmental arrest after one generation. In mammals, orthologs of SPR-5, LSD1, and SPR-1, CoREST, physically associate and absence of CoREST lowers LSD1 demethylase activity. In C. elegans, SPR-5 and SPR-1 interact *in vitro*, and worms deficient in either rescue the egg-laying defect of *sel-12* mutants. These findings suggest an underlying mechanism may exist where SPR-1 regulates SPR-5 function. We hypothesize this interaction is needed to ensure proper epigenetic reprogramming and zygotic development. To test, we compared sterility in *spr-1* vs. *spr-5* mutants. Unlike *spr-5* mutants, *spr-1* worms neither become progressively sterile nor display morphological defects. Utilizing the extreme sterility and developmental delay of *spr-5; met-2* mutants, we evaluated whether these phenotypes were present with *met-2; spr-1*. While *met-2; spr-1* progeny show sterility and developmental delay in one generation, the phenotype is less severe than in *spr-5; met-2*. While ~20% of *met-2; spr-1* progeny are sterile at generation 1 (F1), we observe this measure increase to ~60% by F8, accompanied by a decline in number of progeny. By F10, we find *met-2; spr-1* germlines that phenocopy the disorganized morphology seen in *spr-5; met-2*. Our data suggest SPR-1 regulates SPR-5, but also leaves open the idea that SPR-5 may function with other proteins to reprogram the chromatin landscape. With this, *spr-1* emerges as an epigenetic player with implications for regulating development.

ID #4476

Loss of microtubule acetylation with depletion of monoglycylation leads to ciliary defect-like phenotype in zebrafish embryos

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Microtubule carries out a wide range of functions including structural roles in cilia and flagella by having microtubules with diverse physical properties and dynamic states within every cell. Microtubule post-translational modification provides some diversity necessary for microtubule functions. Past studies suggested that microtubule acetylation and monoglycylation could play a critical role in cilium assembly and maintenance. In this study, we generated a null mutant of microtubule acetylation enzyme, alpha-tubulin acetyltransferase (*atat1*). These *atat1* mutants had no observable morphological or behavioral defects neither in embryo stages nor as adults compared to wild-type littermates. We found that nearly 30 % of *atat1-/-* embryos showed curved body axis phenotype at Prim-5 stage when they had been raised at 35^{o}C. This curved body axis phenotype is a characteristic phenotype of ciliary defects in zebrafish embryos. This phenotype in *atat1-/-* embryos was rescued by overexpression of mouse *atat1-eyfp* mRNA. We found increased microtubule monoglycylation in *atat1-/-* adult testis and embryo pronephric duct. To understand whether loss of microtubule acetylation could be compensated by microtubule monoglycylation to maintain the normal level of cilia under normal condition, we carried out a morpholino knockdown of microtubule monoglycylation enzyme, Tubulin tyrosine ligase-like family, member 3 (*ttll3*). *ttll3* morphant: *atat1*-/- embryos showed curved body axis in 70 % of embryos. Zygotic *atat1-/-* and *ttll3-/-* embryos did not show the curved body axis. Maternally expressed *ttll3* mRNAs could compensate the loss of acetylation in our zygotic *atat1-/-: ttll3-/-* embryos. Due to low fertility and early embryo death occurred in double mutants, we have never been able to study the maternal and zygotic double mutants. Our data suggest that there could be an interplay between microtubule acetylation and monoglycylation in the maintenance of cilia in zebrafish embryos.

ID #4477

Local Role for Steroids in Regenerative Growth in Drosophila

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Drosophila offers an attractive, genetically tractable model system to study regenerative growth. A widely used method has been developed to specifically and temporally drive expression of a pro-apoptotic signal in the imaginal wing disc using the Gal4/UAS system. This pro-apoptotic pulse elicits a regeneration "blastema", a localized zone of proliferating cells, that displays many of the same characteristics as blastemas formed following surgical wounding. Furthermore the same Gal4 pulse can be used to deplete candidate factors in the presumptive blastema (e.g. by RNAi). Thus this system allows us to reproducibly injure fly larvae and screen for genes locally required for regeneration at the same time. The well-conserved Hippo pathway acts through the transcriptional coactivator Yorkie (Yki) to promote regenerative growth. Published work from our lab has linked the Hippo pathway to the Ecdysone pathway through physical interaction between Yki and Taiman (Tai), an ecdysone receptor coactivator. Tai and the ecdysone receptor (EcR) are genetically required for Yki to induce transcription of certain target genes. Thus the bases of the Hippo and Ecdysone pathways intersect through Yki:Tai interaction to form what we term the Yki:Tai transcriptional axis. Tai-dependent Yki targets include genes that are upregulated in regenerating imaginal wing discs, such as the pro-growth microRNA *bantam,* the stem cell factor *piwi*, and the secreted insulin-like factor *dILP8*. Recently, our lab has found evidence that local depletion of the active form of ecdysone, 20-hydroxyecdysone (20HE), impairs regeneration in *Drosophila.* We will test the **hypothesis** that local 20HE production within wing discs promotes tissue regeneration through the Yki:Tai transcriptional axis.

ID #4478
Identification and Characterization of Genes Essential for C. elegans Sperm Guidance
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Fertilization is the fusion of two gametes to initiate development of a new organism. In internally fertilizing animals, sperm must navigate the female reproductive tract to locate oocytes. *C. elegans* oocytes secrete F-series prostaglandins (PGFs) that guide sperm to the fertilization site. These PGFs are synthesized independent of the cyclooxygenase cascade, which is thought to be the sole enzymatic route for prostaglandin synthesis. However, we have shown that cyclooxygenase null mice and worms produce similar PGF isomers, suggesting that a second PGF synthesis pathway exists. The goal of my project is to identify genes critical for *C. elegans* sperm guidance and PGF metabolism. We have devised an *in vivo* screening method using RNAi and a sperm guidance assay. As the female germ line is the site of PGF synthesis, I focused on genes expressed in the adult germline (Reinke et al., 2004). N2 hermaphrodites were administered RNAi by the feeding method. L4 stage worms were added to RNAi plates and kept at 25ºC for 36 hours. RNAi hermaphrodites were anesthetized and incubated with wild-type males stained with MitoTracker Red CMXRos. Mated hermaphrodites were removed from males and allowed to rest for an hour. Fluorescent sperm distribution within the uterus was viewed on plates using a stereomicroscope. In controls, ~90% of sperm targeted the fertilization site. In mutants with sperm guidance defects, sperm were evenly distributed throughout the uterus. I have screened 223 germline genes on Chromosome II. Of these 223 RNAi clones, 38 cause strong sperm guidance defects. These clones correspond to genes implicated in diverse functions. I will validate a subset of these clones using mutational analysis. Liquid chromatography tandem mass spectrometry methods will be used to determine if the mutations impact PGF metabolism. My goal is to identify enzymes and other factors that modulate PGF levels in the germ line.

ID #4479 – Session 1: Transcriptional Regulation and Epigenetics
The regulatory role of Dpy30/Ash2L in epigenetic control of neural 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 closure defects (NTD). Ash2L morphant embryos show a wider neural plate associated with reduced expression of the dorsal neural marker Pax3 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.

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ID #4480

Precocious estrogen signaling during sex determination leads to persistent alterations in ovarian transcription in an environmental model of endocrine disruption, the American Alligator

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As part of the developmental origins of adult disease model, studies investigating the effects of environmental endocrine disruptors (EDCs) have helped elucidate the role of endocrine signaling in shaping the development of the reproductive system. Steroid hormones, particularly estrogens, are critical to sexual development in a broad array of vertebrate taxa, and studies in the American alligator have elucidated the consequences of perturbed estrogen signaling *in ovo* in the embryonic and adult gonad. We have previously reported that alligators from a site with high levels of estrogenic EDCs (AP; Lake Apopka, FL) display a broad suite of reproductive abnormalities, including persistent shifts in ovarian transcription. Because shifts are observed in animals raised from hatching in lab settings, their origins are putatively embryonic. We sought to probe the mechanism underlying these transcriptional changes by exposing alligator embryos from an non-contaminated reference site (WO; Lake Woodruff, FL) to either estradiol (E_2) or a non-aromatizable androgen (DHT) prior to sex determination, and then assessing ovarian transcription of key genes five months later. Comparing unexposed WO and natively-exposed AP animals reveals suppression of three aryl hydrocarbon receptor isoforms (*AHR*), estrogen receptor-β (*ESR2*), and anti-Mullerian hormone (*AMH*), in AP animals, which can be recapitulated by E_2 exposure in WO animals. Furthermore, when ovarian transcription is stimulated via exogenous follicle-stimulating hormone, altered responsiveness of *AHR1B* and estrogen receptor-α (*ESR1*) is observed. These effects appear to be mediated via *ESR1* signaling, as embryos exposed to an *ESR1*-selective agonist, but not an *ESR2* agonist, exhibit suppressed expression of *AHR1A* and *AMH*. Collectively, these findings implicate estrogens as a key regulator during development for ovarian function, and describe a means by which EDCs might contribute to altered reproductive health in adulthood.

ID #4481

Elucidating novel ciliary functions in mammalian development

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The primary cilium is a microtubule based cellular appendage, which over the last decade, has emerged as a vital component of embryonic development. It serves as a facilitator of key signaling events, during embryogenesis and continuing throughout development and adult homeostasis. Importantly, animals lacking cilia related genes exhibit embryonic lethality. Our group is particularly interested in identifying novel cilia-related genes that result in developmental defects leading to embryonic lethality. To accomplish this, we are using the bioinformatics algorithm, INDICiliATOR, which scores potential genes based on the likelihood they participate in ciliary biology using various data collected from cilia databases, transcriptomes and proteomes from a range of species. Using this approach, we identified several novel cilia candidate genes including: *Atxn10, Gdi2, KIAA0753*, and *Rab35*, each reported by the International Mouse Phenotyping Consortium (IMPC) to be homozygous lethal past early gestation. These carefully chosen genes are expected to play roles in lesser understood ciliary function, which could include: the determination of cilia length, ciliogenesis, or novel signaling pathways. We expect that embryonic phenotypes observed in these lines will exhibit more subtlety and specificity than previously described core cilia mutations, with lethality occurring past E9.0. *Atxn10* mutant embryos present with severe cardiac edema and irregular pharyngeal arch formation resulting in lethality by E11.0. Preliminary data has shown that both *KIAA0753* and *Gdi2* lines each exhibit noted variability in observed phenotypes and the time of lethality. To further address the importance of these genes in regulating cilia structure and function, we are generating CRISPR mediated knockout and overexpression cell lines for each gene. These cell lines will be used in future studies to understand the importance of each gene on cilia formation and function during embryonic development.

ID #4482 – Session 2: Organogenesis and Morphogenesis

Nuclear androgen receptor regulates testes organization and oocyte maturation in zebrafish

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Androgens act through the nuclear androgen receptor (AR) to regulate gonad differentiation and development. In mice, AR is required for spermatogenesis, testis development and formation of external genitalia in males and oocyte maturation in females. However, the extent to which these phenotypes are conserved in non-mammalian vertebrates is not well understood. Here, we generate zebrafish with a mutation in the *ar* gene (*ar*$^{uab105/105}$)and examine the role of AR on sexual determination and gonad development. We find that zebrafish AR regulates male sexual determination, since the majority of *ar*$^{uab105/105}$ mutant embryos developed ovaries and display female secondary sexual characteristics. The small percentage of mutants that developed testes displayed female secondary sexual characteristics, exhibited structurally disorganized testes and were unable to release or produce normal levels of sperm, demonstrating that AR is necessary for zebrafish testis development and fertility. In females, we find that AR regulates oocyte maturation and fecundity. *Ar*$^{uab105/105}$ mutant females developed ovaries filled primarily with immature stage I oocytes and relatively few mature stage III oocytes. Two genes whose expression is enriched in wild-type ovaries compared to testes (*cyp19a1a*, *foxl2a*) were upregulated in *ar* mutant testes, while two genes enriched in testes (*amh*, *dmrt1*) were upregulated in *ar* mutant ovaries. These findings demonstrate that AR regulates sexual determination, testes development and oocyte maturation and suggest that AR regulates sexually dimorphic gene expression.

ID #4483 – Session 1: Transcriptional Regulation and Epigenetics

A non-canonical role for Wnt16 in the Wnt signaling network involved in specifying and patterning the early anterior-posterior axis of sea urchin embryos

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In early development of the deuterostome sea urchin embryo, anterior-posterior (AP) specification and patterning depends on integrated information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways, forming an interconnected Wnt signaling network. We have previously shown that a non-canonical signaling pathway involving the Wnt receptor, Fzl1/2/7, antagonizes the progressive posterior-to-anterior down regulation 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. Yet, the exact mechanism by which Fzl1/2/7 signaling antagonizes the other Wnt signaling branches is unclear. Here, we show that maternal *wnt16* is expressed ubiquitously during cleavage stages and that zygotic *wnt16* expression is concentrated in the endomesoderm as early as the mid-blastula stage. We used morpholino and dominant negative interference approaches to analyze the function of Wnt16 during early AP specification and patterning. Our results indicate that Wnt16 antagonizes ANE restriction mediated by Wnt/β-catenin and Wnt1/Wnt8-Fzl5/8-JNK signaling and that this activity depends on a functional Fzl1/2/7 receptor. Our results also indicate that zygotic *wnt16* expression in posterior endomesoderm cells during gastrulation depends on both the Fzl5/8 and Wnt/β-catenin signaling. In addition, we demonstrate that while Wnt16 is unnecessary for the activation and/or maintenance of many genes in the endomesoderm GRN in posterior blastomeres at the beginning of gastrulation, it is essential for gastrulation. Together, our data suggest that Wnt16 activates the Fzl1/2/7 pathway that antagonizes the ANE restriction mechanism mediated by Wnt/β-catenin signaling and Wnt1/Wnt8-Fzl5/8-JNK signaling as well as a role in the morphogenetic movements of gastrulation.

ID #4484 – Session 2: Organogenesis and Morphogenesis
Hedgehog signaling initiates genital tubercle development
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Abnormalities of the external genitalia are among the most common birth defects in humans, affecting approximately 1/250 live births. Development of external genitalia begins with the emergence of paired genital swellings on either side of the embryonic cloaca. The genital swellings then merge to form the genital tubercle, the precursor of the penis and clitoris. Interactions between the endodermal urethral plate epithelium and the surrounding mesenchyme coordinate outgrowth and patterning of the external genitalia.*Sonic hedgehog (Shh)* is expressed in the urethral epithelium and plays an essential role in the growth of the genital tubercle. Deletion of *Sonic hedgehog* results in absence of external genitalia in mice, although Shh mutants still form the initial paired genital swellings, indicating that Shh is not required for initiation of genital outgrowth. In a characterization of the transcriptome of the urethral plate epithelium, we found that *Indian hedgehog (Ihh)*, another hedgehog family member, is co-expressed with *Shh.* To determine if *Ihh* could compensate for the loss of *Shh* to promote initiation of genital swellings in *Shh* mutants, we conditionally deleted both *Ihh* and *Shh* in mice. Using 3D imaging (nanoCT) and cell lineage analysis, we found that the double knockout had a more severe genital phenotype than the *Shh* or *Ihh* mutants. Specifically, we show that *Shh;Ihh* homozygous conditional knockouts fail to initiate genital budding and have an expanded cloaca. Comparison of gene expression in *Shh/Ihh* single and doublemutants shows that *Ihh* can partially compensate for Shh to activate the genital outgrowth circuit. Together these results indicate that coordinated activity of Shh and Ihh is required for initiation of external genital development in mice.

ID #4485
An RNA binding protein link to human intellectual disability alter m6A RNA methylation
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Epigenetic modifications of RNA have been shown to be crucial for regulating gene expression and for our understanding of the etiology of certain diseases. The most prevalent RNA modification, N^6-Methyladenosine (m^6A) is an abundant and reversible post-transcriptional modification linked to RNA splicing, translation, and turnover. Although m^6A influences post-transcriptional fate of mRNAs, the precise role of how m^6A regulate neuronal function and brain development is unclear. Loss of the single *ZC3H14* gene, which encodes an evolutionarily conserved polyadenosine RNA binding protein, leads to a form of inherited autosomal recessive intellectual disability in humans. Loss of the ZC3H14 ortholog, dNab2, within *Drosophila* neurons impairs behavior, short-term memory, and sex bias towards females. Intriguingly, these mutant fly brains exhibit longer poly (A) tails and increased levels of m^6A as compared to control flies. m^6A is required for female-specific splicing of *sex lethal* (Sxl), which determines whether the fly develops as a male or female. Biochemical data shows dNab2 mutant females have low protein expression of female specific sex lethal protein but show elevated expression of the male specific isoforms of Sxl mRNA transcripts compared to dNab2 mutant male flies. These preliminary data led us hypothesis that dNab2 plays a role in m^6A RNA methylation and splicing to regulate neuronal function and sex termination in the dosage compensation pathway of *Drosophila*.

ID #4486

A Yorkie-inhibitory checkpoint downstream of the tumor suppressor dFbw7

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The Hippo signaling pathway is a central regulator of organ growth and tissue homeostasis and is highly conserved between *Drosophila* and mammals. It regulates growth by integrating intrinsic and extrinsic cues to limit nuclear import of the oncogenic transcriptional co-activator Yorkie. In the absence of inhibitory signals, Yorkie enters the nucleus and promotes a transcriptional program of cell proliferation and survival. While the role of the Hippo pathway in growth control is fairly well defined, less is known regarding the extent to which other pathways and checkpoints also impinge on its regulation of Yorkie. Here we describe evidence of an anti-growth checkpoint linking Yorkie and the conserved tumor suppressor dFbw7 (archipelago). dFbw7 is the substrate adaptor of SCF-type E3 ubiquitin ligase that stimulates turnover of Cyclin E and dMyc. Thus, in most contexts, loss of dFbw7 leads to tissue hyperplasia caused by dMyc and Cyclin E hyper-accumulation. However, we find that loss of dFbw7 in a small group of cells in the developing wing epithelial sheets leads to paradoxical undergrowth that is associated with reduced Yorkie protein levels and activity. These effects are phenocopied by overexpression of Cyclin E, which appears to inhibit Yorkie by triggering its endolysosomal routing and turnover. Additional genetic interactions between *dFbw7* and Hippo components provide further support for a Yorkie-inhibitory checkpoint engaged in dFbw7-deficient cells that slows growth and elevates apoptosis. Insight into the functional relevance of this checkpoint could shift current research paradigms and offer potential therapeutic opportunities to the large cohort of Fbw7-deficient tumors in humans. Current work is focused on defining the relationship between dFbw7, Cyclin E, and Yorkie trafficking and turnover in wing disc cells.

ID #4487

Are You What Your Mother Ate?: Early Embryo Environment Shapes Adult Phenotype

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Common daily environmental exposures, such as heavy metals, airborne pollutants, and pesticides, can shape embryonic development and alter adult phenotype. Long-term consequences of sub-lethal environmental exposures are often unknown. The round worm *C. elegans* is an excellent model organism to investigate how embryo environment shapes the adult phenotype. To determine developmental windows when environmental exposures act to alter adult phenotype, we exposed *C. elegans* to sub-lethal doses (0.1-100 μM) during specific larval stages or at adulthood and measured changes in growth, fertility, and energy balance. Environmental exposures include: estrogen mimics Bisphenol A (BPA) and diethylstilbestrol (DES), metals and metalloids (cadmium Cd, arsenic As), biocides (triclosan TRI, tributyltin TBT, fenthion FEN) and airborne pollutants (cigarette smoke CSE, nicotine NIC, benzo-α-pyrene BAP). Some environmental exposures stunted growth (Cd, BAP, TBT), impaired sexual maturity and fertility (Cd, As), while others increased egg-laying (NIC, BPA). Notably, reproductive fitness and longevity were intertwined because hatching and embryo viability were greater in eggs laid early, albeit these parents died sooner than late layers. Most environmental exposures shifted energy balance and altered lipid storage, but only in offspring, while BPA also affected adults. In mammals, environmental exposures are suggested to affect embryogenesis and alter adult phenotype via nuclear hormone receptor (NHR) signaling, specifically PPARγ. *C. elegans* and other nematodes have an exceptionally expanded NHR gene family (~300 vs. ~40 in mammals) but identification of PPARγ homolog proves challenging. Consequently, we created a combined, "double bait" domain homology identification program to detect candidate PPARs in nematode genomes and found strong candidate homologs of PPARγ in worms, with a candidate signaling akin to PPARα, and candidates were validated using RNAi. Funding: Impact Assets.

ID #4488

Growth control by the conserved Aac11/Api5 anti-apoptotic protein

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The *Drosophila* gene *anti-apoptotic clone 11* (*Aac11*), also referred to as *Api5*, encodes a conserved nuclear scaffolding protein that promotes survival of mammalian cells under conditions of serum deprivation and E2F-overexpression, and is frequently overexpressed in human cancer. How Aac11 exerts these effects is not known. Using the developing imaginal discs as a model, we find evidence that Aac11 acts as a pro-growth factor *in vivo*. RNAi depletion and FLP-FRT clonal analysis show that Aac11 is autonomously required for adult organs to achieve their proper size. Transgene driven ectopic expression of Aac11 reciprocally promotes enlarged adult wings. At a molecular level, we find that Aac11 binds to the nuclear hormone receptor coactivator Taiman in a hormone-dependent manner. Aac11 is genetically required for adult overgrowth phenotypes produced by over-activation of the Hippo pathway component Yorkie (Yki): Yki-induced organ enlargement is suppressed by either knocking-down Aac11 or expressing a dominant-negative form of Aac11 with missense mutations in a conserved nuclear hormone receptor binding motif. This growth suppression is not correlated with impaired induction of canonical Hippo-responsive genes but with suppression of a distinct pro-growth program of Yki-induced/Aac11-dependent genes, including the germline stem cell factor *piwi*, the insulin-like peptide 8 (*dilp8*), etc. Use of a temperature sensitive Gal80 transgene to map the temporal requirement for Aac11 indicates that it supports post-mitotic wing growth during the L3-prepupae time interval that coincides with pulses of the steroid hormone ecdysone. Taken together, the data support a role of Aac11 as a steroid inducible-modulator of growth pathways.

ID #4490

Environmental toxicants may alter development via putative PPAR homologs in C. elegans

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Several environmental teratogens, from endocrine disrupting chemicals, like bisphenol A, to combustion pollutants, like cigarette smoke, have been shown to increase alter the expression nuclear hormone receptor PPARγ *in vitro* and *in vivo*. In some model organisms, PPARs are expressed during embryogenesis, posing the possibility that it may function during early development to alter lipid metabolic pathways and ultimately adult phenotype. upon. Given PPARs are involved in many physiological processes, such as reproduction, growth, and energy regulation, the search for functional homologs in other model organisms, such as *C. elegans*, will increase our understanding of its mechanistic role in developmental exposure to environmental teratogens. Environmental exposures are suggested to affect embryogenesis and alter adult phenotype via nuclear hormone receptor (NHR) signaling, specifically PPARγ. Nematodes, such as *C. elegans*, have a greatly expanded NHR gene family, with over seven times the amount of members (~300 vs. ~40) but identification of PPAR homologs, especially PPARγ has been difficult. We hypothesized that a combined domain analysis may detect *bona fide* functional *C. elegans* homologs to human PPARs using bioinformatics. Using this novel "double bait approach" we detected 12 putative candidates for PPARs, with 3 robust candidates for PPARγ. As second bioinformatics approach, using transcriptome data based upon RNA sequencing experiments, we found that putative PPARs are expressed at different stages of the *C. elegans* life cycle. Finally, RNAi knockdown experiments at specific development stages to confirm homolog predictions upon adult and offspring reproduction, growth, and energy regulation. Funding: Impact Assets.

ID #4491

The Drosophila small ovary (sov) gene is differentially required for oogenesis and embryonic centrosome function

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In the germline stem cell niche, cues for stem cell renewal or differentiation arise either cell autonomously or from neighboring somatic niche cells. The *Drosophila* female germline provides an excellent example of this mechanism, as loss of somatic cues leads to deleterious errors in germline development. Here, through clonal knockdown approaches, we demonstrate that the previously uncharacterized *Drosophila small ovary* (*sov*) gene, or *CG14438,* is differentially required in somatic and germline lineages during female germline development. Sov, a nuclear protein with 26 predicted C_2H_2 zinc finger domains, can be found in the somatic escort cells and follicle cells of the germarium. Consistent with its potential role in the piRNA pathway, depletion of Sov leads to transposon desilencing and subsequent apoptosis of somatic cells. As a result, due to impaired Bam signaling and cyst encapsulation by follicle cells, depletion of Sov results in massive tissue degradation, ovarian tumors, and failed oogenesis. Moreover, while Sov depletion in the germline permits oogenesis, the resulting embryos exhibit numerous defects. Consistent with a role in germline development, Sov-depleted embryos produce fewer primordial germ cells. In addition, Sov-depleted embryos display increased nuclear fallout, dysmorphic nuclei during metaphase/anaphase, and aneuploidy, all ultimately resulting in embryonic lethality within the first hours of embryogenesis. To uncover the mechanism underlying these defects, further analysis confirmed that Sov depletion results in centrosome disorganization, detachment, and mispositioning. Taken together, these data suggest that Sov maintains embryonic viability through centrosome regulation.

ID #4492

Determining the role of ldlrap1a in skeletal development and 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 blood stream. Humans with mutations in LDLRAP1 have familial hypercholesterolemia, an autosomal recessive inherited disorder, resulting in abnormally high levels of blood lipoproteins. Evidence exists that LDLRAP1 may also function in chondrocytes, and so we hypothesize Ldlrap1a may also be involved in skeletal development or another cellular function in cartilage. Using CRISPR-Cas9, we generated a zebrafish mutant line for *ldlrap1a*. We have performed in-crosses with a genotyped F_2 *ldlrap1a* generation to generate F_3 embryos. Sequencing of these F_3 mutant embryos revealed a 7bp deletion in exon 3. This causes a frameshift resulting missense and a premature stop codon 37bp into exon 3. We predicted we would observe skeletal defects in mutants, but analysis of F_3 offspring revealed no obvious skeletal defects. This has led us to study the possibility of maternal "rescue" of larval phenotypes by the normal maternal transcripts and proteins. We will cross a homozygous female *ldlrap1a* mutant with a heterozygous male to generate mutants with no functional maternal Ldlrap1a. Since LDLRAP1 has an important role in lipid metabolism in humans, we expanded our study to look at the effects a mutated *ldlrap1a* gene has on cholesterol metabolism in zebrafish. We hypothesize, as in humans, Ldlrap1a functions in zebrafish to clear blood lipoproteins. We plan to do a heterozygous in-cross with the 7bp deletion line. The larvae will receive a high cholesterol or control diet from 4.5dpf until 9.5dpf, and then they will undergo an Oil Red O stain. We predict the zebrafish will exhibit lipid accumulation indicating a function of *ldlrap1a* in cholesterol clearance from the bloodstream. If zebrafish have a lipid clearance defect, this line could be a useful model to study hypercholesterolemia in humans.

ID #4493

Identification of candidate ATP synthase subunits homologs and their phenotypic affects across developmental stages of C. elegans and C. briggsae

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ATP synthase is the critical molecular motor that generates the majority of energy for the majority of organisms. The complete genetic components encoding most of the subunits for this smallest molecular motor, which constitute ATP synthase, are still unknown for most nematodes, including *C. elegans*. To identify homologs for putative subunits of ATP synthase, we compared human sequence to both *C. elegans,* and a related nematode species, *C. briggase.* Usingbioinformatics analysis, our refined homolog searchidentified candidates for many of the previously unidentified 13 components of ATP synthase: Epsilon subunit, alpha subunit, Assembly factor 2, C1 subunit, C2 subunit, C3 subunit, Gamma subunit, D subunit, Delta subunit, F subunit, E subunit, A6L subunit, S subunit. To predict the potential physiological and phenotypes the subunits may affect, we analyzed transcriptome obtained from RNA sequencing using both worm species collected across various stages of the life cycle. Synchronized populations of *C.elegans* and *C. briggsae* were collected at different developmental stages throughout the life cycle using microscope to pick the same number of worms. Gene expression profiles often varied between the two species, *C. elegans and briggsae* across stages. For example ATP5H homolog was expressed in *C. elegans* but not *C. briggsae*, whereas ATP5G3 expression peaked in *C. briggsae* at L2-L3 but was not expressed in *C.elegans*. ATP5J2 gene, which encodes subunit F, had highest expression at larval stages in *C. briggsae* but was expressed at adult stages in *C. elegans*. Ongoing experiments use RNAi to confirm these stage-specific putative ATP synthase components using our Integrated Health Phenotyping (IHP) screen with both *C. elegans* and *C. briggsae* upon energy regulation, growth, and reproduction phenotypes, which are commonly affected when ATP synthase is inhibited. Funding: Impact Assets.

ID #4494

Viktor Hamburger & Planarian Regeneration: Peer Education in an Elementary School Setting

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Science, technology, engineering, art and mathematics (STEAM) education is a current focus in K-12 educational settings to improve participation and cultivate future generation in critical and analytical thinking. While a variety of approaches can be integrated in the elementary school setting, we used a peer mentoring in an experiential setting of the daily science classroom to achieve greater understanding of basic scientific principles, such as regeneration, in young children. Another goal this peer learning activity was to reinforce the scientific concepts of making a hypothesis, and the use of experimental design and control, to answer the question posed by the hypothesis. The primary author, a 8-year-old student, taught her peers (children ranging from 3-9 years of age) to understand the principle of regenerative biology by repeating the experiments of Viktor Hamburger and extending this original research by conducting a group experiment on to test two hypothesi: 1. complex body parts, such as a head, vs. more than simple body parts, like a tail, will take longer time to regrow 2. It will take longer time to regrow multiple body parts than just one body part. Methods. Environment: the classroom, level C, was a mixed age, 7-9 years plus 4 year old, in their school science room, where provided a dissecting stereomicroscope, jars, paint brushes and planria (species). Next she gave a short lecture on regeneration, Dr. Viktor Hamburger, and a summary on his work with planaria. She explained the two hypothesi and taught the proper use of the equipment. All students participated in generating specimens for the experimental and control groups. The outcome was measured by students recalling both orally and in written assessment the scientific concepts of hypothesis, experiment, control, instruments, observation. Overall, the students had greater recall accuracy and retention in questions regarding these concepts more than other lessons taught in the academic year.

ID #4500

Characterization of PTHCre;R26iMyc Mice Through Parathyroid Growth

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Parathyroid glands are responsible for the regulation of serum calcium homeostasis. Parathyroid hormone (PTH), secreted from parathyroid glands, plays an important role in the constant exchange of calcium between the blood and bone tissues. Hyperparathyroidism, usually resulting from parathyroid adenoma, occurs when the parathyroids produce an excessive amount of PTH. In this study we generated a *PTHCre;R26iMyc* mouse model in which parathyroid cell proliferation was increased. Expression of Cre in parathyroid cells leads to *Myc* overexpression, therefore causing an increase in proliferation. This initial study observed the phenotypic effects resulting from an induction of Myc in parathyroid cells: large parathyroids, cell-type abnormalities, poor survival rate, skeletal abnormalities, and improper separation of the parathyroid from the thymus. After characterizing this mouse through parathyroid growth, this preliminary data will determine if this model will be appropriate for future studies concerning parathyroid fate and instability.

ID #4503

Differential Expression of GAD Genes in the Interneurons of the Zebrafish Spinal Cord

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Glutamic acid decarboxylase (GAD) is the enzyme responsible for the synthesis of GABA, the main inhibitory neurotransmitter of the central nervous system. Most vertebrates have two GAD genes, each of which encodes for their respective GAD enzyme; *GAD1* encodes for the GAD67 protein, while *GAD2* encodes for the GAD65 protein. The current understanding in the field is that *GAD1* and *GAD2* are co-expressed in nearly all vertebrate GABAergic neurons but differ in their contribution to total GABA production. Work from our lab has shown that there are at least three GAD genes in zebrafish – *gad1a*, *gad1b* (previously *gad1*), and *gad2*. Additionally, these GAD genes seem to be differentially expressed in the zebrafish spinal cord at one day post-fertilization. There are four subclasses of GABAergic interneurons, termed DoLA, CoSA, VeLD, and KA, that are positioned along the dorsal-ventral axis of the spinal cord. Our work shows that *gad1a* appears to be expressed in the more dorsally-located DoLA and CoSA interneurons, while *gad1b* and *gad2* appear to be co-expressed in the more ventrally-located VeLD and KA interneurons. Thus, we are interested in determining the identities of the GAD-expressing interneurons at this stage. To this end, we have chosen markers for each interneuron subclass in the developing zebrafish spinal cord and have performed whole mount double fluorescent *in situ* hybridization to determine the identities of the interneurons which express *gad1a*, *gad1b*, and *gad2*. Presently, we have determined that *gad1b* is expressed in KA interneurons and are working to determine which subclasses express *gad1a* and *gad2*.

ID #4507

Characterizing TRKIN: A processive minus end-directed kinesin-14 motor involved in maize meiotic drive

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Originally discovered 75 years ago, maize Abnormal Chromosome 10 (Ab10) lines display meiotic drive where the segregation ratios of alleles linked to chromosome knobs are significantly altered from the expected 50:50. It is estimated that meiotic drive in maize has had evolutionary consequences on a genome-wide level by affecting segregation of alleles near knobs. The cytological cause of meiotic drive is neocentromere activity during female meiosis where knobs are directed towards the poles and arrive before centromeres. During female meiosis in plants, only the single lowest of the four cells survives and neocentromere activity favors the knob-containing chromosome to migrate into what will become the functional megaspore. Knobs are composed of two distinct tandem repeat arrays termed knob180 and TR1. We recently showed that Ab10 encodes a kinesin-14 protein family called KINDR that localizes only to knob180 neocentromeres during meiosis. TR1 repeats undergo dramatic neocentromere activity but their motility cannot be explained by KINDR. Here we report the identification of a new kinesin-14 protein, TRKIN (TR1 KINESIN) that is encoded on Ab10 and another chromosome 10 haplotype, K10L2 that also contains TR1 neocentromeres. TRKIN lacks an important conserved motif within its motor domain that is required for microtubule-binding and contains a long 68 amino acid extension C-terminal to its motor domain with a predicted NLS. In vitro, GFP-TRKIN is a minus end-directed processive kinesin motor and the C-terminal extension greatly enhances its microtubule-binding capacity. In maize protoplasts, RFP-TRKIN often shows nuclear localization and several nuclear puncta. TRKIN physically interacts with DNA probes made from TR1 but not knob180 sequences in vitro by EMSA. We currently have an anti-TRKIN antibody in production to observe protein presence and localization in vivo and a *Trkin* Cas9/gRNA transgenic line in production to test if *Trkin* is necessary for meiotic drive.

ID #4509

Using GFP+-GFP- chicken chimera to test a neural crest contribution to taste buds

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Taste cells, the chemoreceptors in taste buds, are specialized cells that transduce gustatory stimuli into neural signals which are conveyed to the central nervous system for the sensation of taste. Taste cell origin and differentiation are fundamental issues for the development of taste organs and taste function. We have recently found species-specific distribution of labeled cells in taste buds in SOX10-Cre mice, a model that specifically labels neural crest lineage in early embryos. In mice there were abundant SOX10-Cre labelled cells, most likely neural crest-derived, within taste buds while in zebrafish, SOX10-Cre labeled cells were not observed within taste buds. Therefore, to get an evolutional insight of this observation, we used a GFP and non-GFP chicken chimera to test the potential neural crest contribution to taste buds in chickens. To generate chimeras, neural fold that contains the progenitors of neural crest from a GFP chicken embryo was dissected and transplanted to a regular non-GFP chicken embryo. This allows us to solidly trace the lineages of neural crest. Embryonic (E) tissues were harvested at E2, E7, E14 and E20.We found that (1) transplanted GFP^+ neural fold were successfully integrated into the host chicken embryo; (2) transplanted GFP^+ neural fold cells were expanded and migrated into the target tissue regions, i.e., primordia of upper beak and lower beak where gustatory tissue developed; (3) GFP^+ cells were extensively distributed in the mesenchyme underlying the epithelium, but no GFP^+ cells were observed within taste buds. Our data suggest that the GFP-chicken chimera model is perfect for long-term lineage tracing studies and there is no contribution from neural crest to chicken taste buds.

ID #4510

Cdc42 effector protein 3 regulates somite segmentation through inhibiting Cdc42 activity

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Tissue rearrangements and cell migration are critical during embryogenesis. Somitogenesis is one process that involves tightly controlled cell shape changes and tissue rearrangements. In addition to the segmentation clock that controls the timing of somite segmentation, we identified a cytoskeletal player, Cdc42 effector protein 3 (CEP3), that is required for somite segmentation per se. CEP3 is specifically expressed in somite tissue in *Xenopus* embryos. Loss-of-function experiments showed that CEP3 is not required for the specification of presomitic mesoderm (PSM), nor the differentiation of muscle cells, but is required for the segmentation process. During somitogenesis, PSM cells will first be mediolaterally elongated, then clefts separating the newly formed somite from the presomitic mesoderm will become visible, and finally the cells will change their shape again and orient along anterior-posterior axis. Live imaging analysis revealed that when CEP3 is knocked down in cells, they adopt a less elongated shape. Despite some differences in cell shapes at different axial levels, cells remain in a continuous sheet. We further showed that the activation of Cdc42 is maintained near the cell membrane in these cells, suggesting that CEP3 negatively regulates the activity of Cdc42. Although there is not a complete mesenchymal-epithelial transition (MET) process in *Xenopus* somitogenesis, our results suggest that Cdc42 also plays a role in the segmentation process, and CEP3 is required to turn down Cdc42 to allow somite separation.

ID #4511

Type I receptor ALK3-mediated BMP signaling in the tongue mesenchyme is required to activate Wnt-ß catenin for the formation of taste papillae.

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The development of taste papillae requires mesenchymal-epithelial interactions via multiple molecular pathways, including bone morphogenetic protein (BMP) signaling. In the BMP signaling cascade, type I receptors (ALK2, ALK3, ALK6) are the main determinants of downstream signaling specificity. Our studies have demonstrated that ALK2 mediatedBMP signaling in tongue mesenchyme plays an important role in regulating tongue shape and size. Here we report that ALK3mediated BMP signaling in tongue mesenchyme exerts important roles in the development of taste papillae. We used transgenic mouse models to constitutively activate (*ca*) or conditionally knock out (*cKO*) the *Alk3* receptor in a mesenchyme-specific manner using *Wnt1-Cre.* At E12.5, when Shh^{+} taste papilla placodes normally emerge, taste papilla placodes were absent in the *Wnt1-Cre/Alk3 cKO* tongue. In contrast to *Wnt1-Cre/Alk3 cKO*, *Wnt1-Cre/caAlk3* mutants did not possess obvious changes in papilla pattern. Our data indicate that high levels of ALK3-BMP signaling is needed for the formation of taste papillae. Tongue organ cultures with Wnt-β catenin signaling activator LiCl showed development of taste papillae in the *Wnt1-Cre/Alk3 cKO* tongue. Our data suggests that ALK3-BMP signaling in tongue mesenchyme activates Wnt-β catenin signaling for taste papilla formation. Further studies are ongoing to explore the mechanism by which ALK3-BMP signaling plays its role in the formation of taste papillae.

ID #4513

Towards CRISPR-Cas9 epigenome editing of the Endo16 enhancer in the sea urchin Lytechinus variegatus.

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The developmental gene regulatory network (GRN) of the sea urchin is well-studied, primarily by knock-downs of transcription factors and so the urchin *Lytechinus variegatus* is well suited to study the functional consequences of direct manipulation of gene expression in cis.

Previous work has identified the knockdown phenotype and both cis- and trans- acting regulators of the endodermal marker *Endo16*. Module "A" of the *Lv-Endo16* promotor is an evolutionary conserved module required for its expression.

We used the CRISPR-Cas9 system to modify the *Lv-Endo16* promoter module A, resulting in a morphologically similar but more severe phenotype than the previously published *Endo16* translation-blocking morpholino. This manipulation will serve as a positive control for silencing the enhancer. We will next validate the molecular phenotype with qPCR and in situ hybridization.

Using these validated guides, we will next attempt to silence *Lv-Endo16* expression with CRISPR-interference by using a deactivated Cas9 fused with a repressor protein called KRAB which condenses the chromatin, making the locus inaccessible to transcription factors. To our knowledge, this technique has not yet been used in invertebrates. When fully developed, CRISPR-i can be used to regulate genes temporally and to explore cis-regulatory architecture and evolution.

ID #4514

Surrounding Progenitors Contribute Differently to Distinct Phases of Taste Bud Development

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Taste buds (TBs) emerge in late embryos (formation phase), and postnatally continue to mature until a taste pore forms (maturation phase), after which they undergo continuous maintenance (maintenance phase). Based on these different phases of TB development (i.e., initial formation, maturation, and maintenance), we examined the commonly held belief that TBs have a sole origin, the surrounding epithelium. We used *K14-Cre* crossed with a nuclear tdTomato to eGFP switch Cre-reporter (nTnG) to label basal epithelial cell lineage, and found that none of the developing TBs appear to be labeled by K14-Cre until embryonic day (E) 18.5, while labeled cells were frequently seen in TBs at birth. By 1w, the number of labeled cells had increased significantly, and at 2w labeling was extensive. After 4w, when TBs are mature and undergo continuous turnover, TBs were almost fully labeled. *Dermo1-Cre/nTnG* mice, labeling mesenchymal cell lineage, were examined as a potential alternative progenitor source, since embryonic TBs did not apparently possess K14 lineage. Interestingly, *Dermo1-Cre*-labeled cells were rarely observed at 1d and 1w, ruling them out as a putative progenitor population for initial development of TBs. However, at 2-4w, labeled cells were seen within many TBs. By 8w, *Dermo1-Cre*-labeled cells were abundant in the majority of TBs. Our data indicate that 1) $K14^+$ epithelial cells contribute to the maturation and maintenance, but not initial formation, of TBs; 2) *Dermo1-Cre* labels a unique population of mature TB cells implicating the presence of $Dermo1^+$ precursors for TB cell renewal. These results suggest that distinct progenitor niches contribute to different aspects of TB formation, maturation and maintenance.

ID #4515

DEVELOPMENT OF SEXUALLY DIMORPHIC ANATOMY IN ANOLE LIZARDS

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Species vary widely in their pattern and magnitude of sexual dimorphism, yet the proximate mechanisms that regulate these differences remain poorly understood. Sexually dimorphic characters present special challenges to our understanding of anatomical evolution because the sexes share the majority of their genomes yet can diverge in size, shape, and anatomical characters during development. Lizards in the genus *Anolis* (anoles) are an often-used model for evolutionary studies of sexual dimorphism and are also an emerging model for comparative developmental analyses. An example of a sexually dimorphic trait in *Anolis* is the dewlap, a colorful throat fan that is usually larger in males than in females and is used frequently by males(butinfrequentlybyfemales) duringcourtship and aggression. Located on the throat and extending down the belly, the dewlap is supported by the second ceratobranchial (C2) cartilage in the hyoid system. We investigated the developmental basis of the sexually dimorphic dewlap apparatus in anoles and identified differences in C2 development between males and females that suggest sex-specific regulation of early skeletogenesis.

ID #4516

SOX10+ Cells Are Progenitors of a Population of Special Taste Bud Cells That Are K8-Low

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In our recent studies using a mouse model, *SOX10-Cre*, to trace migrating neural crest lineages, labeled cells were found within mature taste buds (TBs) in adult mice. In the present study, we aimed to (1) define the time window when *SOX10-Cre*-labeled cells emerge in TBs, (2) characterize the properties of *SOX10-Cre*-labeled TB cells, and (3) explore the *SOX10*-expressing cell niches that contribute to TBs. The distribution of *SOX10-Cre*-labeled cells in neural crest and TBs was analyzed at different stages (E8.5, P1d, 2 wk, 4 wk, 8 wk) by crossing with a tdTomato (RFP) Cre reporter. We found that *SOX10-Cre*-labeled cells were abundant in the connective tissue at all postnatal stages. At P1d, *SOX10-Cre*-labeled cells were observed in fungiform taste buds but absent in circumvallate taste buds. By 2 wk, *SOX10-Cre*-labeled cells were frequently observed in TBs. In mature TBs at 4 wk and in adult mice (8 wk and 16 wk), *SOX10-Cre* labeling was abundant and consistent among TBs in all three types of lingual taste papillae, as well as the soft palate, and labeled cells co-localized with cell markers of Type I, II, and III TB cells. Intriguingly, *SOX10-Cre*-labeled cells within TBs were not apparently labeled by keratin 8, a widely used marker for differentiated TB cells. Cre immunosignals were specifically distributed in migrating neural crest cells in E8.5 embryos, and quantitative RT-PCR analysis showed low Cre expression in tongue epithelium and connective tissue at 2 wk, but was negligible in adult tongue tissues of *SOX10-Cre* mice. Together, our data indicate that *SOX10*-expressing cells serve as precursors for TB maturation and homeostasis and contribute to a unique population of TB cells. Further studies are ongoing to define the *SOX10*-expressing cell population that contributes to TBs, likely from neural crest, TB, or TB-surrounding cells, or some combination of the three.

ID #4517

Analysis of mouse spermatogenic stem cell division dynamics.

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Stem cells are responsible for the replenishment of cells within adult tissues. In the murine testis, remarkably few stem cells generate millions of gametes. This process of spermatogenesis requires an exponential amplification and complex series of differentiation to create thousands of highly specialized sperm cells from a single stem cell division. Unlike other tissues, the turnover rate of the spermatogenic stem cell product is inherently dynamic and varies based on mate availability amongst other factors. Our lab studies the factors that regulate stem cells, specifically the mechanisms regulating stem cell division frequency in response to mating. We've previously shown that the Drosophila male increases the rate of germline stem cell division when repeatedly mated and are now studying if the same effect exists in mammals. However, the rarity of mouse spermatogonial stem cells (SSC), and lack of an apparent niche, makes the in vivo study of cell cycle dynamics difficult. Our initial approach focused on fluorescent microscopy. Male LT-11 mice, transgenic for Id4-GFP, one of several spermatogonial stem cell markers, were either allowed to mate ad libitum, or remain celibate for 20 days, with new females introduced daily. Testes were harvested and fixed for cryosectioning followed by staining with anti-Gfrα1 which labels early stage germline cells and anti-Ki67, to label cycling cells. Counts of GFP/Gfrα1 positive cell revealed 41% of the SSCs were in cell cycle in both mated and unmated mice. Thus no change in the percentage of quiescent cells is induced in mated mice. Future experiments will focus on whether those cells that do cycle, display a faster cycling rate. For this,we are mostly using flow cytometric analysis. We are currently working to dissociate the testis and generate a hoechst profile to assess whether mating can shift cycling cells from G1 to S-G2 or detect the percentage of stem cells that incorporated edu.

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