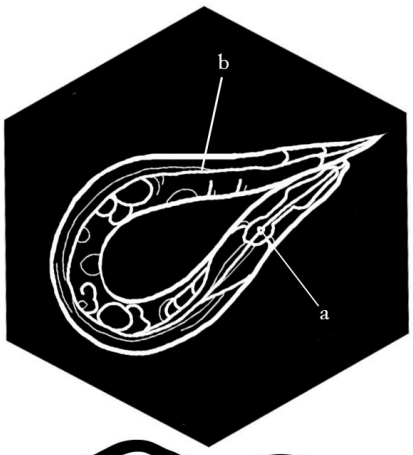
May 18th
to
May 20th



KENNESAW STATE UNIVERSITY®

Society for Developmental Biology

2017 Southeast Regional Meeting

Advancing
the field of
Developmental Biology

Meeting At-A-Glance

Thursday, May 18th
Registration open from 2:30 - 11:00 PM
Atrium open for poster setup at 3:00 PM

3:00 - 6:00 PM	Grant Writing Workshop	Clendenin 1008
6:00 - 6:15 PM	Welcome / Remarks	Social Sciences 1021
6:15 - 7:15 PM	Keynote Address - Mary C. Mullins	Social Sciences 1021
7:15 - 11:00 PM	Dinner / Poster Session I	Science Laboratory Atrium

Friday, May 19th
Registration open from 8:00 AM - 8:00 PM
Coffee Available in Social Sciences Atrium

8:00 - 9:30 AM	Session 1	Social Sciences 1021
9:30 - 10:00 AM	Coffee Break	Social Sciences Atrium
10:00 - 11:30 AM	Session 2	Social Sciences 1021
11:30 - 2:00 PM	Lunch / Poster Session II	Science Laboratory Atrium
	Vendor Exhibits Open	Clendenin 1007
2:00 - 3:30 PM	Session 3	Social Sciences 1021
3:30 - 4:00 PM	Coffee Break	Social Sciences Atrium
4:00 - 5:30 PM	Session 4	Social Sciences 1021
6:15 - 7:15 PM	Keynote Address - Chris V. Wright	Social Sciences 1021
7:30 - 11:00 PM	Dinner / Entertainment	Science Laboratory Atrium

Saturday, May 20th
Registration open from 8:00 AM - 12:00 PM
Coffee Available in Social Sciences Atrium

8:00 - 9:30 AM	Session 5	Social Sciences 1021
9:30 - 10:00 AM	Coffee Break	Social Sciences Atrium
10:00 - 11:30 AM	Session 6	Social Sciences 1021
11:45 AM - 12:00 PM	Awards Announcement / Dismissal	Social Sciences 1021

CAMPUS MAP


Kennesaw State University
Kennesaw Campus
visit the website at www.kennesaw.edu/mobile
Kennesaw State University is a Smoke/Tobacco-Free University
North
Check-in Thursday, 5:00-6:00
Main and
Keynote Sessions
Parking Available,
Second Floor and Above,
Thursday, Friday, and Saturday
Check-in Thursday, 2:30-5:00
Poster Sessions
Grant Workshop
KSU Place Apartments
North Deck
Technology Services
University Village Suites
University Village Apartments
Challenge Course
Jolley Lodge Lot
Jolley Lodge
Central Deck
Zuckerman Museum
Bailey Performance Center
Wilson Annex
Wilson
Music
Prillaman Health Sciences
Visitor Lot
Visual Arts
The Commons
Garden
Bagwell Education Building
Burruss
English
Legacy Gazebo
Social Sciences
University College
Carmichael Student Center
Kennesaw Hall
Campus Green
Pilcher
Sturgis Library
West Deck
Public Safety
Tech Annex
Math & Statistics
Office Annex
Visitor Info & Lot
Siegel Student Recreation & Activities Center
Plaza
Convocation Center
Arboretum
Science
Clendenin
Science Laboratory
East Deck
Church Lot
West Lot
South Housing Lot
Softball Field
Bailey Athletic Complex
Baseball Field
East Lot
Austin Residence Complex
Practice Field
Austin Residence Lot
KSU Center
Owl's Nest
Sports & Recreation Park
Perch
Fifth Third Bank Stadium
Town Point
Admissions
Town Point Lot
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MASTER OF SCIENCE
IN INTEGRATIVE BIOLOGY

KENNESAW STATE UNIVERSITY
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Meeting Program

Thursday, May 18th		
Registration open from 2:30 - 5:00 PM, Clendenin Atrium Registration open from 5:00 - 6:00 PM, Social Sciences Atrium		
Science Laboratory Atrium open for poster setup (Poster Session I) at 3:00 PM		
3:00 - 6:00 PM	Grant Writing Workshop	Clendenin 1008
Walk to Social Sciences Building		
6:00 - 6:15 PM	Welcome/Remarks	Social Sciences 1021
6:15 - 7:15 PM	Keynote Address - Mary C. Mullins	Shaping and Signaling Mechanism of a BMP Morphogen Gradient
Walk to Science Laboratory Building		
7:30 - 8:30 PM	Dinner	Science Laboratory Atrium
8:30 - 10:00 PM	Poster Session I *Abstracts 1-22*	Science Laboratory Atrium

Friday, May 19th		
Registration open from 8:00 AM - 8:00 PM		
Coffee Available in Social Sciences Atrium		
Science Laboratory Atrium open for poster setup (Poster Session II) at 8:30 AM		
8:00 - 9:30 AM	**Session 1: Early development and disease** **Social Sciences 1021**	
8:00 - 8:30 AM	Julia Dallman	Sensory/motor and gastrointestinal phenotypes in a zebrafish model of autism.
8:30 - 8:50 AM	Amy Anderson	N-Modified Creatine as a Treatment for SLC6A8-Related Autism Spectrum Disorders
8:50 - 9:10 AM	David Feliciano	Neurovesicles in Brain Development
9:10 - 9:30 AM	Isaac Raplee	Comprehensive Big Data Bioinformatics Detects Dynamic Changes in Transposons Expression and Epigenetic Regulators during Transformation

9:30 - 10:00 AM	Coffee Break	Social Sciences Atrium
10:00 - 11:30 AM	**Session 2: Cellular mechanisms of development** **Social Sciences 1021**	
10:00 - 10:30 AM	Nanette Nascone-Yoder	Stomach curvature and organ-specific left-right asymmetry genes
10:30 - 10:50 AM	Shuyi Nie	Cell-Matrix Interaction during Neural Crest Migration
10:50 - 11:10 AM	Miguel Salinas-Saavedra	Apico-basal Cell polarity and cell-cell adhesion during metazoa evolution: Insights from early embryogenesis of the ctenophore *Mnemiopsis leidyi*
11:10 - 11:30 AM	Guiquian Chen	Mesenchyme-specific deletion of Nf2 leads to malformation of tongue and taste papillae with fungiform taste buds sustained
Walk to Science Laboratory Building		
11:30 AM - 2:00 PM	Lunch/Poster Session II *Abstracts 23-48*	Science Laboratory Atrium *Lunch sponsored by Fisher Scientific*
	Vendor Exhibits Open	Clendenin 1007
Walk to Social Sciences Building		
2:00 - 3:30 PM	**Session 3: Embryonic patterning and early development** **Social Sciences 1021**	
2:00 - 2:30 PM	Wolfgang Lukowitz	Loss Radialization of apical-basal pattern elements in the *Arabidopsis* embryo
2:30 - 2:50 PM	Jacob Burnett	Ciliogenesis mutants reveal distinct requirements for Hedgehog signaling during mammalian optic cup formation
2:50 - 3:10 PM	Ryan Range	The role of a novel secreted Frizzled-like protein in the Wnt network governing anterior-posterior neuroectoderm patterning in sea urchin embryos
3:10 - 3:30 PM	Athula Wikramanayake	Casein Kinase 1 delta/epsilon mediates anterior-posterior axis formation in the sea urchin embryo, potentially through localized activation of Disheveled
3:30 - 4:00 PM	Coffee Break	Social Sciences Atrium

4:00 - 5:30 PM	**Session 4: Organization and genetic mechanisms of development** **Social Sciences 1021**	
4:00 - 4:30 PM	Lisa Ganser	Models of developmental and neuroplastic perturbation: The zebrafish as a translational model of amphetamine addiction
4:30 - 4:50 PM	Matthew Hale	Embryonic Origins of Altered Ovarian Gonadotropin Responsiveness in an Environmental Model of Endocrine Disruption, the American Alligator
4:50 - 5:10 PM	A. Kelsey Lewis	Penile anomalies induced by the anti-androgenic fungicide vinclozolin
5:10 - 5:30 PM	Jonathon Walsh	Using CRISPR/Cas9 Mutagenesis as an Educational Tool in a Course-Based Undergraduate Research Experience (CURE)
5:30 - 6:15 PM	Coffee Break	Social Sciences Atrium
6:15 - 7:15 PM	Keynote Address - Chris Wright	New ideas on endocrine-cell formation in the pancreas: an organ built from a rapidly expanding plexus-type epithelium
Walk to Science Laboratory Building		
7:30 - 11:00 PM	Dinner/Entertainment	Science Laboratory Atrium
Saturday, May 20th		
Registration open from 8:00 AM - 12:00 PM		
Coffee Available in Social Sciences Atrium		
8:00 - 9:30 AM SO 1021	**Session 5: Genetic regulation and networks during patterning**	
8:00 - 8:30 AM	Tamara Caspary	The cilia protein Arl13b regulates axon guidance in the mouse hindbrain
8:30 - 8:50 AM	Teresa Lee	Longevity and its transgenerational inheritance is enabled by H3K9 methylation

8:50 - 9:10 AM	Christine Larkins	Differentiation of the Perineum Epidermis
9:10 - 9:30 AM	Jialiang Wang	PITX1 Promotes Chondrocyte and Myoblast Differentiation in Mouse Hindlimbs Through Evolutionary Conserved Regulatory Targets
9:30 - 10:00 AM	Coffee Break	Social Sciences Atrium
10:00 - 11:30 AM	**Session 6: Cell signaling pathways during early development** **Social Sciences 1021**	
10:00 - 10:30 AM	Chong Shin	Hepatopancreatic Programming/reprogramming in a Zebrafish Model System
10:30 - 10:50 AM	Amanda Engstrom	LSD1 Inhibition Contributes to Tau-Mediated Neurodegeneration in Alzheimer's Disease
10:50 - 11:10 AM	Xiaofei Bai	A potential role for midbodies in developing tissues of *C. elegans*
11:10 - 11:30 AM	Taylor Hinnant	CRL5 is required in somatic cells for cyst encapsulation during early follicle development
11:30 - 11:45 AM	Results Tabulation	
11:45 AM - 12:00 PM	Awards Annoucements/ Dismissal	Social Sciences 1021

Organizing Committee:
Scott J. Nowak, Lisa Ganser, Martin L. Hudson

In addition to our generous sponsors, the organizers which to acknowledge and thank Elishka Holmquist for graphic design, Laurette Rust, and Melanie Griffin for invaluable advice and assistance. The organizers also thank Mark Anderson, Dean of the College of Science and Mathematics, Kennesaw State University, Charles Amlaner, Vice President for Research, Kennesaw State University, and the Kennesaw State University Departments of Molecular & Cellular Biology and Ecology, Evolution, & Organismal Biology.

Keynote Abstracts

Shaping and Signaling Mechanism of a BMP Morphogen Gradient

Mary Mullins, Joe Zinski, James Dutko, Francesca Tuazon, Benjamin Tajer, Ye Bu, Wei Duo, David Umulis
University of Pennsylvania

The vertebrate embryonic dorsoventral (DV) axis is patterned by a bone morphogenetic protein (BMP) activity gradient during blastula and gastrula stages. This BMP morphogen gradient is shaped by BMP antagonists emanating from dorsal regions that lead to a gradient of signaling with highest levels ventrally. Quantitation of this gradient, defining its range and the dynamics of its formation, as well as its modulation during gastrulation has not been previously investigated at high resolution. We quantified in every cell of the embryo in a temporal developmental series the nuclear intensities of phosphorylated-Smad5 (P-Smad) protein, the BMP signal transducing protein. We use automated algorithms to identify the thousands of individual nuclei present at each embryonic time point, and to measure their corresponding P-Smad intensities. The quantitative dynamics of the gradient will be presented, as well as the role of extracellular modulators in shaping the gradient. A mathematical model-based computational screen was then used to test hypotheses for gradient formation. This systems biology approach revealed an unexpected mechanism, that of a source-sink, whereby a restricted BMP antagonist distribution acts as a BMP sink dorsally that drives BMP diffusion ventrally and gradient formation. BMP signaling in DV patterning requires two ligands, Bmp2 and Bmp7, which have been shown in the zebrafish to function exclusively as a heterodimer. Results will be presented elucidating the requirement for BMP heterodimers and the heteromeric receptor complex in signaling.

This work was supported by NIH R21 OD01796.

New ideas on endocrine-cell formation in the pancreas: an organ built from a rapidly expanding plexus-type epithelium

Christopher Wright
Vanderbilt University

- Pancreas growth does not use reiterative branching-morphogenesis processes, but occurs via a “plexus state” web-like epithelial architecture.
- Plexus-state epithelium resolves to a “standard” epithelial tree later, after loss of endocrine-cell derivation.
- Endocrine-cell birth occurs specifically from within plexus-state epithelium, acting as a birthing niche.
- Epithelial morphogenetic programs and cell-intrinsic fate-determining gene-regulatory networks are coupled via feedback/feedforward loops.
- Lineage-priming to an endocrine-biased state provides an early subdivision of the endocrine/duct bipotency of plexus-state epithelium.

Session Abstracts

SESSION 1: EARLY DEVELOPMENT AND DISEASE

Sensory/motor and gastrointestinal phenotypes in a zebrafish model of autism.

Julia Dallman, David James, Robert Kozol
University of Miami

Autism Spectrum Disorder (ASD) is a common developmental disorder diagnosed by stereotyped interests and deficits in communication. In addition to these diagnostic symptoms, individuals with ASD also have shared comorbidities that include epilepsy and gastrointestinal distress. To shed light on the symptom etiology of comorbidities, we have generated zebrafish models of one of the more common genetic forms of ASD caused by mutations or deletions of one copy of the *SHANK3* gene. While SHANK3 has largely been studied in juvenile/adult mammalian models, in situ hybridization and quantitative PCR show that the gene is expressed at embryonic stages suggesting a functional role at this time and making the developmental accessibility of zebrafish ideal. We used CRISPR/Cas9 to mutate duplicated zebrafish *SHANK3* orthologs, *shank3a/b*. Larvae from several *shank3a/b* mutant allele combinations exhibit robust sensory/motor and gastrointestinal phenotypes. For sensory/motor phenotypes, we focused on the photomotor and vibration-induced escape responses. s*hank3a/b* mutants are significantly more responsive and show reduced habituation to both these stimuli. Such hyperexcitability phenotypes are consistent with epilepsy and altered sensory/motor phenotypes seen in people and provide a basis to determine how *shank3a/b* mutations impact systems-level neural circuits. In addition to sensory/motor phenotypes, we have also quantified gastrointestinal motility. The GI tract produces rhythmic contractions that are an important part of normal digestion. While peristaltic stomach contractions in *shank3a/b* mutants are still rhythmic, they occur at a significantly slower frequency, a phenotype consistent with longer passage time and reflux symptoms seen in people with Phelan McDermid Syndrome. These zebrafish *shank3ab* models provide an experimental model in which to uncover underlying mechanisms of symptom etiology.

N-Modified Creatine as a Treatment for SLC6A8-Related Autism Spectrum Disorders

Amy Anderson, Modi Wetzler, Kristyn A. Robinson, Mary Katherine Sullivan, Susan C. Chapman
Clemson University

Creatine deficiency syndrome is caused by a mutation in the creatine transporter gene *SLC6A8* and results in X-linked intellectual disability and autism spectrum disorder. *SLC6A8* mutations are estimated to be as common as 1-5.4% of all X-Linked Intellectual Deficiencies, with this disorder affecting 42,000 patients in the USA and one million worldwide, but without comprehensive genetic testing, this number is likely underestimated. No treatment modality exists, as supplemented creatine cannot cross the blood brain barrier (BBB) or enter neuronal cells without a functioning transporter. Failure of creatine transport into the brain has significant impacts on energy metabolism, as creatine's phosphylated form is an essential part of the high energy buffering system used to maintain ATP levels in brain tissues. This research introduces a new way to directly screen large numbers of creatine analog compounds in the simple, tractable zebrafish system, in a cost- and time-efficient manner, and has identified potential treatment modalities where none currently exist. We synthesized a new class of N-modified creatine analogs and using high throughput zebrafish screening have accelerated identification of creatine analogs that can cross the BBB, bypassing deficient *SLC6A8* transporters. These analogs were biochemically assayed and showed 87-95% efficiency binding creatine kinase and producing phosphocreatine, compared to native creatine. 48-hour embryos were exposed to 125 uM creatine analogs for 24 hours. Brain tissue was isolated, and the amount of creatine analog in the brain was quantified by mass spectrometry. Analogs that both cross the BBB and interact with creatine kinase will be tested in *SLC6A8* mutant mice before moving to preclinical trials. Other essential molecules in brain (or somatic) function with defective transporters will benefit from this approach, making our study applicable to the broader field of metabolic imbalance resulting from transporter defects.

Neurovesicles in Brain Development

David Feliciano, Mary Morton, Caitlin Seluzicki, Victoria Neckles
Clemson University

Subventricular zone (SVZ) neural stem cells (NSCs) are the cornerstone of the perinatal neurogenic niche. The anatomical limits of the niche are defined by the boundaries of the NSC apical projecting process which projects to the lateral ventricles and a basal projecting process terminates at blood vessels. Microglia are resident immune cells that are found within the perinatal neurogenic niche, and although they regulate NSCs, there are few studies that demonstrate bi-directional communication. Extracellular vesicles (EVs) classified as exosomes and ectosomes are particles that are proposed to transfer miRNA and proteins from donor cells to recipient cells. EVs are proposed to carry neurogenic, angiogenic, and immuno-modulatory molecules. Here, evidence is provided that the EV tetraspanin protein CD9 is expressed within perinatal SVZ NSCs. Neonatal CD9 is localized to apical and basal projecting NSC processes and undergoes time dependent and cell-type specific subcellular localization. Within the first week of life, CD9 positive EVs originating from NSCs are released in close proximity to the basal projecting fiber of NSCs but are actively cleared from the parenchyma. Neonatal EV transplants selectively label SVZ microglia. These results demonstrate that CD9 positive particles originating from SVZ NSCs interact with microglia and may have an immuno-modulatory role in the neonatal brain.

Comprehensive Big Data Bioinformatics Detects Dynamic Changes in Transposons Expression and Epigenetic Regulators during Transformation

Isaac Raplee
University of South Florida

It is widely recognized that all breast cancers start when some cells in an otherwise healthy tissue begin to look abnormal and ultimately result in full-blown cancer. However, in many cases, initial abnormal cells do not always follow the deadly path and cancer does not develop at all. Little is known as to why some patients diagnosed with atypia and ductal carcinoma in situ (DCIS) remain cancer-free while in others the disease progresses to invasive ductal carcinoma (IDC). To identify molecular signatures driving cell fate decisions at atypia and DCIS stages to transformation, we investigated expression of transposonable elements (TEs) as in human atypia, DCIS and IDC. While mutagenic role of TEs in cancer is well documented, we focus on the novel role of LTRs as potential drivers promoting cell de-differentiation during early stages of tumor development with promise as a prognostic tool. To investigate the contribution of TEs in atypia and DCIS, we created a TE Enrichment Set Analysis (TESA) to identify TEs in RNA sequencing datasets across four stages of breast cancer, normal, atypia, DCIS, IDC in humans (n= 8-23). After quality control steps to remove outliers, TEs, compared to transcripts, exhibit substantially less variation in their expression because the first principle component accounted for over 80% expression variation compared to 20% in transcript expression variation ($p<0.05$). Eighty-eight TEs were detected as significant across stages by ANOVA ($\alpha=0.05$, FDR 5%). The majority are LTRs (67%) with the remaining split into DNA TEs (18%), SINEs (11%) and unclassified (4%). Our TESA data complements and provides experimental support that early genomic changes are a mechanism underlying subsequent tumor development. Translational bioinformatics is a technique to identify prognostic molecules for impending invasive breast cancer from biopsies of pre-malignant atypia and ductal carcinoma in situ. Funding: Impact Assets, Hartford CT.

SESSION 2: CELLULAR MECHANISMS OF DEVELOPMENT

Stomach curvature and organ-specific left-right asymmetry genes
Nanette Nascone-Yoder
North Carolina State University

Left-right (LR) asymmetry is a fundamental feature of internal anatomy, yet the emergence of morphological asymmetry remains one of the least understood phases of organogenesis. To address this issue, we investigated the cellular and molecular mechanisms that drive the curvature of the stomach, a classic archetype of vertebrate laterality. In contrast to the prevailing dogma of stomach development, which asserts that the early organ undergoes a series of orthogonal rotations to sculpt its final J-shaped contour, we find that curvature is the result of the contralateral walls of the stomach executing distinct morphogenetic programs. The left wall of the primitive stomach expands more than the right wall, as the left epithelium becomes more polarized and undergoes radial rearrangement. These cellular morphogenetic asymmetries are evident in two different vertebrate models, the frog *Xenopus* and the mouse, and are dependent on LR patterning genes, including *Foxj1*, *Nodal* and *Pitx2*. To identify the downstream genes that orchestrate stomach curvature at the organ level, we have pioneered the use of the Budgett's frog (*Lepidobatrachus*) embryo, a model whose large size facilitates fine-scale manual microdissection of left and right sub-regions of early organs for RNAseq and proteomic profiling. This unique platform has facilitated identification of stomach-specific left- and right-sided genes and thus, new molecules involved in lateralized organ morphogenesis. Ongoing analyses of the expression and function of these genes in the contralateral stomach walls is revealing mechanisms of LR asymmetric organogenesis, with implications for how embryonic LR patterning manifests as distinct types of morphological asymmetries in different contexts.

Cell-Matrix Interaction during Neural Crest Migration
Shuyi Nie
Georgia Institute of Technology

The neural crest is a unique cell population found in developing vertebrates, characterized by its multipotency and migratory capacity. During their migration, they first detach from the neuroepithelium through epithelial-to-mesenchymal transition (EMT), then migrate in a "sheet-like" or "chain-like" manner by remodeling cell-cell adhesion and the extracellular matrix along their path. This migratory behavior of neural crest cells resembles that of cancer metastasis and becomes a good model to study how cancer spread from its primary site. To explore how neural crest cells transit between different phases of migration, we first looked at an important matrix metalloproteinase, MMP14 (or MT1-MMP), which is highly expressed both in many malignant cancers and in migrating neural crest cells. As a transmembrane protease, MMP14 plays critical roles in activating other MMPs (such as MMP2), processing various matrix proteins, and mediate signaling events with other cell surface receptors. By loss-of-function approaches, we found that MMP14 is required in cranial neural crest cell for their migration into the branchial arches. This function in promoting neural crest migration is independent to MMP2. In addition, MMP14 is also required for neural crest EMT, that isolated neural crest cells cannot separate from each other in culture at the loss of MMP14. This may partially mediated by cleavage of cell adhesion molecules, such as Cadherins. Through an exploratory approach, we found that multiple cell adhesion molecules are differentially glycosylated at different stages of neural crest migration. Taken together, neural crest cells control their adhesiveness to each other and to surrounding tissue dynamically to migrate efficiently.

Apico-basal Cell polarity and cell-cell adhesion during metazoa evolution: Insights from early embryogenesis of the ctenophore *Mnemiopsis leidyi.*

Miguel Salinas-Saavedra
University of Florida

Par proteins are conserved components of cellular polarization during early embryogenesis and their role in establishing embryonic asymmetry have been widely studied in bilaterians. In embryos of the cnidarian *Nematostella vectensis* the components of the Par system (NvPar-1, NvPar-3, NvPar-6, NvaPKC, and NvLgl) distribute throughout the microtubule cytoskeleton of pre-blastula stages without any clear polarization along any embryonic axis. However, they become asymmetrically localized at later stages, when the embryo forms an ectodermal epithelial layer in a manner seen in bilaterian animals: NvLgl and NvPar-1 localize in the basolateral cortex, and NvaPKC, NvPar-6, and NvPar-3 at the apical zone of the cell. Interestingly, *N. vectensis* shows a "random" cleavage pattern and it undergoes gastrulation at the animal (not vegetal) pole of the egg. In contrast, Ctenophores (which also gastrulate at the animal pole) develop under a highly stereotyped embryogenesis; begging the question of whether Par genes regulate the cleavage program and help specify the site of gastrulation. By in vivo imaging we characterized the Par protein localization in blastomeres and epithelial cells during the early embryogenesis of the ctenophore *Mnemiopsis leidyi*. mRNA expression of the components of the ctenophore Par system shows that these proteins distribute differently compared to what we have described for *N. vectensis* embryos. This differential localization might be related with the emergence of different junctional complexes during Metazoa evolution.These data will provide a glimpse into the evolution of cell polarity in metazoan embryos.

Mesenchyme-specific deletion of Nf2 leads to malformation of tongue and taste papillae with fungiform taste buds sustained

Guiqian Chen, Mohamed Ishan, Xiushen Wang, Wenxin Yu, Brett Marshall, Xinwei Cao, Marco Giovannini, Hong-Xiang Liu
University of Georgia

Neurofibromatosis 2 (Nf2) has a critical role during embryonic development and is especially important in regulating migration of neural crest cells, from which the tongue mesenchyme arises. Here we report that Nf2 immunosignals were primarily in mesenchyme and nerve fibers of developing mouse tongues. In Nf2 conditional knockouts (cKO) driven by Wnt1-Cre that exclusively labels tongue mesenchyme, we found: (i) a misshapen tongue, e.g., wider posterior region and pointed tip at E12.5-E13.5 and shorter in tongue length from E14.5 through birth; (ii) the absence of circumvallate and foliate papilla and taste buds; (iii) fewer fungiform papillae. In E18.5 Nf2 cKO tongue sections versus control we observed: (a) disorganized epithelial cells with high levels of SOX9 expression; (b) greatly reduced nerve fibers; (c) fewer and atrophied fungiform papillae; (d) significantly reduced Ki67+ cells, and increased apoptosis in epithelium and mesenchyme. Interestingly, numbers of early fungiform taste buds were unaltered in Nf2 cKO. The increased phosphorylation of YAP and reduced activities of transcription factor TEAD1 in Nf2 cKO suggested the involvement of the Hippo-YAP pathway in regulation of tongue development. In organ cultures, Nf2 cKO tongues responded to the disruption of Shh signaling with cyclopamine and activation of Wnt/b-catenin signaling with LiCl for an enhanced papilla formation suggesting that mesenchymal Nf2 regulates Shh and Wnt/b-catenin signaling activities for proper formation of taste papillae in a region- and papillae-type dependent manner. Together, our data demonstrate that the Nf2-mediated Hippo-YAP pathway interacts with Shh and Wnt/b-catenin for its essential role in the development of tongue and taste papillae. Sustained early fungiform taste buds in the non-innervated and atrophied fungiform papillae in Nf2 cKO strongly support the hypothesis that early taste bud induction at embryonic stage is papillae structure- and nerve-independent.

SESSION 3: EMBRYONIC PATTERNING AND EARLY DEVELOPMENT

Radialization of apical-basal pattern elements in the *Arabidopsis* embryo

Wolfgang Lukowitz, Matthew Volny
University of Georgia

The molecular network responsible for establishing and refining the coordinates of the main axis body axis in the early embryo remains poorly understood. Previously, we showed that a GATA-factor, HANABA TARANU (HAN), is required for positioning the inductive boundary between proembryo and suspensor, across which the embryonic root is initiated. Loss of HAN results in a coordinated apical shift of expression domains normally delimited by the proembryo boundary. A significant fraction of han embryos can overcome this early defect to eventually generate a functional root meristem at the boundary between apical and basal tier of the proembryo.

Here, we report that this recovery is due to the activity two functionally equivalent HAN-like genes (*HANL1*, *HANL2*). Loss of all three HAN family genes initially results in *han*-like phenotypes. But as *han* mutants begin to recover, the triple mutants deteriorate further, arresting as oblong, blimp-like structures with small, seemingly isodiametric cells at the center. Several transcripts normally confined to the basal side of the proembryo boundary over time come to surround the triple mutant embryos in concentric layers – for example, *WOX8* or *IAA10*, normally found in the suspensor and distal root, expand to all surface cells, and *WOX5*, normally found in the quiescent center, expands to all sub-surface cells. We interpret this phenotype as radialization of the apical-basal axis.

Ciliogenesis mutants reveal distinct requirements for Hedgehog signaling during mammalian optic cup formation

Jacob Burnett, Floria Lupu, Jonathan Eggenschwiler
University of Georgia

The Hedgehog (HH) pathway plays a role in specification of optic cell fates. HH is expressed in the ventral midline of the eye region, diffuses laterally, and contributes to the establishment of the proximo-distal and dorso-ventral axes within the eye. It is not clear how establishment of these axes ultimately leads to specification of distinct cell fates. We utilized two mouse ciliogenesis mutants *(Ift122-/-* and *Ccrk-/-)* to alter the levels of HH signaling to different degrees. Ift122 mutants exhibited the highest levels of HH signaling within the eye, indicated by HH target gene expression. These mutants failed to specify the retinal pigment epithelium (RPE) or neural retina (NR), while the optic stalk (OS) territory was significantly expanded. Reducing the levels of HH signaling in *Ift122* mutants by simultaneous removal of the HH activator Gli2 led to a partial restoration of RPE and NR fates, and the OS territory was less drastically expanded than in the *Ift122* single mutant. *Ccrk* mutants, by contrast, exhibited a bimodal change in HH signaling with reduced high level responses and ectopic low level responses. *Ccrk* mutants, similar to *Ift122-/-*, exhibited an expanded OS territory, however the extent of this expansion was not as severe. While *Ccrk* mutants showed some NR and RPE specification, the RPE domain was abnormally expanded into the NR region, and the NR territory showed a corresponding reduction. All of these patterning defects in *Ccrk* mutants were fully rescued by simultaneous removal of Gli2. We further elevated the HH pathway in *Ccrk* mutants by simultaneous removal of the HH repressor Gli3, which lead to a complete expansion of the RPE into the NR region and a complete loss of NR fate. Taken together, these experiments suggest that during eye formation, optic progenitors sense subtle changes in the levels of HH signaling and that progressively lower doses of HH signaling are required for proper specification of the OS, RPE, and NR, respectively.

The role of a novel secreted Frizzled-like protein in the Wnt network governing anterior-posterior neuroectoderm patterning in sea urchin embryos

Ryan Range, Anita Khadka, Marina Martinez-Bartolomé, Stephanie Burr
Mississippi State University

The anterior neuroectoderm (ANE) in many deuterostome embryos (echinoderms, hemichordates, urochordates, cephalochordates, and vertebrates) is progressively restricted along the anterior-posterior (AP) axis to a domain around the anterior pole. In sea urchin embryos, three integrated Wnt signaling branches (Wnt/β-catenin, Wnt/JNK and Wnt/PKC) are primarily responsible for this progressive restriction process that begins around the 60-cell stage and terminates by the early gastrula stage. We previously have established that several secreted Wnt modulators of the Dickkopf and secreted Frizzled related protein families (Dkk1, Dkk3, and sFRP1/5) are expressed within the ANE and play important roles in modulating the Wnt signaling network during this process. In this study, we use morpholino and dominant-negative interference approaches to characterize the function of a novel secreted Frizzled related protein, sFzl-like, during ANE restriction. Our results show that ubiquitously expressed maternal sFzl-like and Fzl1/2/7 signaling act together as early as the 60-cell stage to antagonize the ANE restriction mechanism mediated by Wnt/β-catenin and Wnt/JNK signaling. Then, starting from the blastula stage, Fzl5/8 signaling activates zygotic sFzl-like within the ANE territory and it works with the secreted Wnt antagonist Dkk1 (also activated by Fzl5/8 signaling) to antagonize Wnt1/Wnt8-Fzl5/8/JNK signaling in a negative feedback mechanism, thereby defining the outer ANE territory boundary. Together, these data indicate that sFzl-like protects the ANE territory by antagonizing the Wnt1/Wnt8-Fzl5/8/JNK signaling pathway throughout ANE restriction, providing precise spatiotemporal control of the mechanism responsible for the establishment of the ANE territory around the anterior pole of the sea urchin embryo.

Casein Kinase 1 delta/epsilon mediates anterior-posterior axis formation in the sea urchin embryo, potentially through localized activation of Disheveled

Athula Wikramanayake, Wei Wu, Lingyu Wang, Lauren Smith
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Wnt signaling plays a central role in establishing anterior-posterior (AP) polarity in metazoan embryos. A key cytoplasmic component mediating Wnt signaling is the Disheveled (Dvl) protein. In the sea urchin, Dvl is highly enriched and differentially post-translationally modified in a specialized vegetal cortical domain (VCD) of the egg, and the vegetal blastomeres that inherit the VCD during embryogenesis. Functional analysis has shown that localized Dvl activity mediates canonical Wnt signaling in vegetal blastomeres, but the molecular basis of Dvl asymmetric localization and activation remain unresolved. Therefore, identification and functional characterization of proteins interacting with Dvl (DIPs) in the VCD will help us better understand how Dvl partners regulate Dvl activity and Wnt signaling. By applying Dvl Co-immunoprecipitation coupled with mass spectrometry we have identified several potential Dvl-interacting-proteins (DIPs) from isolated egg cortices and 16-cell-stage micromeres. Casein Kinase 1 δ/ε (CK1δ/ε), one of our newly identified DIP candidates, is highly enriched and co-localized with Dvl at the vegetal pole of the sea urchin embryo. Downregulation of CK1δ/ε activity by overexpressing a dominant-negative form of CK1δ/ε resulted in the downregulation of genes expressed in the endomesoderm and the anteriorization of embryos. However, overexpression of CK1δ/ε by injecting synthesized *CK1δ/ε* mRNA into fertilized eggs only induced slight upregulation of endomesoderm genes and mild posteriorization of embryos. Intriguingly, we found that co-overexpressing CK1δ/ε and Dvl induced significantly higher levels of expression of endomesodermal genes compared to expression levels of these genes in embryos overexpressing Dvl or CK1δ/ε only suggesting that CK1δ/ε synergizes with Dvl to positively regulate Wnt signaling.This work establishes CK1δ/ε as a critical regulator of Dvl activation and AP axis specification in sea urchin embryos.

SESSION 4: ORGANIZATION AND GENETIC MECHANISMS OF DEVELOPMENT

Models of developmental and neuroplastic perturbation: The zebrafish as a translational model of amphetamine addiction.

Lisa Ganser
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Both prescribed and illicit use of amphetamines (AMP) has skyrocketed within the last ten years. An increase in diagnoses of adolescent attention disorders, and the therapeutic value of AMP in adults to combat binge-eating disorder and attention disorders, AMP has become a commonly prescribed medication for individuals as young as three - adult age. The abuse of AMP as a "study drug" that increases focus while suppressing appetite has resulted in addictive behavioral changes, especially in users of child-bearing age. Because much of the use and abuse lies within child-bearing aged populations, there is a pertinent need to understand fetal neurodevelopmental effects of gestational exposure to AMP and any neurophysiological changes resulting from AMP addiction in adults. We exposed zebrafish embryos to amphetamine-laden water from 0 - 48 hpf. Measurements of somatic development and behavioral milestones showed significant developmental delays in AMP-exposed embryos vs. controls. Behavioral tests assaying escape response were quantified at 24 and 48 hpf and indicated that AMP-exposed embryos were significantly slower than controls to complete escape response due to an increase in spasticity. Image analyses of embryonic spinal cord at 48 hpf yield significant differences in the ratio of excitatory to inhibitory interneurons among treatment groups, suggesting that signal imbalance may underlie spasticity in the AMP-treated embryos. In the AMP addiction model, we have determined through CPP assays that zebrafish produce addictive behaviors in response to AMP ingestion. Measurable levels of AMP are present in the brain days after ingestion, and AMP-addicted fish display thigmotaxic behavior and severe deficits in body condition compared to controls. Currently, we focus on measuring AMP-induced changes in dendritic arborization and connectivity in the habenula, the area of the zebrafish brain that is analogous to the human dopaminergic reward system.

Embryonic Origins of Altered Ovarian Gonadotropin Responsiveness in an Environmental Model of Endocrine Disruption, the American Alligator

Matthew Hale, Thomas Galligan, Jacqueline Bangma, Brenna Doheny, Jessica Cloy-McCoy, Frannie Nilsen, Louis Guillette, Benjamin Parrott
University of Georgia

As part of the "developmental origins of adult disease" model, studies investigating the effects of environmental endocrine disruptors on wildlife have helped elucidate the role of endocrine signaling in shaping the development of the vertebrate reproductive system. The American alligator has provided utility in this investigation, as populations from a contaminated lake in Florida, Lake Apopka (AP) link developmental exposures to estrogenic contaminants to a suite of reproductive abnormalities. We have previously reported failed ovarian responsiveness to the gonadotropin follicle-stimulating hormone (FSH) in AP alligators, as animals from this site, raised from hatching in laboratory conditions, fail to upregulate gene expression of *CYP19A1* in response to FSH. As this failed response was detected in animals exposed only during development, but not in reference animals, its origins are putatively embryonic. We sought to assess if exposure to an estrogen during gonadogenesis is responsible for this phenotype. To test this, we exposed embryos from a reference site, Lake Woodruff (WO) to estradiol (E2) or an androgen (DHT) during the window of sex determination and gonadogenesis. Hatchlings were then raised for five months in our lab. Upon reaching five months, WO animals, as well as natively-exposed AP animals were administered FSH to assess ovarian gene expression responses and modulation by developmental exposure. Both AP animals and exposed WO animals express CYP19A1 robustly in response to FSH. Developmental exposure did however significantly alter expression of *estrogen receptor-β* (*ESR2*) in FSH-stimulated animals, as well as expression of *aryl hydrocarbon receptor 2* (*AHR2*). Response of the gonadosomatic index (GSI) to FSH was also significantly altered by developmental exposure. These results indicate that, while *CYP19A1* might not be affected, developmental endocrine signals shape gene expression patterning and growth response of the ovary later in life.

Penile anomalies induced by the anti-androgenic fungicide vinclozolin

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In recent decades, there has been a rise of endocrine-related diseases and disorders, including an increased incidence of genital malformations, low semen quality, adverse pregnancy outcomes, neurobehavioral disruption, endocrine-related cancers, earlier onset of breast development, obesity, and type 2 diabetes (UNEP and WHO, 2013). An example of increased genital malformations is seen with congenital penile anomaly (CPA) frequency, which has increased to a rate of 1 in 125, or 0.83%, of male newborns. The most commonly reported CPA is hypospadias, which accounts for 68.3% of CPAs (Nelson et al., 2005). Hypospadias is characterized by an atypical urethral opening along the penile shaft, within the scrotum, or in the perineum. Chordee, or penile curvature, accounts for 8.6% of CPAs, while hypospadias plus chordee make up 5% of CPAs (Nelson et al., 2005). Chordee without hypospadias is a congenital anomaly that usually results in a ventrally tethered penis and normally positioned urethral opening. We found that the endocrine disruptor vinclozolin induces a ventrally-tethered penis, similar to the human CPA chordee without hypospadias. This phenotype is likely due to disruption of steroid hormone signaling.

Using CRISPR/Cas9 Mutagenesis as an Educational Tool in a Course-Based Undergraduate Research Experience (CURE)

Jonathon Walsh
University of Georgia

Lab experience is an integral part of an undergraduate education in the sciences. The majority of students get this experience from lab courses that their institutions offer in their degree program. Some students gain extra experience by working as undergraduate research assistants in laboratories under the supervision of a faculty mentor and/or a graduate student. There has been a recent push to develop and implement course-based undergraduate research experiences (CUREs), which go beyond what is typically expected from a lab course; this type of program combines a general lab course experience with working in a research lab and generating novel, publishable data. We worked to develop and implement a CURE based on CRISPR/Cas9-mediated genome editing technology. By the end of this three semester thesis course, students developed skills in multiple techniques such as transformation, DNA extraction, PCR-genotyping, mammalian cell culture, transfection, and others. Students were expected to gain a deeper understanding of the topic of the project (regulation of ciliogenesis) as well as basic scientific concepts such as genome editing, genotype/phenotype relationships, reversion of mutations, epistasis, synergistic interactions and others. Students involved in the course gained three semesters of experience working in a lab and were able to get firsthand exposure to parts of research that are typically missing from standard lab courses such as troubleshooting experiments, experimental design and re-design, and updating strategies based on newly-acquired data.

SESSION 5: GENETIC REGULATION AND NETWORKS DURING PATTERNING

The cilia protein Arl13b regulates axon guidance in the mouse hindbrain

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Sonic Hedgehog (Shh) signaling is a critical developmental pathway well-established to regulate cell fate specification and proliferation via the Gli transcription factors. Transcription-dependent Shh signaling requires the primary cilium. Additionally, Shh regulates axon guidance through a distinct, transcription-independent mechanism. All Shh signaling is transduced via Smoothened (Smo). *ARL13B* encodes a ciliary GTPase that regulates transcription-dependent Shh signaling and, when mutated, causes the ciliopathy Joubert Syndrome (JS). JS is diagnosed by the molar tooth sign (MTS), a hindbrain malformation caused by cerebellar hypoplasia as well as failure of the superior cerebellar peduncles (SCPs) to cross the midline of the brain. Additionally, JS patients display defects in crossing within the optic chiasm suggesting problems in JS with axon guidance. However, no known mechanism connects cilia genes such as *ARL13B* to the regulation of axon guidance. We aim to test whether *Arl13b* regulates transcription-independent Shh signaling to direct axon guidance in the developing brain. We examined SCP crossing in mouse brains lacking either Smo or Arl13b in projection neurons by performing diffusion tensor imaging (DTI) MRI and retrograde tract tracing. We observed SCPs lacking Smo or Arl13b display significant midline crossing defects in the hindbrain, indicating axon guidance in these projection neurons is Arl13b-dependent and Smo-dependent, suggesting Shh as a guidance cue in SCPs. Our results suggest Arl13b regulates axon guidance in projection neurons that use Shh as a guidance cue, implicating a cilia-associated gene in axon guidance.

Longevity and its transgenerational inheritance is enabled by H3K9 methylation

Teresa Lee, Amanda Engstrom, David Katz
Emory University

Longevity is a complex trait influenced by environmental, genetic, and epigenetic factors. WDR-5, a member of the COMPASS complex, methylates histone 3 at lysine 4 (H3K4). 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 this background is a transgenerational phenotype that takes several 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. This result suggests that H3K9me2 may be inherited transgenerationally and confer longevity in *wdr-5* mutants. To examine this possibility, we mutated *met-2,* the methyltransferase required for all germline H3K9me2, in *wdr-5* mutants. We show that the extended lifespan of *wdr-5* mutants is dependent on met-2, further implicating H3K9me2 in the mechanism of longevity. Moreover, our finding that H3K9me2 is heritable indicates that it may also be involved in the inheritance of longevity in *wdr-5* mutants. To test this possibility, we deleted *met-2* in descendants of *wdr-5* mutants, and find that the loss of met-2 abolishes the inheritance of longevity. We are currently using ChIP-seq to examine global levels of H3K9me2 in *wdr-5* mutants and their long-lived wild-type descendants. Taken together, these data support a model in which germline H3K9me2 facilitates longevity and its epigenetic inheritance.

Differentiation of the Perineum Epidermis

Christine Larkins, Daniel Grunberg, Gabriel Daniels, Erik Feldtmann, Martin Cohn
University of Florida

The perineum, the region between the external genitalia and the anus, undergoes extensive sexual differentiation in response to sex steroids. In males, the anogenital distance and therefore the perineum, is much longer than in females. During embryonic development, the perineum is formed after the cloaca is divided into anorectal and urogenital canals. Cloacal endoderm remains at the midline of the perineum through neonatal stages and contributes to the developing epidermis, a tissue thought to be derived entirely from ectoderm. The fate of these endoderm cells within the epidermis is not clear.

The epidermis is a stratified epithelium that serves as a barrier to maintain homeostasis and protect the organism. In the embryo, a single layer of epithelial cells divides asymmetrically or delaminates to give rise to the multilayer epidermis through a process called terminal differentiation. This process continues in the mature epidermis, as cells within the basal layer differentiate and move through the epidermal layers until they reach the outer layer where they are sloughed off through desquamation. Exactly how the epidermis undergoes stratification and how this process may differ between the sexes is not clear.

Here we sought to examine two aspects of the developing perineum epidermis. First, we used lineage tracing in mice to determine the fate of the endoderm cells within the perineum. We found that endodermal cells obtain an epidermal identity but are lost over time through terminal differentiation and desquamation. Second, we examined sex differences in perineum epidermis development and found that there is sex-specific cell movement in the epidermis, which likely contributes to sexual dimorphism of the perineum skin. These results serve as a foundation for further examination of epidermal development in the perineum region as a means to understand how sex specific epidermal phenotypes occur.

Funding NIH DK105077

PITX1 Promotes Chondrocyte and Myoblast Differentiation in Mouse Hindlimbs Through Evolutionary Conserved Regulatory Targets

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The PITX1 transcription factor is expressed during hindlimb development, where it plays a critical role in directing hindlimb growth and specification of hindlimb morphology. While it is known that PITX1 regulates hindlimb formation, in part, through activation of the *Tbx4* gene, other transcriptional targets remain to be elucidated. We have used a combination of ChIP-Seq and RNA-Seq to investigate enhancer regions and target genes that are directly regulated by PITX1 in embryonic mouse hindlimbs. In addition, we have analyzed PITX1 binding sites in hindlimbs of *Anolis* lizards to identify ancient, evolutionary conserved PITX1 regulatory targets. We find that PITX1-bound regions in both mouse and *Anolis* hindlimbs are strongly associated with genes implicated in limb and skeletal system development. Gene expression analyses reveal a large number of misexpressed genes in the hindlimbs of Pitx1-/- embryos. By intersecting misexpressed genes with genes that have neighboring mouse PITX1 binding sites, we identified 440 candidate targets of PITX1. Of these candidates, 68 exhibit ultra-conserved PITX1 binding events that are shared between mouse and *Anolis* hindlimbs. Among the ancient targets of PITX1 are important regulators of early cartilage and skeletal muscle development, including SOX9 and SIX1. Our data suggest that PITX1 acts as a core regulator in promoting chondrocyte and myoblast differentiation in the hindlimb by direct regulation of several key members of the cartilage and muscle transcriptional networks. We have evolutionarily and genome-widely provided insights into the PITX1-dependent enhancers and targets in vertebrate hindlimb development.

SESSION 6: CELL SIGNALING PATHWAYS DURING EARLY DEVELOPMENT

Hepatopancreatic Programming/reprogramming in a Zebrafish Model System
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Georgia Institute of Technology

Worldwide, liver failure and diabetes mellitus are the leading cause of morbidity and mortality. The therapeutic restoration of hepatocyte and insulin secreting beta-cell mass would support the functions of a failed liver and pancreas. One approach to restoration is the transplantation of exogenous hepatocytes and beta-cells generated from pluripotent stem cells. Despite notable progress, the resulting cells often fail to achieve complete function. Therefore, deciphering the activated signaling pathways and their cross-regulatory interactions during embryogenesis is crucial. A second approach to restoration is the stimulation of endogenous repair mechanisms. Although mammals have a limited capacity for regeneration, they may retain developmental and/or ancestral pathways that are typically quiescent in adults. By studying the recovery in other vertebrates not only with homologous liver and pancreas structure but also with significant capacity for regeneration, we can unveil key repair markers and/or pathways. We use the zebrafish as a primary model system, which offers the functional live imaging ability, high-throughput/regeneration capacity, and single-cell level manipulability, and show that Bmp signaling and antagonistic interplay between Wnt and Notch signaling critically affect beta-cell and hepatocyte regeneration.

LSD1 Inhibition Contributes to Tau-Mediated Neurodegeneration in Alzheimer's Disease
Amanda Engstrom, Amelia Anderson, Rohitha Moudgal, Michael Christopher, Dexter Myrick, Benjamin Barwick, Allan Levey, David Katz
Emory University

Alzheimer's disease (AD) is an irreversible, progressive brain disorder caused by massive neuronal cell death in the cortex and hippocampus. AD is characterized by the accumulation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated Tau (NFTs) – but the molecular mechanisms by which NFTs contribute to neuronal cell death remains unclear. Recent data from our lab has demonstrated that the histone demethylase, LSD1, is necessary for neuronal survival, and its function may be disrupted by NFTs. LSD1 has primarily been characterized for its role in development, and is classically thought to facilitate cell fate transitions. In the adult mouse, loss of LSD1 results in neuronal cell death in the hippocampus and cortex as well as paralysis and learning and memory defects. Loss of LSD1 induces transcriptional changes associated with neurodegenerative pathways, along with the reactivation of stem cell genes, in the degenerating hippocampus. These transcriptional changes significantly correlate with those in AD cases. We have also shown that LSD1 is mislocalized to NFTs in AD cases, and in mouse models of AD. These data suggest that NFTs contribute to neuronal cell death in AD by sequestering LSD1 out of the nucleus and interfering with a continuous requirement for LSD1. To test this model, we removed one copy of *Lsd1* from P301S Tau mice, which contain a human transgene overexpressing an aggregation-prone mutant allele of Tau. If the defects observed in P301S Tau mice are due in part by LSD1 sequestration, then reducing LSD1 levels could accelerate the neurodegenerative phenotypes. Here, we show that this allelic combination results in a synergistic effect on survival, rate of paralysis, and neurodegeneration in the brain and spinal cord. This suggests that aggregated Tau functions pathologically through the loss of LSD1. As a result, prevention of the loss of LSD1 function is a promising therapeutic target to block the progression of AD.

A potential role for midbodies in developing tissues of *C. elegans*

Xiaofei Bai
The University of Tennessee, Knoxville

The midbody forms at the end of cytokinesis and facilitates abscission. Recently, the midbody has been implicated in several morphogenetic processes. To investigate developmental roles of midbody, we examined midbody fate in the invariant lineage of the *C. elegans* embryo. Live-cell imaging revealed unique and tissue-specific patterns of the fate of the midbody and different midbody components. In the first mitosis, symmetric furrowing positions a central midbody that is always internalized by the P1 daughter cell. In the next AB cell division, a highly asymmetric furrow positions a midbody that is engulfed by EMS instead of either AB daughter cell. In two lumen-forming tissues around the 300-cell stage, the intestine and the pharynx, midbodies form after symmetric furrowing and migrate across the cell to the future apical midline. Upon reaching the apical midline, the midbody ring is internalized and disappears; however, the Aurora B kinase, AIR-2, remains on the apical surface after polarization. A similar apical localization pattern is observed for AIR-2 in the pharyngeal primordium. Finally, in cells that form the inner labial sensilla, we observe symmetrical cytokinesis and a midbody migration event that leads to a focal aggregation of AIR-2. AIR-2 persists along the leading edge of developing dendrites as they migrate towards the anterior end of the embryo, anchor at the tips and elongate. Other midbody markers are either internalized and degraded or maintained with AIR-2 in a tissue-specific manner. Inactivating several fast-inactivating temperature sensitive cytokinesis mutants late in embryogenesis causes severe defects in positioning, continuity and shaping of the intestinal, pharyngeal lumen and sensory neurons. These data suggest that the proper execution of cytokinesis, which shows surprising flexibility during development, and specific cytokinesis regulators such as AIR-2, may regulate the final interphase architecture of a terminally dividing cell.

CRL5 is required in somatic cells for cyst encapsulation during early follicle development

Taylor Hinnant, Victoria Hardy
East Carolina University

During oogenesis, follicle cells encapsulate maturing oocytes and are essential for oocyte growth and development. While many signaling pathways have been linked to the control of encapsulation, mechanisms of early follicle development are not fully understood. Cullin-RING E3 ubiquitin ligases (CRLs) control a variety of cellular processes and are necessary for oogenesis in many species. Previous studies in the Drosophila ovary demonstrated that loss of either Cullin 2 (Cul2) or Cullin 5 (Cul5), which encode scaffolding proteins central to CRL complexes, results in abnormal follicles and increased follicle death. It is unknown how Cul2 and Cul5 regulate follicle formation, and whether they function redundantly in this process. We tested whether the Cul5-containing CRL (CRL5) is necessary for early follicle development by analyzing loss-of-function mutants of the ligase complex. Loss of Cul5 or the RING protein Roc2 resulted in fused follicles, ruptured follicular epithelium, and improper encapsulation. Unlike Cul2 mutants, germline differentiation is not altered in the absence of Cul5. Instead, CRL5 is independently required in follicle cells in the posterior germarium for cyst encapsulation. Loss of Cul5 or Roc2 slows follicle cell movement and intercalation as cysts acquire a follicular epithelium, but does not impact follicle stem cell self-renewal or the differentiation of pre-follicle cells. Taken together, our data indicate CRL5 regulates follicle development by promoting the formation of stalks, creating individual follicles. We are currently testing whether Cul5 regulates JAK-STAT, Notch/Delta, or Wnt signaling, which are known to facilitate stalk formation. Our study highlights the role of CRLs in early follicle development, and may lead towards a better understanding and treatment of infertility.

Poster Abstracts

POSTER SESSION I Abstracts 1-22

Presenting author institution indicated

1

N-Modified Creatine as a Treatment for SLC6A8-Related Autism Spectrum Disorders

Amy Anderson, Modi Wetzler, Kristyn A. Robinson, Mary Katherine Sullivan, Susan C. Chapman
Clemson University

Creatine deficiency syndrome is caused by a mutation in the creatine transporter gene SLC6A8 and results in X-linked intellectual disability and autism spectrum disorder. SLC6A8 mutations are estimated to be as common as 1-5.4% of all X-Linked Intellectual Deficiencies, with this disorder affecting 42,000 patients in the USA and one million worldwide, but without comprehensive genetic testing, this number is likely underestimated. No treatment modality exists, as supplemented creatine cannot cross the blood brain barrier (BBB) or enter neuronal cells without a functioning transporter. Failure of creatine transport into the brain has significant impacts on energy metabolism, as creatine's phosphylated form is an essential part of the high energy buffering system used to maintain ATP levels in brain tissues. This research introduces a new way to directly screen large numbers of creatine analog compounds in the simple, tractable zebrafish system, in a cost- and time-efficient manner, and has identified potential treatment modalities where none currently exist. We synthesized a new class of N-modified creatine analogs and using high throughput zebrafish screening have accelerated identification of creatine analogs that can cross the BBB, bypassing deficient SLC6A8 transporters. These analogs were biochemically assayed and showed 87-95% efficiency binding creatine kinase and producing phosphocreatine, compared to native creatine. 48-hour embryos were exposed to 125 uM creatine analogs for 24 hours. Brain tissue was isolated, and the amount of creatine analog in the brain was quantified by mass spectrometry. Analogs that both cross the BBB and interact with creatine kinase will be tested in SLC6A8 mutant mice before moving to preclinical trials. Other essential molecules in brain (or somatic) function with defective transporters will benefit from this approach, making our study applicable to the broader field of metabolic imbalance resulting from transporter defects.

2

Bioinformatic and genetic approaches to understanding the *cnd-1* regulatory network

Wendy Aquino Nunez, Zachery Mielko, Derrica McCalla, Elyse Christensen, Ciara Hosea, Kaylee Bronson, Victoria Owens, Martin L. Hudson
Kennesaw State University

NeuroD1, the vertebrate ortholog of cnd-1, is a basic-Helix-Loop-Helix protein involved in neuronal and pancreatic beta cell fate specification. NeuroD1 loss-of-function mutations have been implicated in human visual impairment, learning disabilities, deafness, and neonatal diabetes. In *C. elegans*, cnd-1 is expressed in many unidentified head neurons and also in D-class motor neurons. Only three genes are known to act downstream of *cnd-1* in *C. elegans*; *unc-3*, *unc-4*, and *unc-30*. All three of these genes are transcription factors that are expressed in D-class motor neurons. However, the remaining downstream targets of *cnd-1* have not been identified. In order to investigate the regulatory role of cnd-1, we performed RNA-extractions from wild-type and *cnd-1* loss-of-function embryos, followed by RNAseq. We assembled a transcriptome outlining differentially expressed genes during embryogenesis and are currently following up by validating candidate *cnd-1* target genes using genetic approaches. In addition, NeuroD1 is known to function in a regulatory cascade with neurogenin and also to positively regulate itself in mammalian neuron specification. We seek to verify this relationship in *C. elegans* in order to better understand the regulatory context of our novel *cnd-1* target genes.

3

***Drosophila* Rbf regulates mitochondrial functions in developing muscles.**

Maria Chechenova, Kaveh Kiani, Anton Bryantsev
Kennesaw State University

The Retinoblastoma tumor suppressor (RB) regulates cell cycle progression through the suppression of E2F/DP heterodimeric transcription factors. Functional inactivation of RB causes tumor progression in a majority of human cancers. Having an understanding of the molecular machinery involved in both uncontrolled cell growth and cell differentiation is important for further development of treatment options for oncological patients. In *Drosophila*, homologues of mammalian RB, Rbf, and its targets, E2f1/Dp, are critical for cell proliferation and apoptosis. In addition, a conservative role of E2f1/Dp in mitochondrial functioning associated with cell differentiation and tissue development was described both in mammals and flies. Here we investigate whether mitochondrial effects of E2f1/Dp functioning in *Drosophila* developing muscles are directly controlled by Rbf. Using RNA interference approach and the UAS/GAL4 system, we reduced Rbf expression in developing muscles down to 27% of the control transcript levels. Such a deficit of Rbf expression resulted in a significant decrease in the expression of the mitochondrial genes accompanied by changes in mitochondrial morphology. Likewise to reported earlier phenotypes for Dp and E2f1 mutants in *Drosophila*, and *Rb1* deficient mouse embryonic fibroblasts, *Rbf* mutants had thin or fragmented mitochondria. Functionally, these flies showed progressive flight impairments suggesting that mitochondria-enriched flight muscles are compromised. Our data suggest that Rbf is involved in the regulation of the mitochondrial program in muscles, which affects proper muscle maturation in flies. Taking into account the conservative function of *Rbf*, we will use *Drosophila* model to analyze molecular mechanism that enable Rbf to control mitochondrial genes.

4

Reprogramming Somatic Cells into iPSCs by Novel Cell Penetrating Peptide-Adaptors

Kelsey Clearman, Martin Hudson, Jonathan McMurry
Kennesaw State University

Human disorders and diseases have been captivating the minds of scientists and physicians as long as they have existed. This captivation has led to the discovery of new technology that may eliminate diseases such as degenerative diseases. Cellular reprogramming and the creation of induced pluripotent stem cells (iPSCs) are examples of this young technology. Many advances of this technology have been accomplished through the use of mRNA, viral vectors, and most recently cell penetrating peptides (CPP). Reprogramming by these mechanisms contain many pitfalls, however, including the introduction and possible integration of foreign DNA into cells, tumorigenic effects, and the inability of protein translocation as a result of inefficient CPPs. However, the creation of a novel CPP delivery mechanism has eliminated the need for mRNA, viral vectors, as well as inefficient CPPs. This novel CPP technology utilizes noncovalent bonds between calmodulin and its binding sequence in the presence of calcium, unlike previous CPPs that relied on covalent bonds. Using this CPP adaptor fusion protein, TAT-calmodulin (TAT-CaM) in association with reprogramming proteins, there should be an overall increase in reprogramming efficiency as well as decreased harm to the cells. Preliminary data shows that Oct4, the main transcription factor involved in pluripotency, contains an internal CPP and can enter cells without the aid of an additional CPP. Oct4 and Oct4 bound to TAT-CaM appear to induce pluripotency around the same efficiency. However, the other reprogramming proteins that will be used, Sox2 and Klf4, cannot enter cells without a CPP adapter and thus will require our TAT-CaM mediator to induce pluripotency. Combined, the three reprogramming factors fused with TAT-CaM are expected to yield a higher efficiency of reprogramming compared to previous methods.

5

Investigating the molecular control of the ecdysone response gene, E74, in *Drosophila* ovarian germ cells.

Lindsay Davenport
East Carolina University

Oogenesis is the process by which an egg develops from precursor cells in the ovary. This process has been widely studied; however, many of the molecular mechanisms that regulate oocyte development and growth remain unclear. The *Drosophila melanogaster* ovary is an exceptional model system for studying the mechanisms of oogenesis. As in humans, germ cells are surrounded by somatic cells which aid proper oocyte development and maturation. Steroid hormones largely drive this process, and in *Drosophila*, the predominant steroid hormone is ecdysone, similar to human estrogen. Ecdysone binds to a heterodimeric receptor which then functions as a transcription factor to promote gene expression. Other factors, including additional transcription factors and chromatin remodeling factors, likely refine this response. Ecdysone signaling is necessary for oogenesis via the regulation of many target genes. One target, *Ecdysone-induced protein at 74EF (E74)*, is required for oogenesis and is highly expressed in ovarian germ cells; however, regulation of E74 expression in the ovary has not been well-studied. To investigate how *E74* expression is regulated in the ovary, we used enhancer mapping to identify regions of the *E74* locus critical for germline expression. Twenty-eight fly lines carrying pieces of the *E74* gene locus fused to a minimal promoter and Gal4 were crossed with flies containing *UAS-lacZ* responder transgene. We identified two 200-bp regions within a large intron of the *E74* locus that are sufficient to drive expression of a reporter. Together, these regions fully recapitulate the endogenous *E74* expression pattern. We then identified several factors, including the chromatin binding factor Trl, as putative regulators of *E74* expression at those sites. *Trl* expression partially overlaps with that of *E74*, suggesting that Trl may be an important modifier of ecdysone signaling in oogenesis. Future studies will characterize the roles of Trl in this process.

6

Understanding animal polarity: Functional studies during early embryogenesis of the sea anemone *Nematostella vectensis*

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How germinal layers are specified during early development of non-bilaterian animals is unclear. In bilaterian animals, rearrangements of the egg's cytoplasm and cortical domains polarize the embryo and direct proper partitioning of maternal determinants into distinct daughter cells often in relationship to a regular cleavage program. In some bilaterian animals, Lethal Giant Larvae (LGL) and PARtitioning-defective proteins (Par) are conserved components of cellular polarization during early embryogenesis. Par proteins and their role in establishing embryonic asymmetry have been widely studied in bilaterian development but not in more basally branching animals. Interestingly, the basally branching cnidarian sea anemone *Nematostella vectensis* shows a "random" cleavage pattern and it undergoes gastrulation at the animal (not vegetal) pole of the egg; begging the question of whether the same molecular mechanisms are conserved for specifying the site of gastrulation. We address this question by characterizing the localization and function of different components of the Par complex during early development of the sea anemone *N. vectensis*. The mRNAs of Par proteins are asymmetrically localized. However, Immunostaining using antibodies made against NvLGL and NvaPKC shows that these proteins distribute throughout the egg and embryo without any clear polarization confirming results obtained when we over expressed them using mRNA injections. In addition, the over expression of the full length and dominant negative version of some Par proteins affect cleavage divisions and gastrulation but do not have a clear effect on embryonic polarity. These data will provide a glimpse into the evolution of cell polarity and the organization of metazoan embryonic germ layer formation.

7

Investigation into the cellular origins of posterior regeneration in the annelid *Capitella teleta*

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Many animals can regenerate, although there is great diversity in relative regenerative capabilities. For example, some animals are able to regenerate their complete body from only a few thousand cells (e.g. planarians), while others can regenerate only a single cell or tissue type (e.g. vertebrates). One of the most intriguing questions in regeneration biology is the cellular source of new tissue that is formed. The polychaete annelid, *Capitella teleta*, displays robust posterior (but not anterior) regeneration following transverse amputation of body segments. However, the source, behavior and molecular characteristics of the cells that form new tissue during regeneration are largely unknown. We hypothesized that the putative primordial germ cell (PGC) niche in *C. teleta* is a source of multipotent progenitor cells. We postulate that following transverse amputation, cells from the PGC niche migrate to the site of injury, and contribute to the regeneration of somatic structures. We used the expression of *CapI-vasa* as a marker of the PGC niche to examine the characteristics of this cell population, and its dynamics in the first days following transverse amputation in juveniles. *CapI-vasa* also marks dispersed cells in the coelomic cavity of juveniles. We investigated the hypothesis that these cells are a migratory population originating from the PGC niche. To test whether there is cell migration into the wound site during *C. teleta* posterior regeneration, we used an indirect method involving incorporation of EdU. Finally, we assessed the relative capacity for posterior regeneration in juveniles with and without the PGC niche, by analyzing nerve extension, cell proliferation and number of regenerated segments as markers of relative regenerative capability.This work is the first study in *C. teleta* that addresses the potential source of cells contributing to regeneration of posterior segments, and establishes essential groundwork for future studies.

8

Epigenetic Contributions to Homologous Chromosome Recognition During Meiosis

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During meiosis, homologous chromosomes must correctly identify one another in order for proper alignment and recombination to occur. Improper pairing can lead to chromosomal rearrangements that can result in defective embryonic development. Currently, little is known about how homologs identify each other to the exclusion of the other chromosomes. There has been evidence supporting the role of DNA Double Strand Breaks (DSB) and strand invasion in homolog recognition. However, mutants lacking DSBs still have the ability to properly align homologs suggesting a DSB independent mechanism exists. In *C. elegans*, Pairing Centers (PC) initiate pairing between homologs. Despite their sequence specificity, some PCs are shared between non-homologous chromosomes further suggesting an additional mechanism for recognition. During meiosis distinct patterns of active transcription are produced on each chromosome and are associated with epigenetic modifications such as the methylation of Lysine 36 on Histone H3 (H3K36me). In humans, H3K36me is recognized by the chromodomain containing protein MRG15. Recently, pairing defects were observed in *C. elegans* lacking the MRG15 homolog, *mrg-1*. The specific role of MRG-1 in homolog pairing and recognition is unknown. Our hypothesis is that histone modifications that result from meiotic transcription, including H3K36me, provide an "epigenetic barcode" used to distinguish chromosomes during homolog searching, and is facilitated through the recognition of H3K36me by the chromodomain of MRG-1. We are examining the role of H3K36me in homolog recognition in germlines lacking *mes-4* and *met-1*, the histone methyltransferases responsible for H3K36me. Our recent data demonstrates that germ cells lacking H3K36me exhibit increased sterility and synaptic delay. Similar observations are seen in *mrg-1* mutants. These results suggest that epigenetic modifications such as H3K36me may play an important role in homologous chromosome recognition during meiosis.

9

SF1 and SF2 boundary functions are essential for *Scr* and *ftz* gene regulation

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Chromatin structure plays important roles in gene regulation. In particular, chromatin boundary elements (CBEs), which are genomic regions that interact with each other in the 3-D nuclear space to form chromatin loops, are known to inhibit or promote transcription by modulating access of enhancers to gene promoters. We have previously identified SF1 and SF2, two CBEs in the *Drosophila* homeotic gene (Hox) cluster, which determine the animal body segment identity. SF1 and SF2 flank and separate a non-Hox gene *fushi tarazu* (*ftz*), from the surrounding Hox genes *Sex comb reduced* (*Scr*) and *Antennapedia* (*Antp*). We hypothesized that the loop formed by SF1 and SF2 insulate *Scr* from the *ftz* early enhancers, while facilitating the late *Scr* enhancers to their promoter. To test this hypothesis, we created SF1 and SF2 knockouts using the CRISPR techniques and investigated the *Scr* and *ftz* regulation in these mutant animals. We found Scr to be ectopically expressed in the ftz pattern in the SF1 knockout animal during early embryogenesis, while in the late embryo the Scr expression is normal. Intriguingly, in the SF2 knockout, ftz is ectopically expressed in the Scr pattern in late embryos, while the early ftz expression is normal. Our evidence further indicates that these ectopic expressions contribute to developmental defects and increased lethality in the knockout animals. These results support our hypothesis that the CBEs organize the 3-D genomic architecture and play critical roles in tissue and stage specific gene regulation during animal development.

10

Retrotransposons in mammalian egg-to-embryo transition

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Retrotransposons profoundly impact mammalian gene expression, notably in germ cells. Previously, we reported LTR retrotransposons are massively upregulated during the egg-to-embryo transition in mice, and initiate synchronous, temporally-regulated expression of multiple genes (Dev Cell 7:597-606). Recent experimental work demonstrates co-option of retrotransposon promoters directly impacts mouse oocyte quality (Cell 155:807-816). We propose that transposons' activity during egg-to-embryo transition is important component of genome reprogramming network, including establishment of novel functional gene modules critical for early development. We hypothesize these networks may vary dramatically among different mammals.

We analyzed RNAseq data of total oocyte and preimplantation embryo transcriptomes to gain insight into transposon contribution to transcriptomes. We analyzed data for three mammalian species, laboratory mice, cows and humans. We programmed pipelines (1) to identify transposons expressed during egg-to-embryo transition, and (2) to discover and map "chimeric" gene transcripts containing alternative transposon-derived exons. Additionally, we applied Gene Ontology (GO)-based pathway enrichment tools to find specific gene network modules.

Our analysis demonstrated profound dissimilarities in classes of transposons expressed during oogenesis and early development among three species, and alternative promoters from transposons drive a large proportion of expressed genes (7%) in all three species. Remarkably, subsets of transposon-driven genes are significantly different among mammals. GO enrichment analysis revealed distinct modules among transposon-driven genes in each species. The profound dissimilarities among gene sets and modules indicate independent origins putatively shaped by natural vs artificial selection. Our findings underscore mammalian oogenesis as an evolutionary "playground" to select for "viable" modules in gene networks.

11

Sex specific dynein requirement in *C. elegans* meiosis

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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. My preliminary results indicate that male and female *C. elegans* have different requirements for dynein components 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 abnormal SYP aggregate formation 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. Understanding meiotic regulation, and sex specific differences in regulation using a genetically tractable organism will help us better understand natural and disease states in humans that lead to an increased incidence of aneuploidy and meiotically based infertility.

12

The Role of Matrix Metalloproteinases in *Xenopus leavis* Neural Crest EMT and Migration

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The neural crest is a unique cell population found in developing vertebrates, characterized by its multipotency and migratory capacity. During their migration, they first detach from the neuroepithelium through epithelial-to-mesenchymal transition (EMT), then remodel the extracellular matrix (ECM) along their path, resembling the behaviors of metastasizing cancer cells. To explore how neural crest cells remodel the ECM during their migration, we looked at an important matrix metalloproteinase, MMP14 (or MT1-MMP), which is highly expressed both in many malignant cancers and in migrating neural crest cells. As a transmembrane protease, MMP14 plays critical roles in activating other MMPs (such as MMP2), processing various matrix proteins, and mediate signaling events with other cell surface receptors. By loss-of-function approaches, we found that MMP14 is required for cranial neural crest cell migration into the branchial arches. This function in promoting neural crest migration requires both the proteolytic activity of MMP14 and its signaling activity through the PEX domain. To determine whether the surrounding tissues hinder the requirement of MMP14 in neural crest migration, we isolated the cranial neural crest tissue and cultured them in vitro. In culture, loss of MMP14 does not inhibit the spreading of neural crest cells, but cells cannot break apart from each other and migrate individually. So similar to different ADAM proteins, MMP14 may also regulate the expression or turnover of cell adhesion molecules, such as Cadherins.

13

Finding key causative genes in muscle wasting

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Systemic wasting of body mass is a hallmark of cancer. Muscle degeneration particularly presents significant complications in cancer treatments, as it precludes administration of chemotherapy and/or surgical interventions. In many cases, progressive muscle weakness can become a primary cause of mortality in cancer patients. Most commonly seen in sufferers of gastric and pancreatic cancers, tissue degeneration proceeds regardless of increased nutritional uptake. The exact mechanisms behind this wasting are not yet understood, but if discovered, may inform the process of clinical treatment development. When tumors are experimentally induced in the gut of *Drosophila melanogaster*, the flies exhibit targeted degeneration of flight muscles, while other muscle types remain relatively intact. To determine molecular factors that mediate such specific muscle degradation, we have analyzed the changes of gene expression in cancer-responsive muscles before and shortly after the onset of tumor. Based on our analysis, we have selected candidate genes whose activity significantly changes in the presence of experimental tumors. These genes belong to various regulatory networks controlling transcription, hormonal signaling, mitochondrial respiration, and proteolytic degradation. Using the advantage of the *Drosophila* model, we then modulate expression of our candidate genes in an on-or-off manner to recapitulate muscle degradation and death. Because of significant evolutionarily conservation of the candidate genes, our study is aimed at revealing novel genetic components in muscle wasting across species.

14

Generating *mef2ca* and *mef2cb* transgenic zebrafish lines using BAC-mediated recombination and CRISPR/Cas9-mediated integration

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The genes *mef2ca* and *mef2cb* (myocyte enhancer factor 2c a and b) are important for craniofacial, muscle, and heart development in zebrafish. The goal of this project is to generate transgenic zebrafish lines expressing fluorescent markers under the control of endogenous regulatory elements for *mef2ca* and *mef2cb*. These lines will allow us to study the pattern of expression of *mef2ca* and *mef2cb* in development and track the function of these factors in specific tissues and cells in embryos. Using BACs (bacterial artificial chromosomes) containing *mef2ca* and *mef2cb*, Phusion PCR products containing the fluorescent reporter genes EGFP and mCherry will be integrated near the start codon of *mef2ca* and *mef2cb* using homologous recombination. Likewise, CRISPR/Cas9 will be used to 'knock-in' a fluorescent transgene into *mef2ca* and *mef2cb* in vivo so that endogenous regulatory sequences drive transgene expression. These lines should provide insight into the dynamic expression of *mef2ca* and *mef2cb* in cells and tissues throughout development of the zebrafish.

15

The role of Wnt Inhibitory Factor-1 in the Wnt signaling network governing anterior-posterior patterning of sea urchin embryos

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The specification and patterning of the anterior-posterior (AP) axis in many metazoan embryos is dependent on a posterior-to-anterior gradient of Wnt signaling. In the sea urchin embryo, patterning of the anterior neuroectoderm (ANE) along the AP axis is dependent on a network involving three interconnected Wnt signal transduction pathways: Wnt/ß-catenin, Wnt-JNK, and Wnt/Ca2+. While much has been learned about the roles of each of these individual Wnt signaling branches in development and disease, our understanding is still limited and little is known about how they interact with one another in any context. Wnt inhibitory factor-1 (Wif-1), one of the least understood secreted Wnt signaling modulators, has been shown to bind Wnt ligands resulting in Wnt/ß-catenin signaling inhibition. In this study, we report that *wif*-1 is zygotically expressed in two different germ layers during early AP and dorsal- ventral (DV) patterning in the sea urchin embryo: the endomesoderm and the dorsal ectoderm. Pharmaceutical manipulations suggest that wif-1 expression is activated by Wnt/ß-catenin signaling in the endomesoderm and a mechanism dependent on Nodal signaling in the dorsal ectoderm. We show that perturbing Wif-1 function disrupts gastrulation and specifically perturbs the correct positioning of the ANE along the AP axis, possibly though the inhibition of the Wnt/Ca2+ pathway. Together, these data suggest Wif-1 may represent an important and direct link between the gene regulatory networks that control AP and DV patterning in the early sea urchin embryo.

16

The Eph receptor/ephrin pathway is required for AIY interneuron development and food-seeking behavior

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In order to survive, an organism must be able to receive, integrate, and respond to sensory stimuli. However, the cellular basis of sensory perception and response is difficult to study in complex animals such as humans, and is therefore poorly understood. The nematode *Caenorhabditis elegans* is a relatively simple organism yet displays many distinct behaviors, making it an ideal system to understand the relationship between gene function, cell shape, cell physiology, and behavioral output. Much of the thermosensory and chemosensory information that the nematode receives from its sensory neurons is processed via a pair of interneurons called AIYL and AIYR. In wildtype animals, the AIY cell bodies lie just posterior to the pharynx, and extend an anterior process that contacts its contralateral partner at the base of the nerve ring. The AIYL and R processes then diverge and extend around the nerve ring, ultimately making contact again on the dorsal side of the animal via a gap junction. We previously showed that the Eph receptor tyrosine kinase VAB-1 is required for AIY cell body placement and ventral AIYL/R contact. Conversely, the ephrin EFN-4 is required for dorsal AIYL/R connectivity. We have extended these studies and show that the AIYL/R ventral contact is mediated via the ephrin gene efn-1. In addition, we show that this connectivity requires both vab-1 kinase activity and also a non-kinase dependent vab-1 function. To integrate AIYL/R morphology and function with behavior, we are using WormLab software to image and analyze *EphR/ephrin* mutants both on and off food. Wildtype animals search for food using long "runs" interspersed with reversals and ~170-degree "omega" turns. We find that *vab-1* mutants display a strong circling locomotion, both on and off food. We are currently investigating neuromuscular junction morphology in *EphR* and *ephrin* mutants to see if this correlates with dorsal versus ventral locomotion bias.

17

Fin-folds and autopods share a conserved Shh-Gremlin-Fgf regulatory network

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The morphological transition from fins to limbs involved the loss of the fin-fold (dermal rays) and expansion/ remodeling of the distal endoskeleton to form an autopod (hands/ feet) with digits. Recently, we've made observations of an autopodial-like pattern of HoxD expression in paddlefish fin-folds and functional studies in zebrafish demonstrating a role for 5'HoxA/D genes in dermal ray formation. These insights led us to ask if other components of autopod regulatory networks are also involved in fin-fold development. The gene regulatory networks that integrate limb bud outgrowth and patterning have been partially characterized in tetrapods, revealing molecular interactions between the posterior limb bud mesenchyme (i.e., the zone of polarizing activity, ZPA) and the distal limb bud ectoderm (the apical ectodermal ridge, AER). In this network, ZPA-derived Sonic Hedgehog (Shh) acts through LIM-homeodomain transcription factors (LHXs) to induce the BMP antagonist Gremlin. Gremlin, in turn prevents BMP inhibition of AERderived Fgfs, which maintain ZPA-Shh, which are required for proper patterning of the digits. According to Thorogood's influential "clock" model, delay in conversion of the AER to a fin-fold prolongs the signaling influence of the AER on the endoskeletal mesenchyme, resulting in expansion of fin radials and a reduction of the dermoskeleton. Limbs, which lack a dermoskeleton, reflect the extreme of this hypothesis in that the AER (and its proliferative cues) persist through autopod formation. Herein, we test this hypothesis in the American paddlefish, *Polyodon spathula* through a survey of expression of Shh-Gremlin- Fgf transcriptional network components and use these data to evaluate predictions of clock-based models of appendage evolution.

18

Abnormal cardiac patterning and development in *akirin* mutant embryos

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Akirin is a highly conserved nuclear transcription co-activator that is essential for proper Twist-regulated gene expression during the embryonic myogenesis program. While Akirin has previously been shown to co-regulate the patterning of the skeletal musculature, recent studies have implicated Akirin as a crucial regulator of the *tinman* locus during specification and patterning of the cardiomyoblasts, the muscle cells that will form the dorsal vessel or heart. akirin mutants display a highly disorganized dorsal vessel, marked by missing cardiomyoblasts, and highly aberrant morphology. We are currently employing fixed embryo and live-imaging techniques to image heart formation in akirin mutant embryos, as well as dorsal vessel contraction in akirin mutants. Our results indicate that *akirin* mutant hearts are patterned abnormally from the onset of cardiac specification, and that the migration of cardiomyoblasts appears to be negatively affected as a result of a loss of Akirin. Given that Akirin is a highly conserved protein among metazoans, it is likely that these results provide a novel mechanism for cardiac specification and patterning that is similarly conserved from insects to mammals.

19

Receptor ALK2, but not ALK3, mediates the regulatory role of BMP signaling in taste organ formation in a mesenchyme-specific manner

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Taste organs, among many epithelial appendages such as feathers, hair follicles, and teeth, require mesenchymal-epithelial interactions for proper development. Among the many molecules and pathways, regulatory roles of bone morphogenetic proteins (BMPs) are important. However, little is known about the involvement of receptor(s) mediating BMP signaling in taste organ development. In the present study, we used mouse models with constitutively activated (ca) BMP receptors in a mesenchyme-specific manner driven by Wnt1-Cre to examine the roles of type I BMP receptors ACVR1 (ALK2) and BMPR1A (ALK3) in the development of tongue and taste papillae. Wnt1-Cre driven caAlk2 and caAlk3 mutants at embryonic day (E) 10.5-11.5 had no observable morphological differences in the embryos compared to littermate controls. At E12.5-P1, caAlk2/Wnt1-Cre tongues were smaller, misshapen, and missing the pharyngeal region compared to littermate controls. In contrast, no obvious change was seen in caAlk3/Wnt1-Cre mice. We found that in caALK2/Wnt1-Cre tongues, fungiform papillae and early taste buds formed in the smaller tongue at E18.5. Immunoreactions on sections using the cell markers E-Cadherin, Vimentin and Desmin showed that both the epithelium, mesenchyme and muscles were disorganized in caAlk2/Wnt1-Cre mouse tongue at E14.5 and E18.5 compared to the littermate control and caAlk3/Wnt1-Cre mutants. Furthermore, caAlk2 and caAlk3 driven by K14-Cre (epithelium-specific) did not lead to an apparent phenotypic changes in taste organs. Our data indicate that BMP signaling plays an important roles in the development of taste organs in a receptor- and tissue-specific manner.

20

Gastrointestinal Motility Issues Associated with Autism Spectrum Disorders

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Autism Spectrum Disorders (ASDs) are currently estimated to affect 1-2.6% of children world-wide. Along with the behavioral and developmental issues associated with ASD, gastrointestinal (GI) distress is a commonly reported but poorly understood co-occurring symptom. As a first step towards determining the mechanisms behind ASD related GI disorders, we are using a zebrafish ASD model to gain insight on how genetic variants with high autism relatedness impact GI function. We focus on the high-confidence ASD gene *SHANK3*; deletions that include *SHANK3* are causal for Phelan-McDermid Syndrome (PMS), a form of ASD with GI distress reported in nearly 50% of cases. The parallels between zebrafish and human GI physiology provide a basis for understanding mechanisms that underlie ASD associated GI distress. As with many zebrafish orthologs of human genes, the human *SHANK3* gene is duplicated in Zebrafish. Retention of the two gene copies has been shown to reflect sub-functionalization; consistent with this, *shank3a* is expressed at higher levels in the brain than *shank3b* at early developmental (in situ and qPCR), while *shank3b* is expressed in a more ventral domain corresponding to the upper GI tract. Using a *shank3* loss-of-function zebrafish I have compared changes in GI motility between *shank3ab* mutants and WT fish; tracking and quantifying differences in the frequency of peristaltic movements within the GI tract. Data collected thus far shows that *shank3* zebrafish mutants display a significantly slower frequency of peristaltic contractions compared to their wild type counterparts. This is a highly penetrant phenotype since a single *shank3b* mutant allele is sufficient to significantly reduce the peristaltic frequencies. Our motility findings may relate to GI distress common to ASD in people based on unpublished clinical studies showing delayed digestive transit times in PMS patients

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mPing as a tool for activation tagging in zebrafish

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The goal of this project is to demonstrate the successful in vivo transposition of the mobile element mPing, from *Oryza sativa* (rice), in zebrafish. mPing is a 430-bp, class II miniature inverted-repeat transposable element (MITE), which is mobilized by two enzymes: ORF1, which contains a DNA recognition domain, and TPase, which contains a catalytic DDE domain. mPing, like many invertebrate transposons, has yet to be tested for activity in a vertebrate organism, yet may serve as an effective tool for transposon mutagenesis in vertebrates, such as zebrafish. A single *iTol2* expression vector, containing beta actin promoter-driven mCherry (interrupted by an mPing derivative called mmPing20x), will be co-injected with mRNAs for Tol2 transposase and ORF1-T2A-TPase (Pong transposase). The expression vector also contains a cmlc2:EGFP transgenesis marker labelling cardiac cells, to verify transgenesis. The rate of successful transposition will be determined in injected F0 fish by measuring the ratio of mCherry-positive fish to the number of fish with cardiac EGFP expression. We will establish whether it is possible to remobilize the mmPing20x elementin subsequent generations via injection of ORF1-T2A-TPase mRNA into individuals carrying the transposable element. The results of this study will form the basis for future research to use mmPing20x containing a *Xenopus*-derived EF2α enhancer as an activation tag in zebrafish as a tool for gene discovery.

22

Identification of Akirin interacting partners during embryonic myogenesis.

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The specification and differentiation of muscle precursor cells, or myoblasts, by the action of the Twist mesodermal and muscle transcription regulator is a key event in the formation of the *Drosophila* larval musculature. Myoblast population dynamics are tightly controlled by gene expression moderated by this myogenic transcription factor to determine somatic cell fates. Despite the primary importance of myoblast mechanics for building and patterning the musculature, the identities of many molecular players involved in this process remain unknown. Recently we have determined that Akirin, a highly conserved nuclear protein, appears to play a critical role in the regulation of Twist-dependent gene expression during mesodermal specification and muscle development. We hypothesize that Akirin serves as a cofactor to promote interactions between regulatory transcription factors and chromatin remodeling activity to impact gene expression across varying targets. Using a genetic interaction screen in Drosophila, we have begun to identify Akirin interacting proteins that participate in the process of muscle specification, patterning, and development. Our screening method has identified a number of proteins that genetically interact with Akirin during muscle patterning in the embryo. Double heterozygous mutant embryos for *akirin* and one of these potential partners demonstrate a host of deranged or misshapen muscle phenotypes. Thus far we have uncovered a small number of predicted gene products that appear to be involved in general transcription initiation, as well as components of chromatin remodeling complexes. By generating an interactome of its potential partners, we will gain crucial insight into Akirin's mechanism of molecular action during myoblast specification and muscle patterning.

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Signed, Sealed, and Delivered: CPP Technology Delivers Active Protein Cargo

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TaT-CaM is a recently developed cell penetrating peptide-linker construct that has been shown to successfully penetrate cells with protein cargos of various sizes and properties. This technology uses the TaT cell penetrating peptide (CPP) covalently attached to calmodulin (CaM) which binds specifically to target peptides in high calcium. Advantages include tight but non-covalent binding between CaM on the TaT CPP and its calmodulin-binding-site-tagged cargo in high Ca+2 buffer conditions, followed by release of cargo from the CPP after delivery into cells in which cytoplasmic Ca+2 concentration is low. Cellular membrane penetration and localization of various proteins with specific trafficking signals has been demonstrated previously. Here we explored the ability of enzyme cargo delivered by the TaT-CaM system to retain activity after cell penetration. Confocal imaging of fluorescently labeled catalase demonstrated successful penetration of BHK cells. Imaging of cells transfected with peroxisomal marker CellLight® Peroxisome-GFP and subsequently incubated for as little as 20 minutes with the TaT-CaM-catalase complex (TaT-Cat) indicate that delivered catalase localizes to the peroxisome. Cell lysates of TaT-Cat treated cells have significantly more catalase activity than control cells, demonstrating successful delivery of active enzyme. We are exploring the effect of the delivered catalase on cells subjected to oxidative stress, in this case treatment with H2O2.

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Teratogenic effects of ethanol during in vitro maturation alter gene networks that persist through later stages of development

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Although the deleterious developmental effects of chronic alcohol consumption during pregnancy are well known, on the contrary, the consequences of acute alcohol consumption near conception are controversial. During this developmental period changes in epigenetic modification occur genome-wide, thus ethanol exposure may interfere with formation of proper epigenetic marks. Given the pluripotency of the embryo such misprints may be inherited throughout the body potentially leading to pathologies seen in Fetal Alcohol Syndrome via cellular metabolism. The objective of this study is to identify genes whose expression is altered by ethanol exposure near conception using in vitro egg maturation (IVM) techniques. Changes in gene expression were detected by microarrays (Affymetrix 430 v2.0) and bioinformatics analysis (Gene Ontology, VLAD, MetaCyc etc) in GV, metaphase II oocytes, 2-cell, 8-cell and blastocysts exposed to ethanol (0, 0.05, 0.1% v/v) during oocyte maturation only (FGO stage exposed). These levels mimic blood alcohol concentration after moderate and high (legal intoxication) drinking. Ethanol exposure decreased genes regulating the initiation of translational (Eif2, Eif3, Eif4, and Eif5 family members) from oocytes throughout pre-implanation embryonic development, when typically de novo RNA translation is observed. Many maternal transcripts persisted at the 2-cell stage embryos exposed to ethanol compared to control also suggesting deficiency in mRNA turnover. Likewise, DNA methyltransferases and histone demethylases were also decreased after ethanol exposure from the egg-to-embryo transition. Many genes involved in cellular stress such as mitochondrion transport and heat shock proteins, were increased after ethanol exposure. In summary, ethanol exposure near conception can induce changes in gene expression that persistent to pre-implantation stage altering mitochondrial, translational control, and epigenetic regulators.
Funding: Impact Assets, CT.

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ZNF845, a C2H2 zinc-finger transcription factor, is required for cnidocyte (stinging cell) development in the sea anemone *Nematostella vectensis*

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Cnidocytes, (stinging cells) are one of the few clear examples of a truly novel cell type. Found only in cnidarians (corals, jellyfish, hydroids, etc), cnidocytes vary in both morphology and function across taxa and are therefore an important diagnostic feature of this group. Development of the explosive organelle (the cnidocyst) requires the expression of several cnidarian-specific structural proteins but expression of these novel genes seems to be regulated by conserved families of transcription factors. In the sea anemone *Nematostella vectensis*, cnidocytes differentiate from a SoxB2-expressing progenitor cell, which also gives rise to neurons. Previously, we have shown that transcription factors from two gene families which are conserved across metazoans (PaxA and Mef2) are required for terminal differentiation in two lineages of cnidocytes in *N. vectensis*. In this study, we show that ZNF845 (a C2H2 zinc finger transcription factor) is expressed throughout the ectoderm during embryogenesis, in a pattern consistent with cnidocyte development and that knockdown of *ZNF845* results in loss of cnidocytes. We further demonstrate that *ZNF845* is downstream of *SoxB2* and upstream of *PaxA*/*Mef2* as knockdown of the former, but not the latter, results in loss of *ZNF845*-expressing cells. Finally, we show that *ZNF845* is not expressed in the *SoxB2*- or the *PaxA*/*Mef2*-expressing cells, suggesting *ZNF845* specifically labels an intermediate, cnidocyte-specific, progenitor cell between the *SoxB2*-expressing "neural" progenitor cell and the differentiating cnidocyte. In the hydrozoan *Hydra vulgaris*, ZNF845 labels a population of hydrozoan-specific stem cells (I cells) which give rise to cnidocytes, neurons, gland cells, and gametes. These results suggest that *ZNF845* may have had a role in specifying progenitor cell identity in the stem cnidarian but that its function may have become specific to cnidocyte progenitor cells in the lineage leading to anthozoans (corals and sea anemones).

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Wnt16-Fzl1/2/7-NFAT signaling antagonizes the restriction of the anterior-posterior neuroectoderm in the sea urchin embryo

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Anterior neuroectoderm (ANE) specification, positioning, and patterning is a crucial event in body plan formation in all deuterostomes. Studies from diverse metazoan embryos indicate that Wnt/ß-catenin signaling is essential for the specification and patterning of the neuroectoderm along the primary axis. In early development of the sea urchin embryo, ANE positioning depends on integrated information from the Wnt/ß-catenin, Wnt/JNK, and Wnt/PKC pathways, forming an interconnected Wnt signaling network. We have previously shown that Fzl1/2/7-PKC pathway antagonizes the down-regulation of the ANE GRN by Wnt1/Wnt8-Fzl5/8-JNK signaling in the anterior ectodermal half of early cleavage and blastula staged embryos, allowing for the proper positioning of the ANE territory around the anterior pole. Yet, the exact mechanism by which Fzl1/2/7-PKC signaling antagonizes Fzl5/8-JNK signaling during this process is still unclear. Hence, our research aims to better characterize the Fzl1/2/7 pathway and the GRN it activates to identify possible interactions between these different Wnt signaling branches. Using a candidate gene approach in combination with whole-transcriptome differential screens, we identified a candidate Wnt ligand, Wnt16, a potential transcriptional effector, NFAT, an intracellular signal transduction modulator, Siah1, and several transcription factors in the GRN activated by Fzl1/2/7 signaling. We use morpholino knockdown assays to demonstrate that these regulatory factors are necessary to antagonize the ANE restriction mechanism mediated by Fzl5/8-JNK signaling. Our results indicate that Wnt16 and NFAT are necessary for ANE specification and activation of the putative GRN activated by Fzl1/2/7 signaling. Together, our data suggest that Wnt16 activates the Fzl1/2/7 pathway during ANE positioning and that NFAT acts downstream of Fzl1/2/7-PKC signaling as its transcriptional effector necessary to antagonize Fzl5/8-JNK signaling mediated down regulation of ANE GRN.

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Exploring the Regulation of *ftz-f1* Expression in the *Drosophila* Ovary

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Drosophila melanogaster oogenesis is regulated by the steroid hormone ecdysone. One known transcriptional target of ecdysone is *ftz-f1*, a nuclear hormone receptor involved in many vital biological processes, including tissue formation. To test whether *ftz-f1* is an ecdysone target in oogenesis, our research sought to identify regulatory elements within the *ftz-f1* gene locus that contain putative Ecdysone Receptor (EcR) binding sites and are sufficient to drive expression in the ovary. Flies containing transcriptional reporter constructs encoding small regions (tiles) of the *ftz-f1* locus, a minimal promoter, and a Gal4 reporter were used to compare ovary cell expression patterns. We computationally identified potential binding sites for EcR in the *ftz-f1* locus. A small region within the large intron of the *ftz-f1* locus, containing an EcR binding site, is sufficient to drive expression in mid oogenesis nurse cells and oocytes. Additional tiles that contained EcR binding sites showed specific expression in either the late follicle cells or terminal filament cells. Multiple drivers of operculum expression exist within the *ftz-f1* locus including tiles that do not have EcR binding sites. One region of the *ftz-f1* locus which does not contain an EcR binding site was sufficient to drive expression in discrete populations of somatic cells. Individual tiles within this region correlated to niche cell expression including terminal filament cells, cap cells, and escort cells. Ongoing research aims to further analyze the *ftz-f1* specific regions that correspond to discrete patterns of cells expression in order to learn more about the factors that could influence the regulation of *ftz-f1* expression.

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Screening for Genetic Factors that Determine Muscle Specialization

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Here we study a very basic question of how similar tissues come to express different genes, which can be seen in muscles destined to perform different functions. In our model organism, the fruit fly (*Drosophila melanogaster*), the large flight muscles in the thorax contract very frequently and can work for hours without fatigue. In contrast, small muscles in the abdomen sparingly contract, and do not support extensive physical load. These two muscle types demonstrate significant differences at the morphological as well as molecular levels. Specifically, flight and abdominal muscles express distinct muscle genes that are important for the same function, muscle contraction. We have characterized a reporter system made on the basis of differentially expressed muscle genes, *Act57B* and *Act88F*. This system will be used in genetic screening to identify and potentially unravel important genetic factors controlling the selectivity of gene expression in muscle specialization. We hypothesize that members of chromatin remodeling complexes can be involved in controlling selective gene expression in different muscle types.

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Genetic suppressors of mutant separase may elucidate membrane trafficking role of *C. elegans* separase

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Successful cell division depends on coordinated regulation of chromosome segregation and cytokinesis. Chromosome segregation requires equal partitioning of sister chromatids duplicated in S-phase and held together by the cohesin complex during mitosis. Separase regulates multiple processes during mitotic exit and cytokinesis. The canonical role of separase, the cysteine protease, is to cleave the kleisin subunit of cohesin, allowing chromosome segregation during mitotic and meiotic anaphase. Separase has also been shown to have a non-canonical role in regulating cortical granule exocytosis (CGE) during meiotic division in Caenorhabditis elegans embryos. A temperature sensitive separase multination (sep-1(e2406)), which results in a single missense mutation (C450Y) within the N-terminal domain of SEP-1, does not localize to cortical granules and is unable to promote exocytosis but results in minimal chromosome segregation defects.

We have performed a genetic suppressor screen to identify separase regulators that rescue lethality of *sep-1(e2406)*. We found multiple intragenic suppressors that alter residues in the N-terminus of SEP-1. These residues are likely to affect structure stability and binding to other proteins. Consistent with previous observations, we have also identified a substantial number of *pph*-5 mutant suppressors. These suppressor mutations are found both in the TPR and phosphatase domain of the highly conserved phosphatase PPH-5 and may provide insight into the phosphoregulation of separase function during exocytosis.

We have also identified multiple novel *sep-1(e2406)* suppressors which belong to independent complementation groups, greatly expanding the potential for elucidating separase regulation during membrane trafficking.

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Identification of novel *kal-1* transcriptional regulators

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Kallmann Syndrome (KS) is a rare genetic condition that alters olfactory sensation and also hypothalamic neuron migration, which ultimately inhibits reproductive development. We hypothesize that transcription factors required for the expression of known KS genes may be KS loci in their own right. The human *kal-1* gene is strongly conserved between vertebrates and invertebrates and when mutated, leads to X-linked KS. Because this gene is not found in rodents, we are using a *C. elegans* model of X-linked KS to identify transcriptional regulators of the *kal-1* gene. Our reporter gene and loss-of-function analyses identify the bHLH gene *cnd-1* as a *kal-1* transcriptional regulator. As such, we anticipate that a loss-of-function in *cnd-1* will affect ventral enclosure in the same pathway as *kal-1*. Finally, we have performed a deletion analysis of the *kal-1* promoter to narrow down which regions are required for *kal-1* transcription at various stages of development and in what specific cell type. These data are being corroborated at single-cell level using *kal-1-GFP* and histone mCherry co-lineaging data.

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Conserved targets of ISL1 in genital development and binding at shared limb-genital enhancers in chicks

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The early development of limbs and external genitalia involves expression of many of same genes. In addition, ChIP-seq experiments in mice have indicated that many enhancers are active in both tissues. The *Isl1* gene encodes a transcription factor that is required for the initiation of hindlimb buds in mice. Conditional *Isl1* knockouts also show severe impairment in development of the genital tubercle - the embryonic precursor to the penis and clitoris. Similarities in external genital development across amniotes suggests derivation from their last common ancestor over 300 million years ago. Using ChIP-seq against ISL1 in the genital tubercles of mice and chicks, we reveal a set of conserved enhancers targeted by ISL1 in both species. These genital tubercle binding regions are significantly associated with genes involved in limb development. Furthermore, ChIP-seq against ISL1 in chick early hindlimb buds suggests extensive enhancer sharing between ISL1-expressing hindlimb and genital tubercle cells.

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Regulation of Stem Cell Lineages in *Drosophila* Testes by Notch Signaling

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Spermatogenesis in *Drosophila melanogaster* testes is predicated on the proper interaction between germline cells and their microenvironment somatic cyst cells. Germline stem cells (GSCs) and somatic cyst stem cells (CySCs) are located at the apical tip of the testis where they go through asymmetric division to produce new stem cells and daughter cells. The daughter cells for the GSCs called gonialblasts are fully enclosed by the CySCs daughter cells, the cyst cells. The gonialblasts undergo transit amplifying divisions to produce clusters of precursor cells called spermatogonia that eventually develop into spermatids to produce fertile sperm. Throughout this process, the surrounding cyst cells grow in size and co-differentiate with the enclosed germ cells. The Notch signaling pathway relies on the membrane-bound ligand expressed by the signal-sending cell binding to the Notch transmembrane surface receptor on the signal-receiving cell. This signaling event leads to activation of Notch target genes in the receptor-expressing cell. Notch signaling appears to play a role in the early stages of spermatogenesis. Antibodies against Notch signaling components localize to the apical tip of the testes. Knockdown of Delta in the germline leads to germline loss while hyperactivation of Notch signaling in cyst cells results in failure of cyst cells and germline to differentiate properly. These cyst cells simultaneously express both early and late stage molecular markers and the germline fail to develop into spermatids. We hypothesize that Delta signals from the germline to the Notch receptor on the encompassing somatic cyst cells to prevent germline and cyst differentiation. We propose that activation of Notch in cyst cells prevents their premature differentiation and subsequent germline differentiation.

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The ETS-1 transcription factor in *Xenopus* heart development: Implications for a multi-hit model for HLHS

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Hypoplastic left heart syndrome (HLHS) is one of the most severe human congenital heart defects (CHDs), affecting ~3% of all infants born with congenital heart disease. Although there is strong evidence implicating a genetic etiology, only a few diseasecausing genes have been identified. Progress towards understanding HLHS has been hindered by the lack of genetically engineered animal models. Previously, we have found that loss of transcription factor Ets-1 in *Xenopus* leads to an embryonic lethal cardiac phenotype reminiscent of HLHS: a hypoplastic outflow tract and a thickened ventricular chamber with diminished chamber volume. Here, we further characterized the cardiac phenotype in ETS-1 knockdown embryos. We demonstrate that the HLHS phenotype can be rescued by grafting wildtype cardiac mesoderm progenitor tissue during early stages of cardiac development. Furthermore, loss of ETS-1 causes dysregulation of genes involved in normal endocardial/myocardial signaling. Specifically, knockdown of ETS-1 results in an increase in *BMP10*, *ErbB2* and *Myocardin* expression during early heart development. These results indicate that loss of ETS-1 causes an HLHS-like phenotype through a multi-hit model involving the cardiac neural crest and endocardium, suggesting the possibility of early intervention for the prevention of HLHS.

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Transposon expression signature in *Nematostella vectensis* development

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Previously we have discovered massive orchestrated upregulation of Long Terminal Repeat (LTR) transposable elements (TEs) during the egg-to-embryo transition in mice. To expand these observations, we focus on elucidating TE expression in oogenesis and early development of phylogenetically distant species across all Metazoa. Here, we report unprecedently detailed analysis of TE expression in starlet sea anemone (*Nematostella vectensis*), a representative of Cnidaria, a sister phylum of Bilateria.

N. vectensis is among the simplest model organisms whose cells are organized into tissues. It is an established model organism in evo-devo studies, such as comparison to the development of more complex Bilateria, and is useful in our understanding of early embryonic development. Repetitive sequences comprise approximately 30% of *N. vectensis* genome, most prominent are tandem repeats and DNA transposons. We report differential expression analysis of TEs during *N. vectensis* development using RNA-seq data. We found Kolobok-1_NV, DNA transposon belonging to enigmatic Kolobok superfamily of eukaryotic DNA TEs, as the highest expressed transposon. DNA TEs of Harbinger and piggyBac families, and several retrotransposons of Penelope family, are also among the highly expressed transcripts in early *N. vectensis* development, particularly at the gastrula and early planula stages. Our findings underscore the notion that eukaryotic species' genome variability depends upon unrestricted expression of recently "co-opted" TEs during early development. Funding: Impact Assets, Farmington, CT.

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Parathyroid Cell Fate Instability and Cell Cycle Length

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The parathyroid is the organ responsible for maintaining calcium homeostasis in the body. During mouse development, the parathyroid develops in tandem with the thymus in the 3rd pharyngeal pouch, where the dorsal cells differentiate into parathyroid and the ventral cells differentiate into thymus. Although the fate of thymus cells is quite stable, parathyroid cell fate has been shown to be unstable with parathyroid cells transdifferentiating to thymus cells at low frequencies during late fetal stages. It is unclear whether the parathyroid program is spontaneously shut off and the thymus program subsequently is activated or whether spontaneous activation of the thymus program turns off the parathyroid program. Preliminary data suggests that induction of the master thymus regulator, FoxN1, in parathyroid cells is sufficient to downregulate the parathyroid program; however, we still do not know if the endogenous thymus program has been activated in these cells. Proliferation has been linked to DNA methylation. The thymus program may remain in an unmethylated state and be available for activation due to low proliferation of parathyroid cells during late fetal development. One aim of this research is to determine the length of the cell cycle in the developing parathyroid. In order to devise a strategy to manipulate the cell cycle in parathyroid cells, we are exploring a transgenic mouse line that expresses CyclinD1 under the Keratin5 promoter in hopes that the parathyroid cell proliferation is increased in these mice during embryonic development.

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Comprehensive Big Data Bioinformatics Detects Dynamic Changes in Transposons Expression and Epigenetic Regulators during Transformation

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It is widely recognized that all breast cancers start when some cells in an otherwise healthy tissue begin to look abnormal and ultimately result in full-blown cancer. However, in many cases, initial abnormal cells do not always follow the deadly path and cancer does not develop at all. Little is known as to why some patients diagnosed with atypia and ductal carcinoma in situ (DCIS) remain cancer-free while in others the disease progresses to invasive ductal carcinoma (IDC). To identify molecular signatures driving cell fate decisions at atypia and DCIS stages to transformation, we investigated expression of transposonable elements (TEs) as in human atypia, DCIS and IDC. While mutagenic role of TEs in cancer is well documented, we focus on the novel role of LTRs as potential drivers promoting cell de-differentiation during early stages of tumor development with promise as a prognostic tool. To investigate the contribution of TEs in atypia and DCIS, we created a TE Enrichment Set Analysis (TESA) to identify TEs in RNA sequencing datasets across four stages of breast cancer, normal, atypia, DCIS, IDC in humans (n= 8-23). After quality control steps to remove outliers, TEs, compared to transcripts, exhibit substantially less variation in their expression because the first principle component accounted for over 80% expression variation compared to 20% in transcript expression variation ($p<0.05$). Eighty-eight TEs were detected as significant across stages by ANOVA ($\alpha=0.05$, FDR 5%). The majority are LTRs (67%) with the remaining split into DNA TEs (18%), SINEs (11%) and unclassified (4%). Our TESA data complements and provides experimental support that early genomic changes are a mechanism underlying subsequent tumor development. Translational bioinformatics is a technique to identify prognostic molecules for impending invasive breast cancer from biopsies of pre-malignant atypia and ductal carcinoma in situ. Funding: Impact Assets, Hartford CT.

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Determining the development of the parietal eye in brown anoles.

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Circadian rhythmicity controls several physiological and behavioral responses in animals, and in people specifically, disturbances of circadian rhythms underlie mood disorders such as depression and seasonal affective disorder. The proper functioning of the circadian axis in humans and most other vertebrates is dependent on the pineal organ, a neuroendocrine gland that acts as the main synthesizer of melatonin, and the coordination of this hormone's production relies on perception of the photoperiod. However, the mechanisms governing this link between photoreception and melatonin production are not well understood. Because light is perceived in lizards through an additional parapineal structure that is not present in humans, the parietal eye, this extracranial organ offers a unique opportunity to study mechanisms of the pineal complex, as it is easily accessible and can be easily manipulated. As a first step toward better understanding these pineal processes, this project establishes *Anolis sagrei*, the brown anole lizard, as a new model organism by using a histological approach to characterize a timeline of parietal eye morphogenesis, providing a foundation for identifying molecular instrumentation mediating development. The expectation is that, because the parietal eye develops a cornea, lens, and retina similar to those of the lateral eye, the parietal eye and lateral eye will display similar molecular mechanisms of development that illuminate their relationships to the pineal gland.

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Molecular Genetic Analysis of Genes Involved in Tail Bud Development in a Hermaphroditic Vertebrate (mangrove killifish) and Phenotypic Validation in Medaka

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Kryptolebias marmoratus (mangrove killifish) is a hermaphroditic vertebrate that inbreeds to genomic isogeny amenable to efficient and robust forward mutagenesis. Two recessive mutants were previously identified by genetic screening as *shorttail* (*stl*) and *balltail* (*btl*) phenotypes during embryonic development. Total RNAseq of mutants, siblings, and their wild type progenitor (99.97% homozygous) uncovered ENU induced missense mutations in the homologous genes *noto* (*stl*) and *msgn1* (*btl*). In situ hybridization patterns of notochord (*col9a1b*), somites (*hsp90aa*), spinal cord (*sox3*), and tail bud (*spt*) native markers in these mutants, revealed suppression of notochord, somite, and spinal cord by *stl*, whereas *btl* suppresses somites, while expanding notochord expression in the developing tail. Neither mutant affected the expression of *spt*, suggesting these genes are specifically involved in regulating the formation of tail between trunk and terminal posterior axis margins. Further analysis of *noto* and *msgn1* expression amongst these mutants demonstrates *stl* suppresses *msgn1* in the developing tail. To validate the above genetic analysis, we injected morpholinos and analogous mutant mRNA alleles in one cell stage embryos, followed by rescue with wild-type mRNA to phenocopy our results in medaka. Likewise, tail bud cell fate was marked in wild-type versus morpholino injected medaka embryos with Kaede fluorescence to uncover a crucial role during tail bud development in the formation of axial (notochord) and paraxial mesoderm (somite). We propose a model whereby *noto* initially organizes stem cells and *msgn1* positively regulates the formation of somites while suppressing notochord in the developing tail.

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Nociception, and the Experience of Pain Signaling Due to Nerve Damage

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Neuropathic pain is difficult to repair and alleviate. Pain is experienced through the integration of neuronal circuits involved with nociceptive signaling. These harmful stimuli are encoded and processed through a specific group of mechanosensory TRPA1 channels. Those who suffer from neuropathy-based pain attempt to relieve pain symptoms with prescription analgesic medications. Most of these medications come with detrimental side effects including a triggering of the brain's reward and addiction pathways. Taking these drugs for an extended period of time results in dependency problems. In an effort to understand the neurophysiological changes associated with neuropathic pain sensation and to understand how these channels respond during pain stimuli, I will closely examine the effects of suprathreshold stimuli on the TRPA1 channel and manipulation with the putative neuropathic analgesic, THC (delta-9-tetrahydrocannabinol). TRPA1 channel activity will be assayed using the zebrafish model through electrophysiological recording, behavior recoding, and anatomic changes in TRPA1 neuron anatomy and connectivity. Specifically, I will use electrophysiological methods to record noxious signal modulation in zebrafish from the TRPA1 channel containing cranial nerve VIII while in the presence of THC.

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Application of Tol2-based Activation Tag Constructs for Zebrafish Mutagenesis

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Transposons are segments of DNA that can move from one region to another within the genome. The *Tol2* transposon from Medaka fish has successfully been used for transgenesis, integrating foreign DNA, into a wide variety of vertebrates. Our goal is to develop Tol2 into a mutagenesis tool for gene discovery. Mutagenesis by transposon insertion, called transposon tagging, enables the discovery and analysis of gene function by causing mutations. Activation tagging, a type transposon tagging, is when a strong enhancer is positioned within the transposon. Activation tagging is used to learn about the function of genes by inducing overexpression. This is significant because many genes may otherwise be hard to study because of lethality or redundancy. Activation tagging has never been used for zebrafish, but it is commonly used for gene discovery in plants.

Zebrafish can serve as vertebrate development models, therefore activation tagging within zebrafish allows for the discovery of genes that are important for vertebrate development. A Tol2-based activation tag, with a *h2afx* promoter sequence inserted in the middle of Tol2 terminal inverted repeats (TIRs), was engineered using various molecular biology techniques (PCR, digestion, and sequence analysis). Additionally, a DNA construct encoding Tol2 transposase, which will allow transposition of the activation tag to occur, was produced. The integration of both constructs into zebrafish embryos is being performed to measure transposition rates and look for altered gene function. To develop more active constructs for zebrafish mutagenesis, yeast transposition studies are also being performed in order to identify methods to increase transposition rates.

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Elucidating the Role of Securinin Regulating Separase during Cortical Granule Exocytosis

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Meiosis is a tightly regulated process leading to the production of haploid gametes. A key player in this process is separase (SEP-1). Known for its canonical role in chromosome segregation, studies suggest that SEP-1 has an additional function in vesicular trafficking during cell division. We hypothesize that cell cycle machinery known to control SEP-1 activity for chromosome segregation also controls its localization to the cortex and subsequent exocytic activity. Following spindle attachment and chromosome alignment during the meiotic M phase, the anaphase promoting complex (APC/C) is activated, resulting in the degradation of SEP-1 inhibitory chaperone securin (IFY-1) and entry into anaphase I. We have observed that SEP-1 localizes to cortical granules and regulates their exocytosis during anaphase I, which is necessary for eggshell formation. Before it appears on cortical granules, SEP-1 localizes to cytosolic filaments near the plasma membrane. We have shown that SEP-1 colocalizes with IFY-1, on filaments during prometaphase, and both disassociate from these structures during anaphase I. Inhibition of APC/C activity prevents SEP-1 and IFY-1 from leaving the filaments. These data suggest that degradation of IFY-1 may regulate SEP-1 localization to vesicles. To address whether IFY-1 degradation is required to allow SEP-1 translocation onto vesicles, we generated a non-degradable IFY-1 (IFY-1DM::GFP). IFY-1DM::GFP is not completely degraded following anaphase I onset, remaining on chromosomes and in the cytoplasm into anaphase II. Expectedly, IFY-1DM::GFP causes embryonic lethality. Interestingly, IFY-1DM::GFP causes polar body extrusion failure, which could be related to defects in cortical granule exocytosis. We will investigate how IFY-1DM::GFP affects SEP-1 localization to cortical granules. This will provide insight into how key regulatory components of the cell cycle control SEP-1 localization to promote timely cortical granule exocytosis during anaphase I.

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Characterization of a Piglet Model of Traumatic Brain Injury Utilizing Non-Invasive Magnetic Resonance Imaging and Histological Assessment

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Traumatic brain injury (TBI) is a major cause of death and disability in the United States, chiefly affecting children ages 0-4 years. TBI at such a young age may lead to long-term neurological deficits. Recent failures in translatable research suggest a more human-like animal mode, like a piglet, is necessary for developing an effective therapy. Magnetic resonance imaging (MRI) and histological assessments are pertinent in the comprehensive understanding and treatment of TBI at the tissue and cellular levels. We hypothesized that controlled cortical impact (CCI) would result in a concussive piglet TBI model with substantial changes in lesion and hemisphere volume coupled with histological changes that persist over time. TBI was induced in six male piglets, with MRI scans conducted 24 hours and 12 weeks post-TBI. Histological changes were observed by quantifying NeuN+ neurons, GFAP+ astrocytes, and Iba1+ microglia in the cortical peri-lesion area through 12-weeks post-TBI. Lesion size was significantly reduced comparatively at 12 weeks with a significant change midline shift as compared to 1 day post TBI. There was a significant ($p<0.01$) decline in NeuN+ neurons beginning 1-week post-TBI. GFAP+ astrocytes increased significantly ($p<0.0001$) from normal starting 1 day post-TBI. Lastly, Iba1+ microglia increased significantly ($p<0.05$) at each timepoint. The observed directional change in midline shift and decrease in lesion size can be attributed to attenuated swelling and significant brain atrophy. The noted histological changes suggest there was significant cell death and there was a significant upregulation in GFAP+ astrocytes and Iba1+ microglia, which suggests TBI leads to gliosis and an inflammatory response that mounts over time. The characterization of key cytoarchitectural changes in the CCI TBI piglet model will enable more robust and predictive assessments of novel therapeutics that will likely lead to more success in human clinical trials.

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Determining the role of *ldlrap1a* in zebrafish skeletal development

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A line of mutant zebrafish containing a jaw mutation named *b1187* was discovered during a forward genetics screen. This mutation is characterized by fused joints and abnormal shaping in cartilage and bone in the craniofacial region of zebrafish. To find the gene behind the *b1187* mutation multiple genes were sequenced. Although there were no differences between mutant and wild-type sibling cDNA, the phenotype was closely linked to the *ldlrap1a* gene locus (low density lipoprotein receptor adaptor protein 1a). This led to a reverse genetics approach using the CRISPR/Cas9 system to create of a line of zebrafish with a mutated *ldlrap1a* gene. ldlra*p*1a is known to be involved in cholesterol signaling, however it may also have a role in skeletal development. A F0 generation containing an *ldlrap1a* mutation was generated and was then crossed with wild-type siblings to create three separate F1 generations. The F1 generations were screened using PCR and T7 endonuclease digest to identify approximately half of the offspring who were heterozygous mutants for the *ldlrap1a* gene. Fin clip samples were taken from all three individual heterozygous carriers and a wild-type zebrafish and these samples were sent for sequencing. Of the three heterozygote carriers, one appeared to have a favorable 7 base pair deletion. This sequenced fish was then crossed to a wild-type zebrafish to create an F2 generation. In-crosses between F1 mutant carriers and histological stains of offspring are in progress. If we observe jaw abnormalities resembling the b1187 mutation we could conclude that *ldlrap1a* is not only involved in cholesterol homeostasis, but may also be involved in craniofacial development.

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Casein Kinase 1 delta/epsilon mediates anterior-posterior axis formation in the sea urchin embryo, potentially through localized activation of Disheveled

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Wnt signaling plays a central role in establishing anterior-posterior (AP) polarity in metazoan embryos. A key cytoplasmic component mediating Wnt signaling is the Disheveled (Dvl) protein. In the sea urchin, Dvl is highly enriched and differentially post-translationally modified in a specialized vegetal cortical domain (VCD) of the egg, and the vegetal blastomeres that inherit the VCD during embryogenesis. Functional analysis has shown that localized Dvl activity mediates canonical Wnt signaling in vegetal blastomeres, but the molecular basis of Dvl asymmetric localization and activation remain unresolved. Therefore, identification and functional characterization of proteins interacting with Dvl (DIPs) in the VCD will help us better understand how Dvl partners regulate Dvl activity and Wnt signaling. By applying Dvl Co-immunoprecipitation coupled with mass spectrometry we have identified several potential Dvl-interacting-proteins (DIPs) from isolated egg cortices and 16-cell-stage micromeres. Casein Kinase 1 δ/ε (CK1δ/ε), one of our newly identified DIP candidates, is highly enriched and co-localized with Dvl at the vegetal pole of the sea urchin embryo. Downregulation of CK1δ/ε activity by overexpressing a dominant-negative form of CK1δ/ε resulted in the downregulation of genes expressed in the endomesoderm and the anteriorization of embryos. However, overexpression of CK1δ/ε by injecting synthesized CK1δ/ε mRNA into fertilized eggs only induced slight upregulation of endomesoderm genes and mild posteriorization of embryos. Intriguingly, we found that co-overexpressing CK1δ/ε and Dvl induced significantly higher levels of expression of endomesodermal genes compared to expression levels of these genes in embryos overexpressing Dvl or CK1δ/ε only suggesting that CK1δ/ε synergizes with Dvl to positively regulate Wnt signaling.This work establishes CK1δ/ε as a critical regulator of Dvl activation and AP axis specification in sea urchin embryos.

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Interactions of Akirin and Muscles Wasted during myogenesis

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We have recently identified the highly conserved nuclear co-factor Akirin as an essential partner during the process of Twist-mediated gene activation during *Drosophila* embryonic myogenesis. *akirin* mutants display multiple defects in muscle patterning, with missing, misattached, and/or duplicated muscles. Live imaging data indicates that the muscles that do form are morphologically thinner and weaker than those observed in wild-type sibling embryos, and that these muscles rapidly deteriorate prior to hatching. These *akirin* mutant phenotypes were reminiscent of *muscles wasted* (*mute*) mutants; the specification, positioning, and patterning of mute mutant muscles initially form, but rapidly degenerate as the embryo nears hatching. Despite these phenotypic similarities, a connection between the two had yet to be described. Using a combination of confocal-based live imaging of developing embryos, as well as analyzing whole-mount fixed embryos, we have confirmed a genetic link between these loci. akirin/mute double heterozygous mutant embryos display a profound disorganization of the embryonic muscle pattern, with severely degenerated muscles, large numbers of unfused myoblasts, and abnormal patterning and formation of muscle groups in pre-hatching embryos. While a direct interaction between these two gene products is currently under investigation, these data strongly indicate a potential interaction during the myogenic process.

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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 that include intellectual disability and craniofacial anomalies. PSS is associated with mutations in genes encoding factors in the PHF21A protein complex, including KDM1A (lysine-specific histone demethylase 1A), ZMYM2 (zinc finger protein 198), and ZMYM3 (zinc finger protein 261) proteins. It is hypothesized that these protein complexes are involved in craniofacial development of zebrafish in a way that reflects their function in humans. Previously, F0 founder fish carrying mutations in *zmym2* and *zmym3* were generated at the 1-cell stage. Founders were screened by PCR and T7 endonuclease digest which identifies mutations in the DNA and were used to generate F1 lines. The F1 generation was screened by using tail fin DNA in PCR and T7 endonuclease digest. F1 zebrafish were sequenced and frameshift mutations were identified. Zebrafish with confirmed frameshifts will be out-crossed to produce an F2 generation. The F2 generation, of which 25% are expected to be homozygous mutants, 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 work in this project will be used to identify the roles of *zmym2* and *zmym3* in zebrafish development, and how a loss of function of these factors may underlie the defects seen in PSS.

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Identification and Characterization of a Voltage-Gated Proton Channel in *Helisoma trivolvis*

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Voltage-gated proton channels (HV1) are transmembrane proteins that conduct protons across the cellular membrane in response to a change in membrane potential. They have a wide variety of biological functions from protists to humans; their role in acid extrusion in neurons gives them potential importance in neuronal development. The first published report of a voltage activated proton current was from neurons of the snail *Helix aspersa*. This channel exhibited extremely fast activation, but whereas most HV1 subsequently characterized are open orders of magnitude more slowly. Here we report the identification and characterization of HV1 in ganglia and brain tissue of the related species *Helisoma trivolvis*. We used animal HV1 sequences to BLAST the *H. trivolvis* genome and identified a likely HV1 coding sequence. We isolated RNA from *H. trivolvis* brain and ganglia tissue, cloned the putative HV1 gene using PCR primers designed against the coding sequence, and subcloned the gene into the mammalian expression plasmid (pCA-IRES-eGFP). Patch-clamp of the gene product expressed in HEK-293 cells shows that this channel is a bona fide HV1 (HtHV1) with hallmark characteristics of voltage and pH dependent activation, and near-perfect selectivity for protons. At comparable pH, HtHV1 shows similar very fast activation to the original Helix report. Preliminary transcriptome analysis shows that *H. trivolvis* neurons differ in HV1 RNA expression. We outline our immunostaining approach to determine HtHV1 localization, and compare HtHV1 protein expression, in different *H. trivolvis* neurons.

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Characterization of conserved regions of Drosophila Akirin: Development of tools to inhibit chromatin remodeling

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Akirin is small, highly conserved nuclear protein found throughout the metazoa. It lacks known protein domains, defined catalytic activities and the ability to bind DNA. However, it has the ability to influence gene expression by linking transcriptional regulation to chromatin remodeling. Regions at the N- and C-termini are highly conserved and may play a role in the function of Akirin. Akirin physically binds to Twist, a highly conserved basic-helix-loop-helix (bHLH) transcription factor involved in regulating developmental processes. In this study, we sought to determine if conserved regions of Akirin interacted with Twist, and in so doing, could they be used as inhibitors of chromatin remodeling? Our novel cell-penetrating peptide (CPP)-adaptor system was used as a mechanism for delivering Akirin peptides into living cells. The conserved regions (CR) of Akirin were fused with calmodulin binding sites for purification and binding to CCP-adaptor, TAT-CaM. Additionally, isolated constructs were examined for Twist binding. Penetration assays were performed with CPP-adaptor/Akirin complexes to determine the ability of CRs to localize in the nucleus. All Akirin constructs showed high affinity to the CPP-adaptor, TAT-CaM. AkirinCR3 was able to bind to Twist. All constructs were successfully delivered across the plasma membrane. AkirinCR3 may constitute all or part of the Twist binding site, and hence may be an effective inhibitor of Akirin-Twist interactions and chromatin remodeling.

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