Society for Developmental Biology
2013 Mid-Atlantic Regional Meeting

The College of William & Mary
April 19-21, 2013

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Conference Schedule:

Conference check-in, all talks, as well as continental breakfast and lunches will be held in the Integrated Science Center (ISC). The poster session and banquet will be held in the Williamsburg Hospitality House. A campus map with directions between the Hospitality House and the Integrated Science Center can be found in the link for Travel & Directions.

Friday, April 19th

4:00-6:00 pm **Conference Check-in** (ISC 1119)

6:00-7:45 pm **Session I – Genomics & Epigenetics** (ISC 1127)

6:00 – 6:15 pm	**Welcome from SDB** (Matt Wawersik & Margaret Saha)
6:15 – 6:45 pm	**Featured Speaker: Valerie Reinke (Yale School of Medicine):** *Mechanisms of tissue-specific regulation of piRNA-rich genomic regions*
6:45 – 7:00 pm	**Ying Zhang (NCI-Frederick):** *Characterization of SPRY2 cis-acting elements responsive to FGF signals*
7:00 – 7:15 pm	**Julia Wang (Johns Hopkins):** *Tandem targeting of Dre-mir216A/B microRNA clusters using TALENS*
7:15 – 7:30 pm	**Amanda Monahan (University of Maryland Baltimore County):** *SOCS36E attenuation of Jak-STAT signaling is required for precise cell fate determination in Drosophila*
7:30 – 7:45 pm	**Aurora Esquela-Kerscher (Eastern Virginia Medical School):** *Overlapping microRNA networks during nematode development*

7:45-8:15 pm **Break** (ISC 1119)

8:15-9:45 pm **Session II – Specification & Differentiation** (ISC 1127)

8:15 – 8:45 pm	**Featured Speaker: Sally Moody (George Washington University):** *FOX N' SOX: On becoming neural*

8:45 – 9:00 pm **Daniel McIntyre (Duke University):** *Short range Wnt5 signaling specifies posterior ectodermal fate in the sea urchin*

9:00 – 9:15 pm **Joseph Zinski (University of Pennsylvania):** *Dynamics and shaping of the BMP signaling gradient in DV axial patterning of the zebrafish gastrula*

9:15 – 9:30 pm **Peng-Fei Xu (University of Virginia):** *Building a vertebrate embryo using a combindation of morphogenetic gradients*

9:30 – 9:45 pm **Niteace Whittington (Georgetown University):** *The levels of Sox21 alters its function in neurogenesis*

Saturday, April 20th

8:00 - 9:00 am **Continental Breakfast** (ISC lobby – 1st floor)

8:00 – 8:45 am **Funding opportunities in Developmental Biology** (ISC 1127)

9:00 – 10:45 am **Session III – Neural development** (ISC 1127)

9:00 – 9:30 am **Featured Speaker: Lisa Tanneyhill (University of Maryland):** *A path forward: Dismantling cellular junctions during cranial neural crest cell EMT*

9:30 – 9:45 am **Paaqua Grant (George Washington University):** *A novel, maternally expressed gene, SMCR7L, is important for early neural development in Xenopus*

9:45 – 10:00 am **David Wotton (University of Virginia):** *Defective neuroepithelial cell polarity in the absence of TGIF function*

10:00 – 10:15 am **Matthew Anderson (NCI-Frederick):** *FGF3 regulation of BMP signaling is required for neural tube closure*

10:15 – 10:30 am **Benjamin Thiede (University of Virginia):** *A gradient of retinoic acid signaling regulates tonotopic development in the chick cochlea*

10:30 – 10:45 am **Joyce Fernandes (Miami University):** *Motor neurons signal myoblast proliferation*

during Drosophila myogenesis using the EGF pathway

10:45 – 11:00 am **Break**

11:00 – 12:30 pm **Session IV – Evo-Devo/Eco-Devo** (ISC 1127)

11:00 – 11:30 am	**Featured Speaker: Eric Engstrom (College of W&M/Hampton University):** *HAM proteins and cell division: A pivot point in angiosperm evolution?*
11:30 – 11:45 am	**Veronica Hinman (Carnegie Mellon University):** *The evolution of developmental gene regulation networks for endosomal specification among echinoderms*
11:45 – noon	**Alys Cheatle (Carnegie Mellon University):** *Modularity in DNA binding preference of a Tbrain Transcription factor may allow for more versatile transcriptional responses and increased evolvability*
noon – 12:15 pm	**Senel S. Tektas (University of Delaware):** *Wntless: Structure to Function*
12:15 – 12:30 pm	**Theresa Grana (University of Mary Washington):** *Diversity of early development in diverse nematode species*

12:30 – 2:30 pm **Education lunch** (ISC 1127): Lunch provided for all attendees: Topics include: (1) Discussion of "CourseSource," a new initiative stemming from *Vision and Change, and (2)* Discussion of: why are education sessions are so poorly attended? What do we want from education sessions and the SDB education and outreach committee?

3:30 – 6:30 pm **Poster session** (Hospitality House – Williamsburg Ballroom)

7:00 – 10:00 pm **Banquet** (Hospitality House – Empire Ballroom)

Sunday, April 21st

8:00 - 9:00 am **Continental Breakfast** (ISC lobby – 1st floor)

8:30 – 8:45 am **Microscopy Techniques** (ISC1127)**: Scott Olenych (Zeiss)** *Light sheet fluorescence microscopy – Lightsheet Z.1*

9:00 – 10:30 am **Session V – Morphogenesis & Organogensis** (ISC 1127)

	9:00 – 9:30 am	**Featured Speaker: Marnie Halpern (Carnegie Institute for Science):** *Formation and function of left-right asymmetry in the zebrafish brain.*
	9:30 – 9:45 am	**Tim Jackson (University of Pittsburgh):** *Extracellular ATP regulates contractile mechanical tone of early embryonic epithelia*
	9:45 – 10:00 am	**Maria Kaltcheva (NCI-Frederick):** *Interdigit BMP signaling is essential for programmed cell death and implicated in digit formation*
	10:00 – 10:15 am	**Audrey Hendley (Johns Hopkins University):** *Ablation of p120 catenin in embryonic mouse pancreas disrupts acinar cell, islet and duct development*
	10:15 – 10:30 am	**Heather Szabo-Rogers (University of Pittsburgh):** *Amplitude of growth factor signaling tunes craniofacial morphology*
	10:30 – 10:45 am	**Amanda Dickinson (Virginia Commonwealth University):** *Retinoic acid: a master regulator of craniofacial development*
10:45 – 11:00 am	**Break** (ISC 1119)	
11:00 – 12:30 pm	**Session VI – Stem Cells & Regeneration** (ISC 1127)	
	11:00 – 11:30 am	**Featured Speaker: Steve DiNardo (University of Pennsylvania):** *Resolving zig and zags: Assigning planar cell polarity during epithelia morphogenesis*
	11:30 – 11:45 am	**L.A. Naiche (NCI-Frederick):** *Defining the origins of hemogenic endothelium, the source of hematopoietic stem cells, by lineage tracing TBX4*
	11:45 – 12:00pm	**Jenny Lenkowski (University of Michigan):** *TGFβ signaling in regeneration of the zebrafish retina*
	12:00 – 12:15 pm	**Rajeswari Banerji (Lehigh University):** *Development of a model for Roberts Syndrome in zebrafish regenerating fins*

	12:15 – 12:30 pm	**Noelle Dwyer (University of Virginia):** *The vertebrate-specific kinesin, Kif20b, is required for cytokinesis of polarized cortical stem cells and normal cerebral cortex size*
12:30 – 1pm	**Awards & Final Announcements** (ISC1127)	

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Oral Presentation Abstracts

Session I – Genomics & Epigenetics

Featured Speaker: Valerie Reinke (Yale School of Medicine)

MECHANISMS OF TISSUE-SPECIFIC REGULATION OF piRNA-RICH GENOMIC REGIONS

Dionna Kasper, Guilin Wang, Kathryn Gardner, Timothy Johnstone, Valerie Reinke
Department of Genetics, Yale University School of Medicine, New Haven CT 06520

The conserved Piwi/piRNA pathway safeguards genomic integrity and is essential for germline development and gametogenesis. Piwi is an RNA-binding and cleavage protein associated with the class of small RNAs termed Piwi-interacting RNAs, or piRNAs. The molecular mechanisms regulating germline-specific expression and function of piRNAs are poorly understood. piRNA genes are highly clustered in the genome, especially in *C. elegans*, where >15,000 piRNA loci are concentrated in two regions on a single chromosome. Amazingly, piRNA loci exhibit synchronized expression in the germ line, despite being interspersed among hundreds of coding genes with diverse expression patterns. piRNA clustering is evolutionarily conserved, indicating that clustering is a key aspect of their coordinated expression, yet how this regulation is implemented is a mystery. Using genomic technologies, we have identified factors that promote piRNA expression in germ cells. Strikingly, these factors appear to function by demarcating the chromosomal domains from which piRNAs are expressed, rather than regulating individual piRNA genes. Our data suggest a model in which a tissue-specific epigenetic domain is established that is generally permissive for piRNA expression. Ultimately, knowledge of how piRNAs are generated will facilitate our understanding of how they function to protect genome integrity and gametogenesis in the germ line to ensure fertility and robust fetal development.

CHARACTERIZATION OF SPRY2 CIS-ACTING ELEMENTS RESPONSIVE TO FGF SIGNALS

Ying Zhang, Mark Lewandoski, Cancer and Developmental Biology Lab, National Cancer Institute, Frederick, MD 21702

A challenge in developmental biology is to understand how signaling pathways regulate downstream genes. The Fibroblast Growth Factor (FGF) family is one of the first signaling pathways discovered to act during development, but ironically, compared to other pathways, we know little about the downstream mediators and FGF-responsive elements (FREs) in target genes. Therefore, we are investigating potential FREs in Spry2, which encodes an important modulator of FGF signaling and is expressed in FGF signaling centers during development. We have constructed a Spry2-luciferase construct that contains 8 kb of Spry2 sequences upstream of the initiation codon, which is fused to luciferase. This construct is responsive to exogenous FGF in NIH3T3 cells. We then generated a series of transgenic mouse lines with a similar transgene but with Spry2 sequences driving lacZ activity. This construct recapitulates endogenous Spry2 expression in most regions from embryonic day E7.0 through E11.5. These include the primitive streak (PS), presomitic mesoderm, somites, limb buds, branchial arches, anterior neural ridge and the mid-hindbrain organizer. We are performing the appropriate genetics to determine FGF-responsiveness. Thus far we have found that transgenic expression is reduced in the PS when Fgf4 and Fgf8 are inactivated in that tissue with TCre-activity and in the midhindbrain junction when Fgfr1 is inactive with En1Cre. Also, limb bud Spry2-lacZ activity is absent in mutants lacking the apical ectodermal ridge, a source of FGF activity. By using different Fgf4 and 8 loss-of-function alleles, we found that Spry-lacZ activity is more sensitive to FGF loss than the endogenous Spry2. Thus we have generated useful FGF-reporter mouse lines and are refining our analysis to determine the minimal sequences that act as FREs. We will use these sequences in DNA-centered techniques to determine the trans-acting proteins that target gene activation via the FGF signaling cascade.

TANDEM TARGETING OF DRE-MIR216A/B MICRORNA CLUSTERS USING TALENS

Julia Wang, Bidyut Ghosh, Michael Parsons, Steven D. Leach
Institution of Genetic Medicine. Johns Hopkins.

MicroRNAs have been shown to be involved in a variety of biological processes. Recently, *in situ* hybridization using a locked nucleic acid (LNA) probe demonstrates that *dre-mir216* is expressed specifically in the exocrine pancreas of 5-day zebrafish larva. *Dre-mir216* has two family members in the genome: *dre-mir216a* and *dre-mir216b*, located 300bp apart. Here, we made use of the powerful Transcription activator-like (TAL) effector nucleases (TALENs) system to study the functions of this microRNA family. We simultaneously injected two pairs of TALENs targeting each individual locus, aiming for bi-locus mutagenesis. When injected individually, each TALEN pair achieved 100% efficiency in introducing targeted small in-del mutations. In comparison, when both pairs of TALENs were co-injected, approximately 50% of the resulting fish carried mutations in one or both loci. Currently, F1 fish embryos that have targeted mutations in both *dre-mir216* loci are being raised. F1 mutantswill then be crossed to achieve homozygosity, allowing us to generate fish deficient in both *dre-mir216a* and *dre-mir216b*. In summary, TALEN technology facilitates targeting of genomic regions which may be challenging to target by traditional methods. By co-injection of multiple TALEN pairs, multi-locus mutagenesis can be achieved in a single generation.

SOCS36E ATTENUATION OF JAK/STAT SIGNALING IS REQUIRED FOR PRECISE CELL FATE DETERMINATION IN DROSOPHILA

Amanda J. Monahan and Michelle Starz-Gaiano
UMBC, Baltimore, MD

Proper cell fates are established via precisely regulated gene expression. Often, genetic control is linked to a higher-order signal transduction cascade, such as the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway. Border cell migration, during Drosophila oogenesis, provides a powerful model to study JAK/STAT regulation. The Drosophila egg chamber consists of the oocyte and 15 sister cells encased by a monolayer of somatic epithelial cells (the follicle cells). At mid-oogenesis, STAT is activated in a subset of anterior follicle cells. The 4-6 follicle cells with the highest level of activated STAT are fated as the border cells, which collectively migrate to the oocyte. Hypo- or hyper-activated STAT results in abnormally low or high numbers of invasive cells respectively, which results in poor migration. Thus, STAT signaling must be precisely regulated to achieve proper cell fates and developmental outcome. Apontic (APT), a downstream transcriptional target of STAT, regulates the expression of miR-279, which binds the STAT 3'UTR. miR-279 directed negative regulation is sufficient to facilitate detachment, but is not adequate to create a boundary between migratory and non-migratory fates. We determined Suppressor of Cytokine Signaling at 36E (Socs36E) is necessary to regulate these fates. Ectopic levels of Socs36E impede migration of the presumptive border cell population and promote normally non-motile cells to become invasive. Conversely, its loss expands the range of STAT activation, triggering additional invasive cells. We, also, found *Socs36E* genetically interacts with *apt, miR-279*, and *Cullin-2*. Our data suggest STAT and APT regulate Socs36E, which is thought to function in an E3 ubiquitin ligase complex to attenuate STAT. We have found Socs36E is a critical component in a genetic circuit required to establish a boundary between the motile border cells and their non-invasive epithelial neighbors via STAT attenuation.

OVERLAPPING MICRORNA NETWORKS DURING NEMATODE DEVELOPMENT

Jeanyoung Jo, Holly Lewis, Kenya Madric, and Aurora Esquela-Kerscher
Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, Virginia

MicroRNAs (miRNAs) are small non-coding RNAs that regulate important developmental events associated with cellular growth and differentiation. The most thoroughly characterized miRNAs, *lin-4* and *let-7*, direct cell fate determination during the larval transitions in *Caenorhabditis elegans* (*C. elegans*) and act as key regulators of temporal gene expression. Distinct *lin-4* and *let-7* family members display overlapping expression patterns in the developing hypodermis and reproductive system in nematodes, which implied that combinations of miRNAs across families - sharing little sequence homology to one another - control common developmental events. Indeed, our deletion studies indicated that the *lin-4* homologue, miR-237, and the *let-7* family members, miR-48 and miR-84, function in an overlapping network, likely with chromatin remodeling genes, to direct cell cycle progression in the germline as well as formation of the egg-laying structures. Our work also suggested that members within the *lin-4* miRNA family are biologically distinct despite sharing a common "miRNA seed". The "seed" region, consisting of nucleotides 2-8 of the mature miRNA sequence, is considered crucial for miRNA activity and target specificity. However, we found that the *lin-4* homologue, miR-237, failed to functionally compensate for *lin-4* using an *in vivo* rescue assay, which challenges this notion. Use of the simple animal model *C. elegans* to understand miRNA regulation during larval development has provided our lab with valuable insight into the biological roles miRNAs play in cancer progression pathways in humans.

Session II – Specification & Differentiation

Featured Speaker: Sally Moody (George Washington University)

FOX N' SOX: ON BECOMING NEURAL

Sally A. Moody, Department of Anatomy & Regenerative Biology, George Washington University, Washington DC, USA, 20037.

The nascent neural ectoderm in the vertebrate embryo forms in response to signaling factors from the Organizer that prevent the expression of epidermis-specific genes. In this environment, several neural-specific transcription factors are expressed in the presumptive neural ectoderm at the beginning of gastrulation. We found that one of these, FoxD4, a Forkhead transcription factor, is a major regulator of neural fate. Knock-down of FoxD4 in Xenopus embryos dramatically reduces the size of the neural plate and diminishes the expression of at least 11 other early neural transcription factor genes. Increased expression of FoxD4 enlarges the neural plate by: q) up-regulating neural transcription factors that promote an immature, proliferative neural state; 2) delaying the expression of neural plate stem cell genes; and 3) down-regulating neural progenitor and differentiation genes. We found that FoxD4 regulates some neural genes by transcriptional activation and others by transcriptional repression. Using deletion mutants we have mapped the activation domain to an acid domain in the N-terminus of the protein, and identified two regions in the C-terminus that contribute to transcriptional repression. Because these functional domains are highly conserved in mammalian FoxD4, we are studying whether mouse FoxD4 plays a similar role in the transition of embryonic stem cells to neural stem cells.

SHORT-RANGE WNT5 SIGNALING SPECIFIES POSTERIOR ECTODERMAL FATE IN THE SEA URCHIN Daniel C. McIntyre[1], N. Winn Seay[2], Jenifer C. Croce[3] and David R. McClay[1]
[1] Biology Department, Duke University, Durham, NC, USA; [2] Harvard Medical Schools, Boston, MA, USA; [3] UPMC Université Paris 06, UMR7009, CNRS, Biologie du Développement, Observatoire océanologique de Villefranche-sur-Mer, 06230 Villefranche-sur-Mer France.

In most animals, borders between tissues are conserved sites of patterning, often acting as organizational centers that direct structural assembly. In some cases, the formation and function of a boundary has been extensively studied – for example, the midbrain-hindbrain boundary in vertebrates. Yet there are prominent boundaries whose establishment and function(s) remain unknown. The border between the posterior ectoderm and the endoderm is one such site. There, two germ layers meet and establish a life-long relationship that also later serves, in deuterostomes, as the anatomical site of the anus. In the sea urchin, a prototypic deuterostome, the posterior ectoderm-endoderm boundary is established prior to gastrulation, and ectodermal cells at the boundary are thought to provide patterning inputs to the underlying mesenchyme. These signals are necessary for production of the larval endoskeleton. Our results show that a short-range Wnt5 signal from the endoderm actively patterns the adjacent boundary ectoderm. This signal activates a unique sub-circuit of the ectoderm gene regulatory network including the transcription factors IrxA, NK1, Pax2/5/8, and Lim1, which are ultimately restricted to sub-regions of the border ectoderm (BE). Wnt5 also activates the skeletal patterning signal VEGF. Surprisingly, perturbations of Nodal and BMP2/4, earlier shown to be activators of ectodermal specification and the secondary embryonic axis, instead restrict the expression of these genes to sub-regions of the BE. A detailed examination showed that endodermal Wnt5 functions as a short-range signal that activates only a narrow band of ectodermal cells, even though all ectoderm is competent to receive the signal. Thus cells in the BE integrate positive and negative signals from both the primary and secondary embryonic axes to correctly locate and specify the border ectoderm.

DYNAMICS AND SHAPING OF THE BMP SIGNALING GRADIENT IN DV AXIAL PATTERNING OF THE ZEBRAFISH GASTRULA
Joseph Zinski[1], Wei Dou[2], David Umulis[2], Mary Mullins[1].
[1]Department of Cell and Developmental Biology, University of Pennsylvania. [2]Department of Agricultural & Biological Engineering, Purdue University.

It is well established that a gradient of Bone Morphogenetic Protein (BMP) signaling patterns the Dorsal-Ventral (DV) axis of the vertebrate embryo. However, quantitation of this gradient, definition of its range and the dynamics of its formation, as well as its modulation during gastrulation have not been investigated. We show in zebrafish that this gradient changes not only in intensity, but in shape over the time period when DV tissues of the trunk and head are patterned. We show that these changes in shape are at least in part due to region-specific repression of BMP signaling by the extracellular BMP antagonist Chordin (Chd). To measure these changes, we quantified the nuclear intensities of fluorescently stained Phosphorylated Smad5 (P-Smad) protein in every cell of the embryo at 30-minute time-intervals throughout early gastrulation. We used automated algorithms to identify the thousands of individual nuclei present at each embryonic time-point, and to measure their corresponding P-Smad intensities. In WT embryos, we show that the P-Smad gradient first intensifies rapidly in the ventral half, then diminishes in the lateral regions. The gradient steepens, forming a tighter peak around the ventral-most point. We show that during DV patterning, Chd only inhibits BMP signaling laterally. Though Chd primarily inhibits BMP signaling, it has been shown to enhance BMP signaling by transporting BMP ligand in Drosophila DV patterning and crossvein formation. However, we found that no regions appeared to decrease in P-Smad intensity due to the loss of Chd. This indicates that Chd only plays an inhibitory role in DV patterning in the zebrafish gastrula. We are the first to quantify the shape of the BMP gradient during vertebrate DV patterning. Furthermore, by comparing the WT and Chd loss-of-function gradients, we determined the spatiotemporal effect of the BMP antagonist Chd on the signaling gradient.

BUILDING A VERTEBRATE EMBRYO USING A COMBINATION OF MORPHOGENETIC GRADIENTS

Peng-Fei Xu, Karine Ferri, Christine Thisse and Bernard Thisse
Cell Biology Department, University of Virginia, Charlottesville, USA

We have previously shown that in zebrafish, the entire embryonic margin acts as a global and continuous organizer. The organizing properties result from the combined activity of BMP and Nodal morphogenetic gradients and the gradual variation of their ratio of activity observed from the ventral to the dorsal domains of the margin is the crucial parameter that controls the identity of the embryonic structures formed. By recapitulating, within the field of uncommitted blastomeres of the animal pole, the continuous variation of BMP/Nodal ratio of activities observed at the embryonic margin, we are able to induce the formation of a complete secondary embryo that contains all tissues and organs of a wild-type embryo and that develops at the animal pole from animal pole cells. Analysis of the respective contribution of the BMP and Nodal pathways to the formation of the secondary embryo reveals that Nodal signalling results in the formation of a blastopore where an ectopic gastrulation occurs leading to the formation of radially symmetrical structures of dorsal identity. Adding a BMP secreting centre adjacent to the domain stimulated by Nodal breaks the symmetry of the blastopore lip, inducing ventral and lateral tissues to form and in addition that polarizes gastrula cell movements. Our analysis reveals that the antero-posterior orientation of the ectopic embryonic axis depends only on the position of the BMP secreting cells relative to the blastopore induced by Nodal and is completely independent of the primary embryo. Altogether, our study establishes that, artificially imposing these morphogenetic gradients to receptive, yet uncommitted cells, is sufficient to turn on and control the zygotic developmental pathways responsible for the formation of a whole embryo and supports that the main function of the maternally provided spatial determinants is to induce and/or stabilize the morphogenetic gradients of BMP and Nodal.

THE LEVELS OF SOX21 ALTER ITS FUNCTION IN NEUROGENESIS

Niteace C. Whittington, Doreen D. Cunningham, and Elena S. Casey
Department of Biology, Georgetown University, Washington DC

Neurogenesis, the progression from neural progenitor to committed neuron, is a tightly regulated process that is fundamental for development of the central nervous system (CNS). Members of the SoxB transcription factor family play critical roles in this process. Whereas the SoxB1 transcriptional activators (Sox2 and Sox3) are required for induction and maintenance of a proliferating neural progenitor population, the closely related SoxB2 proteins function as repressors and are proposed to inhibit SoxB1 targets to control the progression from progenitor to neuron. To determine the mechanism of action of the SoxB2 proteins, we are characterizing the function of the SoxB2 protein, Sox21, in primary neurogenesis in the African clawed frog *Xenopus laevis*. Our gain of function assays showed that rather than promoting differentiation, both *Xenopus* and chick Sox21 expand the neural progenitor domain and prohibit neuronal differentiation, indicating that Sox21 enables progenitors to stay in the cell cycle longer by interfering with the ability of proneural protein Neurogenin (Ngn) to induce its downstream targets and ultimately neuron formation. Our loss of function assays demonstrated that Sox21 is required for neural progenitor induction by *noggin* and is consequently required for neuron formation in ectodermal explants. However in whole embryos, while the decrease in Sox21 reduced neuron formation, progenitors remained unaffected. Together our gain and loss of function data suggest that Sox21 plays more than one role in neurogenesis. Since Sox protein target specificity and function are dependent on partner protein interactions, we propose that when expressed at different levels, Sox21 interacts with different partners and therefore has different functions during neural development.

Session III: NEURAL DEVELOPMENT

Featured Speaker: Lisa Tanneyhill (University of Maryland)

A PATH FORWARD: DISMANTLING CELLULAR JUNCTIONS DURING CRANIAL NEURAL CREST CELL EMT

Lisa A. Taneyhill and Andrew T. Schiffmacher
University of Maryland, Department of Animal Sciences, College Park, MD 20742

Neural crest cells are a population of migratory cells that play a critical role in embryogenesis. Initially stationary in the dorsal neural tube, premigratory neural crest cells undergo an epithelial-to-mesenchymal transition (EMT) that involves the dissolution of cellular junctions and cytoskeletal rearrangements in order to facilitate their emigration from the neural tube. Importantly, this EMT is co-opted during human diseases such as organ fibrosis and cancer cell metastasis, making the neural crest an outstanding in vivo model to study molecular mechanisms associated with EMT. Neural crest cells then migrate along stereotypical pathways and eventually differentiate to form the peripheral nervous system, portions of the heart, skin pigment cells and craniofacial skeleton. As such, defects in neural crest formation can have drastic developmental consequences and can cause several human congenital and hereditary malformations, diseases and cancers. Our prior work has shown that premigratory neural crest cells down-regulate components of adherens junctions, including Cadherin6B (Cad6B), through transcriptional repression to promote EMT and migration. Importantly, misregulation of this process causes defects in the generation of migratory neural crest cells. Like other cadherins, Cad6B possesses a long protein half-life. Its depletion from premigratory cranial neural crest cells, however, occurs more rapidly than normal protein turnover and is correlated with EMT. This observation implies that methods besides transcriptional repression likely also exist to remove Cad6B from premigratory neural crest cells. We now demonstrate that Cad6B proteolysis works in concert with transcriptional repression to down-regulate Cad6B during cranial neural crest cell EMT. Our results highlight the importance of using multiple mechanisms to control Cad6B expression and localization in order to ensure appropriate neural crest cell EMT and migration to pattern the vertebrate embryo.

A NOVEL, MATERNALLY EXPRESSED GENE, SMCR7L, IS IMPORTANT FOR EARLY NEURAL DEVELOPMENT IN *XENOPUS*

Paaqua A. Grant[1], Diana L.E. Johnson[1], Sally A. Moody[2]
[1]The George Washington University, Columbian College of Arts and Sciences, Dept. of Biological Sciences.
[2]The George Washington University, School of Medicine and Health Sciences, Dept. of Anatomy and Regenerative Biology.

Maternal factors, such as mRNAs, which are localized asymmetrically in the cytoplasm of eggs, are important for proper development in many organisms. In *Xenopus*, these factors specify the body axes and delimit the germ layers. We identified nearly 100 transcripts that are highly enriched in animal blastomeres of fertilized *Xenopus* embryos. One of these, SMCR7L, is a novel, vertebrate specific gene whose function is poorly understood. At blastula stages it is expressed in animal cap ectoderm, at gastrulation/neural plate stages it is dorsally enriched in the ectoderm and by tail bud stages it is highly enriched in the neural tube. Knockdown of SMCR7L with high doses of MOs results in developmental arrest at gastrulation, whereas low doses result in down-regulation of neural genes. In contrast, injection of SMCR7L mRNA into a single blastomere of 8-cell embryos expands the domains of early neural and neural crest genes, and ectopically induces *geminin* in ventral ectoderm. These data indicate an early role in neural fate specification.

DEFECTIVE NEUROEPITHELIAL CELL POLARITY IN THE ABSENCE OF TGIF FUNCTION

Anoush Anderson[1], Ken Taniguchi[1],Tiffany Melhuish[1], Ann Sutherland[2] and David Wotton[1].
[1] Department of Biochemistry and Molecular Genetics, and Center for Cell Signaling;
[2] Department of Cell Biology, University of Virginia, Charlottesville, VA.

Tgif1 and Tgif2 are transcriptional co-repressors that limit the response to Transforming Growth Factor β (TGFβ) signaling by complexing with the TGFβ-activated Smad transcription factors. Deletion or mutation of human *TGIF1* is associated with holoprosencephaly (HPE), a severe genetic disease affecting craniofacial development. We have recently shown that conditional deletion of both Tgif1 and Tgif2 in mice increases TGFβ/Nodal signaling and causes HPE, in part by disrupting the Sonic Hedgehog (Shh) signaling pathway. In addition to defects in Shh signaling and forebrain patterning, conditional Tgif mutant embryos appear to have disrupted neuroepithelial cell polarity, which primarily affects the ventral part of the neural tube. In the ventral neural tube of Tgif mutant embryos at E8.5-E9.5 the subcellular localization of aPKC is defective, cell-cell junctions are disrupted and an isolated neural tube forms between the notochord and the remaining dorsal neural tissue. These defects appear to be dependent in part on excess TGFβ/Nodal signaling, but are independent of the Shh pathway. We are currently analyzing how this isolated neural tube forms, and testing whether it affects dorso-ventral neural tube patterning. Our working model is that in the absence of Tgif function, excess TGFβ/Nodal signaling drives loss of neuroepithelial cell polarity, resulting first in ventral neural tube disorganization, followed by the formation of an isolated ventral neural tube, as cell polarity is restored. We are now testing this model and trying to identify Tgif target genes that may be responsible for driving this phenotype.

***FGF3* REGULATION OF BMP SIGNALING IS REQUIRED FOR NEURAL TUBE CLOSURE**

Matthew J. Anderson[1], Thomas Schimmang[2], Mark Lewandoski[1]
[1]Cancer and Developmental Biology Laboratory, NCI Frederick, NIH
[2]Institute For Biology and Molecular Genetics, University of Valladolid, Valladolid Spain

Fibroblast growth factors (FGF) are well known for regulating early mesoderm and somitogenesis during axis extension. However, axis extension also requires a coordination of morphogenesis between all primary lineages, particularly the early mesoderm and neural tube (NT); defects in one often affect the other. However, little is known about the signals through which these two tissues communicate. We have uncovered a new role for FGFs in signaling from the mesoderm to the NT for the normal development of both tissues and normal axis extension.

Adult *Fgf3* null mice ("mutants") have axis extension defects with fewer and malformed caudal vertebrae, due to embryonic defects in somitogenesis. These phenotypes are secondary to a NT closure defect (NTD), first evident at the 25-somite stage, preceding mesoderm malformation by ~ 16 hours. Furthermore, *Bmp4*, shown previously to inhibit NT closure, is upregulated and caudally expanded in the mutant dorsal NT, beginning at the 24-somite stage. Thus, we hypothesize that FGF3 repression of *Bmp4* expression in the NT is required for NT closure. To test this we performed three genetic manipulations. First, we increased BMP signaling by deleting a single allele of the BMP antagonist *Noggin* resulting in a greater severity in the NTD and a subsequent greater caudal truncation. Second, we attenuated BMP signaling via homozygous deletion of the receptor gene *BmpRIb,* partially rescuing the NTD and subsequent caudal malformations. Finally, we deleted NT *Bmp4* expression in mutants, which rescued the NTD, confirming the role of BMP signaling in the etiology of the *Fgf3* mutant. This work represents the first identification of a mouse FGF-ligand that signals to the NT thereby regulating both NT closure and normal axis extension, Furthermore this is a significant advance in our understanding of the etiology of NTDs, which occur in approximately 1% of human births.

A GRADIENT OF RETINOIC ACID SIGNALING REGULATES TONOTOPIC DEVELOPMENT IN CHICK COCHLEA

Benjamin Thiede[1], Zoe Mann[2], Yuan-Chieh Ku[3], Michael Lovett[3], Matthew Kelley[2], Jeffrey Corwin[1] (presented by J. Corwin)
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Frequency tuning depends on the basilar membrane's resonance properties and the responses of hair cells (HCs). In the chick cochlea, there is a longitudinal gradient of HC phenotypes. Proximal end HCs contain 250 or more stereocilia that reach a maximum length of 1.5 um, while distal-end HCs contain 50 or fewer stereocilia that reach a maximum length of 5.5 um. At locations between those ends, the number and length of stereocilia grade from HC to HC. We sought to discover what signals pattern the development of this gradient of HC phenotypes. Using transcriptome sequencing we sought to identify components of signaling pathways that are differentially expressed along the tonotopic axis of the E6.5 chick cochlea. We used qPCR to check these results and to evaluate expression at other ages. To test whether treatments would affect phenotypes that are linked to HC position, we cultured cochleae from E6.5 embryos for 6 days in a control medium or media supplemented either with all-trans retinoic acid (RA) or citral, a synthesis inhibitor. We used Scanning EM to assess stereocilia length and number, qPCR to assess expression of the B1 subunit of the BK channel, and immunostaining to assess Calbindin expression (phenotypes that all differ along the longitudinal axis).
In our analysis of the sequence data we discovered opposing longitudinal gradients of mRNA expression for an enzyme involved in the synthesis of RA and an enzyme involved in RA degradation. RALDH3, a RA synthesis enzyme, is expressed in a proximal-to-distal descending gradient, while CYP26C1, a RA metabolizing enzyme, is expressed in a distal-to-proximal gradient. However, a reverse (distal-to-proximal) gradient of RALDH3 is expressed at E10 and persists in the P14 cochlea. When we cultured E6.5 cochleae in control medium for 6 days they developed the expected longitudinal gradient of HC phenotypes. In contrast, cochleae we cultured with RA developed HCs with distal-like phenotypes throughout the sensory epithelium. Cochleae cultured with the RA synthesis inhibitor, citral, developed HCs with more proximal-like phenotypes. RA is a candidate morphogen that may influence the longitudinal patterning of HC phenotypes in the developing chicken cochlea. The evidence for a RA gradient we observed suggests that it may have an important role in the development of tonotopy.

MOTOR NEURONS SIGNAL MYOBLAST PROLIFERATION DURING *DROSOPHILA* MYOGENESIS USING THE EGF PATHWAY

Joyce Fernandes and Kumar Vishal, Miami University, Oxford Ohio.

Myogenesis of the indirect flight muscles of the *Drosophila* thorax takes place during the first 24 hours of the 4- day pupal phase. Muscle precursor cells that originate in the wing imaginal disc undergo several rounds of proliferation and fusion to give rise to the largest muscles of the adult fruit-fly. The myoblasts usually fuse with organizer cells to generate the nascent fibers that in turn serve as targets for continued fusion. Denervation experiments showed that a burst of myoblast proliferation that normally occurs coincident with peak fusion is eliminated (Fernandes and Keshishian, 2005), along with the reduction in expression of *dumbfounded,* an IgSF molecule expressed in muscle founder cells which is known to promote fusion. Taken together, these results formed the basis of a working model in which the innervating motor neuron signals to founder cells to regulate myoblast proliferation. Presence of MAP Kinase in myoblasts and nascent fibers prompted us to examine a role for the EGF signaling pathway. Targeted expression of dominant negative EGF receptors in the founder cells resulted in a 41% reduction of nuclei in adult muscle fibers ($P<0.005$), and defects in fiber number and pattern in 95% of animals. Expression of dominant negative EGF receptors in myoblasts resulted in a less severe phenotype (32% reduction in nuclear number; fiber number is altered in 60% of animals). When the expression of EGF ligand Spitz was blocked in motor neurons, fiber number and pattern was altered in 100% of experimental animals. Current studies will examine proliferation rates and the process of fusion during the pupal phase in the above genetic manipulations. The nerve dependence of adult myogenesis in *Drosophila* has the potential to serve as a model for vertebrate skeletal myogenesis.

Session IV: Evo-Devo / Eco-Devo

Featured Speaker: Eric Engstrom (Hampton University and College of William and Mary)

HAM PROTEINS AND CELL DIVISION: A PIVOT POINT IN ANGIOSPERM EVOLUTION?

Eric Engstrom, Hampton University and College of William and Mary

Vascular plants grow discontinuously throughout their life-spans, repeatedly initiating new shoot and root systems. This capacity for continuing organogenesis and growth throughout their lifespans, termed indeterminate growth, or simply indeterminacy, permits plants to adaptively regulate their development in response to dynamic environments, which, as sessile organisms, they cannot relocate away from in response to adverse conditions. Indeterminacy, in turn, requires retention of populations of pluripotent stem cells, meristems, which are distinguished from differentiating cells by their rates of progression through the cell cycle. HAIRY MERISTEM (HAM) proteins, members of the GRAS family of transcriptional regulators, are essential for maintenance of indeterminate growth in flowering plant shoots. Specific cellular/molecular functions of HAM proteins underlying meristem maintenance are largely unknown. However, recent studies have identified genes encoding cell cycle regulatory proteins, including *CYCLIN B*, as transcriptional targets of HAM function. Consistent with a role for HAM proteins in regulating cell cycle progression, recently acquired complete null loss-of-function *ham* mutants exhibit phenotypes consistent with impaired cell cycle progression both within and outside of the shoot meristem. Molecular phylogenetic analyses of HAM proteins suggest that new functions for HAM proteins were acquired early in flowering plant evolution, suggesting the intriguing possibility that HAM protein evolution may have played a significant role in the evolution of the unique organization and characteristics of flowering plant meristems.

THE EVOLUTION OF DEVELOPMENTAL GENE REGULATORY NETWORKS FOR ENDOMESODERMAL SPECIFICATION AMONG ECHINODERMS.

Brenna McCauley, Veronica Hinman
Carnegie Mellon University. Department of Biological Sciences

The early development of the sea urchin is a model system for understanding how cells become specified and take on separate identities during embryogenesis. The recent focus in the sea urchin field is to take a systems approach to this question, to as completely as possible describe the gene regulatory network (GRN) for this process, with the goal of providing an explanation of the causal link between the genome and development. We are undertaking a similar systems approach to define the GRN for early specification in another distantly related echinoderm, the sea star *Patiria miniata*. The goal of this comparative work is to understand how biological systems can reengineer GRN topologies for changed developmental outcomes. I will discuss our recent work that focuses on determining how early and maternal processes control endoderm and mesoderm specification in sea stars. Sea urchin and sea stars have overall very similar early development but with specific differences that require a restructuring of the GRN. For instance sea stars do not produce a micromere cell lineage which in sea urchins is central for establishing the remaining endomesoderm. We find that positive transcription factor interactions are highly conserved, but that repression, quantitative responses, and signaling interactions are more evolutionary labile.

MODULARITY IN DNA BINDING PREFERENCE OF A TBRAIN TRANSCIPRTION FACTOR MAY ALLOW FOR MORE VERSATILE TRANSCRIPTIONAL RESPONSES AND INCREASED EVOLVABILITY

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In the starfish (Pm), Tbrain (Tbr), a t-box transcription factor, carries out a variety of ancestral roles in the endomesoderm and ectoderm. However, in the sea urchin (Sp), Tbr has a singular and well-defined role in the skeletogentic gene regulatory network (GRN). The DNA binding regions of these proteins contain differences in critical DNA-contacting amino acids, suggesting a protein level change could be in play, but it is thought that this type of change must be incredibly rare owing to the potentially lethal pleiotropic effects of altering a multifunctional protein. However, novel techniques have allowed for far more sensitive assays of transcription factor function. One such technique, Protein-Binding Microarrays (PBMs), has indicated that DNA binding is more complex than originally indicated, in that the same transcription factor can recognize multiple binding sites, and only a subset of these might be conserved among closely related paralogs (Badis and Bulyk 2009). Here we first demonstrate that this type of complexity also applies to orthologous transcription factors. While both Tbr orthologs recognize the same primary motif, only PmTbr also has a secondary binding motif. While affinity for the primary binding site is relatively conserved, affinity for the secondary binding motif is more evolutionarily labile. We have verified the effects of binding specificity and affinity changes *in vivo* using a dual OtxB1/2 CRM reporter system. The secondary site is able to substitute for the primary site to produce almost identical levels reporter expression when Tbr levels are sufficiently high. However, reduced Tbr levels elicit a more drastic decrease in expression from the secondary site-containing reporter compared to wild type; we predict such a response is advantageous when a rapid transcriptional response is required during development, whereas more gradual changes in other genes' expression may be able to occur simultaneously through utilization of primary binding sites. We have uncovered a layer of TF binding divergence that could exist for many pairs of orthologous TFs. As an ortholog gains binding sites or loses binding sites, it could result in a disproportionate gain or loss in complexity of developmental territory patterning, resulting in significant evolutionary changes.

WNTLESS: STRUCTURE TO FUNCTION

Senel S. Tektas and Erica M. Selva, Department of Biological Sciences, University of Delaware

Wingless (Wg) is the *Drosophila* founding member of the Wnt family of secreted signaling molecules that regulate cellular signaling pathways conserved from *Drosophila* to humans. Wnt signal transduction is required for numerous phases of organismal development and tissue homeostasis and when aberrant, can drive cell transformation that results in cancer. Wntless (Wls) is a multipass transmembrane protein that is a conserved component of the Wg/Wnt pathway. It acts within Wg/Wnt-producing cells to promote Wg secretion and downstream pathway activation. Wls functions as a chaperone that binds to Wnt/Wg in the endoplasmic reticulum (ER) to escort it through the secretory pathway for cell surface deployment, a necessary step for downstream signaling. One puzzling feature of Wnt signaling ligands is the covalent addition of palmitoyl-moieties within the ER that renders them lipophilic and unable to traverse the secretory pathway or be released into the extracellular space as a soluble protein. Therefore, defining structural features of Wls required for Wg binding, secretory pathway escort and cell surface deployment could expend our understanding of complex Wnt signaling mechanisms. The overall objective of this project is to define the Wg binding domain within Wls and confirm the existence of a recently identified homo-oligmerization property of Wls protein and ultimately determine its function in Wg signaling. Using an immunoprecipitation approach, we have mapped the minimal Wg binding domain of Wls to a region roughly within amino acids 113 to 137. Immunoprecipitation studies confirm Wls does homo-oligomerize and both its first trans-membrane span, as well as residues between amino acid 137 to 223 plays a role in this process. Furthermore, oligomerization is required to create the Wg binding pocket. Finally, we have shown that N-glycosylation at asparagine 58 is required for secretion of soluble Wls truncations into the cell culture media and thus, is likely require for Wls membrane targeting.

DIVERSITY OF EARLY DEVELOPMENT IN DIVERSE NEMATODE SPECIES

Theresa M. Grana
University of Mary Washington

Across the diversity of nematodes numerous developmental schemes are used to generate the basic worm body plan. Comparison of the development of both distant and closely related nematodes continues to shed light on the plasticity of early development versus constraints on development. For example, comparison of basal and more derived nematode species has shown a trend from indeterminate early development to less variance in early cleavages. Comparisons of early development of the relatively closely related species *C. elegans* and *C. briggsae* have shown that cell fate specification can occur through distinct means. Here, I describe the development of a set of seven nematode species representing members of clades 9B, 9D, 9E and 11*, for which there is no published description of development. Found locally on the UMW campus, the surrounding counties, and other places my students have traveled, several of these are new species and others are new strains of formally described species. Cleavage patterns, nuclear positioning, and rate of development will be compared to published studies of members of the same clades and subclades. This and future studies will shed additional light on the evolution of development.

Session V – Morphogenesis & Organogensis

Featured Speaker: Marnie Halpern (Carnegie Institution for Science)

FORMATION AND FUNCTION OF LEFT-RIGHT ASYMMETRY IN THE ZEBRAFISH BRAIN

Marnie E. Halpern, Tagide deCarvalho, Erik Duboué, Lucilla Facchin, Elim Hong and Abhignya Subedi
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A sequence of developmental events results in left-right (L-R) differences in the epithalamus of the zebrafish larval forebrain, with distinct sidedness in neuroanatomical and molecular properties. The consequences of this are widespread in the nervous system, as epithalamic asymmetry directs pre-synaptic olfactory input and determines how efferent connections are made with a midbrain target. We are currently examining the significance of this circuitry and how it is established. A variety of behavioral assays reveal that larvae and adults with either L-R reversal or loss of epithalamic asymmetry differ significantly in their responses compared to controls. In particular, larvae with reversed directionality show a prolonged recovery to an aversive stimulus. L-R reversed adults exhibit behaviors indicative of increased fearfulness or anxiety, and elevated levels of the stress hormone cortisol. The results indicate that development of a localized asymmetry has a more extensive influence throughout the brain and that altering its directionality leads to unexpected changes in behavior.

EXTRACELLULAR ATP REGULATES CONTRACTILE MECHANICAL TONE OF EARLY EMBRYONIC EPITHELIA

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Cell signaling and mechanical forces play vital roles in morphogenesis and organogenesis. However, pathways that trigger the generation of forces shaping embryonic tissues are not well understood. We previously utilized nano-perfusion assays to demonstrate that cell lysate can induce F-actin remodeling and epithelial contractions. This study identifies ATP as the active component in lysate, participating in a G-protein coupled receptor (GPCR) pathway to regulate contractile tone of epithelium during gastrulation. After testing a series of candidate factors, ATP was identified as the primary candidate in lysate causing contractions. ATP was nano-perfused over the animal cap of *Xenopus laevis* embryos. Acute exposure produced spatiotemporally-specific epithelial contractions while chronic exposure caused exogastrulation. Using the ATP hydrolyzing enzyme apyrase, embryos were rescued from the effects of ATP or lysate exposure on blastopore closure. Incubation with apyrase alone resulted in slightly faster blastopore closure, implicating a role of endogenous extracellular ATP regulating gastrulation. Using various inhibitors of F-actin and non-muscle myosin II, we showed actomyosin contractility is a key target in the response to ATP. Of the possible receptors for extracellular ATP, we demonstrated that GPCRs of the P2Y family were likely candidates by expressing Gγ-SaaX mutant mRNA in embryos, which reduced or abolished contractility. Temporal expression of P2Y-family proteins, analyzed using PCR, indicated P2Y2R as the target receptor. Two morpholino oligonucleotides specific to alloalleles A and B (P2Y2R-MO) were injected into epithelial precursors at the 8-cell stage to knock down P2Y2R expression. Nano-perfusion of ATP over P2Y2R-MO injected embryos had diminished or no contractions. This strongly suggests that ATP induces epithelial contractility through the P2Y2R GPCR, offering an example of a robust pathway controlling embryonic ectodermal contraction mechanics.

INTERDIGIT BMP SIGNALING IS ESSENTIAL FOR PROGRAMMED CELL DEATH AND IS IMPLICATED IN DIGIT FORMATION

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Shaping of the embryonic limb involves many processes including growth, differentiation, and programmed cell death (PCD). Furthermore, these processes integrate complex information from multiple signaling cascades such as the Bone Morphogenetic Protein (BMP) and Fibroblast Growth Factor (FGF) pathways. Our previous work has shown that BMP signaling regulates interdigit (ID) PCD indirectly by modulating the secretion of FGFs from the apical ectodermal ridge, which act as cell survival factors to the ID mesenchyme. Nevertheless, this indirect model does not exclude a direct role for BMPs in PCD. Due to lack of genetic evidence, it is still unclear whether ID BMPs directly trigger PCD. To test whether BMPs act as direct triggers of ID PCD, we inactivated gene encoding the receptor BMPR1A within the ID tissue with Osr1-Cre. The resulting mutants are syndactylous and show a decrease of embryonic ID PCD.To test redundancy between BMPR1A and BMPR1B in PCD we inactivated ID *Bmpr1A* in a *Bmpr1B* $^{null/null}$ background. This compound mutant has a further decrease in PCD. During this analysis we serendipitously discovered a potential role of the ID in digit formation. *Bmpr1B* $^{null/null}$ digits are short with abnormal development of their phalanges. The defect is completely rescued in digit one when we inactivate *Bmpr1A* in the ID and to a limited degree in digits two through five. Marker analysis for RNA expression of various components of the BMP signaling pathway revealed a dramatic upregulation of *Growth Differentiation Factor 5 (GDF5)*, a potential alternate ligand, in the ID mesenchyme. Currently, GDF5 has not been implicated to be involved in limb PCD. To fully understand the role of ID BMP signaling on normal limb development, we are also inactivating in the ID mesenchyme *Bmp2, 4,* and *7* with Osr1-Cre. Preliminary analysis of these mutants reveals a syndactylous phenotype. This work will clarify the role of ID BMP signaling in directly regulating PCD and digit formation.

ABLATION OF p120 CATENIN IN EMBRYONIC MOUSE PANCREAS DISRUPTS ACINAR CELL, ISLET, AND DUCT DEVELOPMENT

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To examine the role of p120ctn in mouse pancreas development, we ablated p120ctn selectively in developing pancreatic epithelium using an early Pdx1:cre driver. Expression of Cre in this line is ubiquitous in multipotent pancreatic progenitor cells at E10.5, and thus targets all lineages of the developing pancreas – acinar, islet, duct, and centroacinar. Pdx1:cre^{Tg}; p120$^{f/f}$ homozygotes display striking morphological and organizational anomalies at P0 in all pancreatic lineages. Loss of p120ctn early in development results in a profound expansion of tubular epithelia, loss of acinar cell mass, and disruption of islet localization within the embryonic pancreas. The expanded tubular epithelium is accompanied by the continued expression of transcription factors Sox9 and Aldh1 in this compartment. A dramatic tubulogenesis phenotype becomes evident at E12.5 marked by increased epithelial tubule lumen diameter. Despite the loss of p120ctn, epithelial cell-cell junctions appear normal. We show that *in vivo* substitution by p120ctn family members ARVCF, δ catenin, and p0071 is a likely mechanism for the maintenance of epithelial integrity during development in p120ctn null pancreas.

AMPLITUDE OF GROWTH FACTOR SIGNALING TUNES CRANIOFACIAL MORPHOLOGY

Brian J. Cusack and Heather L. Szabo-Rogers

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Human orofacial clefting (OFC) is the most common human congenital anomaly, occurring in approximately of 1/700 live births and has a complex multifactorial, multigenic origin. While significant work has been done to identify the genomic regions associated with increased risk for human OFC, the molecular and morphogenetic consequences are unexplored. The previously identified human OFC risk genes are enriched in effectors of the transforming growth factor beta, hedgehog, Wnt and Fibroblast growth factor signaling pathways. There are no clear functional consequences within the human loci for these genes associated with OFC and traditional mouse models do not have overt craniofacial phenotypes. We hypothesized that the OFC risk-genes are modulating the output of these signaling pathways which results in abnormal morphogenesis. We chose to modify these pathways in the zebrafish embryo during three distinct time periods of craniofacial development: neural crest migration, patterning and differentiation. We found both dose and stage dependent changes to facial morphology and in particular to the shape and size of the ethmoid plate- the surrogate zebrafish palate. Interestingly, we found that muscles can still develop in the absence of jaw cartilage. We conclude that careful dissection of the timing and amplitude of pathway activation will provide insights into human OFC.

RETINOIC ACID: A MASTER REGULATOR OF CRANIOFACIAL DEVELOPMENT

Amanda Dickinson, Virginia Commonwealth University

My lab has uncovered multiple roles for retinoic acid during craniofacial development. First we have defined a role for retinoic acid signaling in activating transcription of homeobox genes Lhx8 and Msx2 during upper lip and primary palate development in *Xenopus laevis*. These genes are both expressed in overlapping domains with retinoic acid receptor gamma (RARγ) and RALDH2. Together, decreased lhx8 and msx2 mimics decreased retinoic acid signaling and embryos form a median cleft in the upper lip and primary palate. Inhibition of RARγ and decreased Lhx8/Msx2 function result in decreased cell proliferation and failure of dorsal anterior cartilages to form. These results suggest a model whereby retinoic acid signaling regulates Lhx8 and Msx2, which together direct the tissue growth and differentiation necessary for the upper lip and primary palate morphogenesis. Secondly, we have found that retinoic acid also regulates the transcription factor RAI1 (retinoic acid induced 1) in *Xenopus*. Mutations to this gene cause Smith-Magenis Syndrome (SMS), a rare genetic disorder characterized by craniofacial defects including cleft palate. We have performed the first characterization of RAI1 during development in a vertebrate model system. Decreased RAI1 in *Xenopus* closely resembles the human condition showing both orofacial and brain defects. Finally, we have found that retinoic acid regulates neurotrophic and guidance factors and decreased signaling results in defasiculation of the trigeminal nerve. In summary, we have formulated the hypothesis that retinoic acid is a master regulator of multiple tissues during orofacial development. Our work in trying to understand how retinoic acid modulates gene expression has the potential to lead to a better understanding of the complex nature of this region of the embryo and thus the etiology of many human orofacial diseases.

Session VI: Stem Cells & Regeneration

Featured Speaker: Stephen DiNardo (University of Pennsylvania)

RESOLVING ZIG AND ZAGS: ASSIGNING PLANAR CELL POLARITY DURING EPITHELIAL MORPHOGENESIS

Kynan T. Lawlor, Daniel Ly, Seth Donoughe, Bob Simone and Stephen DiNardo
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Directional cues are necessary to coordinate tissue morphogenesis during development. The polarization of cells along the plane of a tissue, termed planar cell polarity, is one way in which such directional cues are specified. Two groups of genes – the Dachsous (Ds) and Frizzled (Fz) systems – play key roles in the establishment, maintenance and propagation of such polarity. While progress has been made in understanding the function of these two systems, unresolved issues remain. Primary among these is whether these systems act in parallel or in sequence, and how they engage effectors. Studies in Drosophila have provided much insight into resolving these issues. In most tissues commonly studied, however, the expanse of the tissue sometimes presents difficulties with interpretations. In contrast, we have been investigating the establishment of planar polarity within the embryonic epidermis, which is more confined, experiencing much less proliferation. With an advantage that issues of growth control, which is a second distinct output of Ds signaling, do not arise, study of planar polarization within the embryonic epidermis should provide an excellent complementary system to other tissues. Indeed, we have described several polarized events across this epithelium, including junctional remodeling and the asymmetric positioning of f-actin bundles. I'll report some of our progress on using this tissue for unraveling the contributions from Ds and Fz to polarization.

DEFINING THE ORIGINS OF THE HEMOGENIC ENDOTHELIUM, THE SOURCE OF HEMATOPOIETIC STEM CELLS, BY LINEAGE TRACING *TBX4*.

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Differentiation is normally thought of as a linear process, proceeding from less differentiated to more differentiated cell fates, and reversal of this process is generally thought of in the context of oncogenic transformation or manipulations such as iPS cells. However, during normal development, hematopoietic stem cells (HSCs) arise from apparently differentiated, functional endothelial cells. This remarkable transdifferentiation occurs in the endothelial walls of specific embryonic vessels, called the hemogenic endothelium. A significant unanswered question is the difference between hemogenic and non-hemogenic endothelium, which both express markers of fully differentiated endothelium. The vasculature of the placenta and umbilical cord, which are hemogenic tissues, originates in a transient embryonic organ called the allantois. We have observed that the bulk of the allantois is formed from cells that express T-box transcription factor *Tbx4* or their descendants (the *Tbx4* lineage), but the allantois-derived endothelium arises from two different lineages. The endothelium of the umbilical cord and proximal placenta are derived from a distinct population of cells that are not part of the *Tbx4* lineage, while the endothelium of the distal placenta is derived from the *Tbx4* lineage. This spatial distribution correlates with observed hemogenic fate and non-hemogenic fate, respectively. Despite abundant *Tbx4* lineage endothelium in the hemogenic placenta, the *Tbx4* lineage does not populate either circulating blood or HSC niches. These results indicate that the endothelium can be functionally sorted into two populations: a hemogenic lineage that has never expressed *Tbx4,* and a non-hemogenic *Tbx4* lineage. Because the *Tbx4* lineage arises several days prior to hematopoietic activity, isolation of these lineages allows us to investigate the underlying factors that determine the ability of endothelium to retain the potential for future stem cell transdifferentiation.

TGFβ SIGNALING IN REGENERATION OF THE ZEBRAFISH RETINA

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Zebrafish have a remarkable ability to regenerate central nervous system tissue, and in particular the neural retina. Müller glia are the radial glial cells of the retina; in zebrafish, they act as tissue-specific stem cells that are capable of regenerating all retinal neurons. Following acute light lesion of the cone photoreceptors, Müller glia activate, dedifferentiate, and divide to form rapidly proliferating neurogenic progenitors that replace the destroyed photoreceptors. Proliferation in mammalian Müller glia is inhibited by canonical TGFβ signaling during development *in vivo* and *in vitro*. We previously showed dynamic regulation of canonical TGFβ signaling pathway members in a microarray analysis of adult zebrafish Müller glia during the first 36 hours after destruction of photoreceptor neurons. We are now testing the hypothesis that regulation of TGFβ signaling is necessary for the proliferative response of Müller glia to photoreceptor destruction in adult zebrafish. Our current research focuses on the TGFβ signaling transcriptional co-repressors *tgif1* and *six3b* that are upregulated in Müller glia within 8 hours post lesion. We found that fish homozygous for the *tgif1*^{fh258} allele express only a truncated Tgif1 protein and have reduced proliferation following destruction of photoreceptor neurons. We examined expression levels of several genes critical for retina regeneration. Only genes known to be regulated by TGFβ signaling were misregulated in *tgif1*$^{fh258/fh258}$ fish relative to wild-type siblings. Furthermore, *tgif1*^{fh258} mutant fish have significantly less photoreceptor regeneration than wild-type fish, and *tgif1*^{fh258};*six3b*^{vu87} double mutants show a synergistic genetic interaction. Our data suggest that there are multiple mechanisms in place to down-regulate TGFβ signaling during

photoreceptor regeneration. We are continuing to compare the activation, dedifferentiation, and proliferation of Müller glia in these mutant fish to wild-type fish.

DEVELOPMENT OF A MODEL FOR ROBERTS SYNDROME IN ZEBRAFISH REGENERATING FINS

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Roberts syndrome /SC-phocomelia is a rare genetic disorder affecting human development. It is characterized by severe growth deficiency leading to craniofacial abnormalities, limb malformation, cleft palate, ear malformation, corneal clouding and mental retardation. Recently it has been reported that mutations in *ESCO2* causes Roberts syndrome. ESCO2 is an acetyltransferase that is required for the establishment of sister chromatid cohesion during S phase of cell division. *ESCO2* activity in cell cycle is well established but its role in regulating gene expression is still not known. Using zebrafish regenerating fin model it is possible to understand the underlying molecular mechanisms of the developmental abnormalities seen in Roberts syndrome. The overall goal in this project is to find the role of *ESCO2* in regulating gene expression that is separate from its role in cell cycle. Interestingly we find that *esco2* regulates expression of gap junction gene *cx43*. During fin regeneration, mutation in *cx43* causes the short fin (*sof*^{b123}) phenotype which is characterized by short fins due to defects in length of the bony fin rays. Previously it has been shown by our lab that Cx43 is required for both cell proliferation and joint formation during fin regeneration. In this present work it has been demonstrated that knock down of *esco2* recapitulates similar phenotypes caused by knock down of *cx43* gene in regenerating fins. Therefore morpholino mediated *esco2* knock down in regenerating fin shows decrease in fin length and segment length. Using in situ hybridization we find that *cx43* expression depends on *esco2* function. Together, our findings reveal that *esco2* knockdown recapitulates the *sof*^{b123} and *cx43*-knockdown phenotypes, and appears to function upstream of *cx43.*

THE VERTEBRATE-SPECIFIC KINESIN, KIF20B, IS REQUIRED FOR CYTOKINESIS OF POLARIZED CORTICAL STEM CELLS AND NORMAL CEREBRAL CORTEX SIZE

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Most studies of cytokinesis are performed in isolated single cells. Yet in developing organisms, cells must divide within a tissue, maintaining structure and patterning. Furthermore, stem cells divide symmetrically or asymmetrically by cytokinetic partitioning of cell fate determinants. However, the mechanisms of cytokinesis in developing tissues are poorly understood. A mouse mutant in the kinesin Kif20b may help elucidate the unusual requirements for cytokinesis in the developing brain. Mammalian neural stem and progenitor cells reside in a highly polarized epithelium, and their cytokinesis is polarized. The cytokinetic furrow ingresses from basal to apical, and the midbody forms and mediates abscission at the apical membrane. We found that mutation of *Kif20b* in mice disrupts cytokinetic midbodies of cortical neural stem cells and causes reduced cerebral cortex size. Kif20b was previously shown to be involved in late cytokinesis, during abscission, but its precise role was not defined. Kif20b protein normally localizes to cytokinetic midbodies of dividing cortical stem cells, but is undetectable in *Kif20b(magoo)* mutant cells. In the mutant brains, cytokinetic furrows and midbodies form, but the number and positioning of midbodies at the apical membrane is abnormal. Surprisingly, binucleate cells are not increased in *magoo* mutant brains, but apoptosis is increased. Apoptosis is highest at early ages when cortical stem cells divide symmetrically. Possible roles of Kif20b in cytokinesis and in postmitotic differentiation will be discussed. In conclusion, Kif20b is required for organization of cytokinetic midbodies of cortical stem cells, and for normal cerebral cortex growth. Loss of Kif20b appears to increase abscission failures and apoptosis. We speculate that regulation of cytokinesis mechanisms may play a role in controlling organ size, and that abnormalities of cytokinesis may be an unappreciated cause of human developmental disorders.

POSTER ABSTRACTS

Epigenetics and Genomics

1. MOLECULAR DETERMINATION OF TERMPERATURE-SENSITIVE EMBRYONIC LETHAL MUTANTS BY WHOLE GENOME SEQUENCING IN *C. elegans*

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A large set of temperature-sensitive embryonic lethal and zygotic defective mutants were previously established through EMS mutagenesis (Hirsh and Vanderslice, 1976 and Cassada *et al*., 1980). Although the approximate map position for each is known, the mutated genes remain unknown after more than 30 years since their isolation. We selected a few strains for re-characterization of the phenotype and identification of the mutant gene. Strains that reproduced the embryonic lethality by shifting to the non-permissive temperature of 24°C were pursued further. After a single out-cross with the Hawaiian strain, homozygotes were re-isolated and pooled and genomic libraries of each were constructed. These libraries were subjected to WGS. By identifying all known Hawaiian single nucleotide polymorphisms (SNPs), gene candidates were identified based on the absence of Hawaiian SNPs and the presence of coding sequence changes. These genes are thus the likely candidates responsible for the embryonic lethality phenotype. Using RNAi, we are currently testing each candidate, to determine whether they phenocopy the embryonic lethality. Additionally, from our sequencing results, it appears that some mutations previously thought to be in separate genes may be allelic. We are currently confirming this by complementation tests.

2. EPIGENETIC MODIFIER CONTROL IN STEM CELLS

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Stem cells are necessary for normal mammalian development as well as general maintenance of tissues in adult organisms. Mouse embryonic carcinoma and embryonic stem cells serve as good in vitro models of stem cells and early embryonic events. Stem cell characteristics are inextricably linked to which genes are expressed and which ones are repressed in cells at a given time. Incorrect gene expression can lead to severe developmental defects or embryonic arrest. Many factors control gene expression but epigenetic modifier genes encoding proteins for histone modifications, DNA methylation, and chromatin remodeling are an important regulatory group. Loosely packaged chromatin generally leads to greater accessibility of DNA sequences and gene expression while condensed chromatin often results in loss of accessibility and gene repression. There are many epigenetic modifier genes in the mammalian genome which help to precisely control which genes are “on” or “off” in cells at a specific stage. While several of these genes have been characterized as important or even essential in early mammalian development, the role of many more chromatin genes in stem cells has yet to be elucidated. Using siRNA in an in vitro model of mouse stem cells, we have identified chromatin modifier genes influencing stem cell characteristics including growth, stem cell gene expression, anchorage independent growth, and alkaline phosphatase stain. Further characterization of the role of these epigenetic modifiers in stem cells could impact our understanding of stem cell gene control as well as have implications for therapeutic uses of stem cells.

3. INVESTIGATING THE PHYSICAL INTERACTION BETWEEN HISTONE REPRESSOR A AND ANTI-SILENCING FUNCTION1 A IN *ARABIDOPSIS*

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Histone Repressor A (HIRA) is a protein that regulates development in both plants and animals. In animals, mutations in *HIRA* cause early embryo lethality in mice and may contribute to DiGeorge syndrome in humans, a cranio-facial disorder. The mammalian HIRA is known to physically interact with ANTI-SILENCING FUNCTION1 A (ASF1A). The physical interaction between HIRA and ASF1a is required to form a protein complex that permanently represses genes that cause cells to divide. Very little is known about HIRA or ASF1A in plants. As in mammals, mutations in HIRA cause embryo lethality. Mutations in ASF1A also cause developmental abnormalities (but not lethality) in plants. The goal of this project is to determine if HIRA and ASF1A physically interact in plants as they do in animals.

4. FUNCTIONAL CHARACTERIZATION OF THE UPSTREAM REGULATORY REGIONS OF X-MSR, A GENE INVOLVED IN VASCULAR AND NEURAL DEVELOPMENT

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MSR, also known as the APJ receptor in mammals, is a G-protein coupled receptor that functions in the development of the vertebrate vascular and nervous system. The *X-msr* gene is broadly expressed throughout the developing vascular system beginning early during embryogenesis; later in development it is expressed in discrete regions of the nervous system. It is also implicated in suppressing neuronal differentiation in ES cells. While its role in vascular and cardiac development is relatively well studied, the role of *X-msr* in neural development remains poorly understood. To address the transcriptional regulatory mechanisms by which *X-msr* is expressed in neural tissue we cloned 2.2 kilobases of the upstream region and created transgenic lines to drive expression of GFP. We have compared expression of endogenous *X-msr* with that of the GFP transgene throughout development and found that the expression patterns are significantly different. Unlike the endogenous gene, the GFP transgene was strongly expressed throughout the nervous system and in the ciliary margin of the retina. This suggests the possible absence of a transcriptional repressor in the 2.2kb upstream region, an element which we are attempting to identify. Using bioinformatic and transgenic analyses of *X-msr*, we are also conducting promoter analysis to identify the transcription factors and corresponding binding sites responsible for expression in the ciliary margin and other neuronal locations.

5. ANALYSIS OF miRNA EXPRESSION IN *brd2* KNOCK DOWN ZEBRAFISH (*DANIO RERIO)* EMBRYOS

Alison Presti and Angela DiBenedetto, Villanova University

This research project assays genome-wide microRNA (miRNA) expression in *brd2* knock-down zebrafish (*Danio rerio*) embryos using miRNA microarray technology in order to identify potential miRNA targets of Brd2 transcriptional regulation. Brd2 is a transcriptional co-regulator that functions in formation of chromatin complexes affecting signal-dependent transcription and is implicated in proliferation, apoptosis, cell cycle control and homeotic regulation in development. However, nothing is known about potential miRNA targets, which are powerful post transcriptional gene regulators and have key roles in development. By binding to the 3'UTRs of specific target mRNAs, they repress translation of and/or destabilize that mRNA. A single miRNA may bind any gene transcript carrying a complementary sequence in its 3'UTR, and thus can potentially control hundreds of target mRNAs. Genome-wide miRNA expression was analyzed in *brd2*-knockdown zebrafish embryos. Anti-Brd2-specific antisense morpholinos capable of blocking *brd2* mRNA translation were microinjected into the embryos to create *brd2*-knockout embryos, while control morpholino-injected emabyros served as comparisons. At 48 hours of development, total embryonic RNA from was extracted and used to synthesize fluorescently tagged and amplified miRNA complementary DNA (cDNA) probes for each treatment. 246 known zebrafish miRNAs were assayed by hybridization on the microarray, and hybridization intensity of cDNA probes were compared. Preliminary results indicate 85 differentially expressed miRNAs in *brd2* knockdowns that are significantly different ($p \geq .01$) with strong signal intensities. Biological and literature based criteria were used to prioritize potential Brd2-regulated miRNA, and choose candidates for follow up and conformation by RTqPCR. Because miRNAs have the potential to affect many cellular processes, abnormal miRNA expression is known to be associated with many diseases in humans, such as cancer, heart disease, neurological diseases, and immune function disorders. Gaining a better understanding of potential upstream regulators of miRNA expression will thus enable an increased understanding of both fundamental biological processes and therapeutic applications.

Specification and Differentiation

6. THE ROLE OF THE CENTROSOME IN THE ANTEROPOSTERIOR AXIS ESTABLISHMENT OF *C. ELEGANS* EMBRYOS

Dominic Castanzo, Eva Jaeger, Margaret Williams, Dr. Rebecca Lyczak
Ursinus College

Polarity establishment in the single-cell *C. elegans* embryo results in an asymmetric division, thus determining the anteroposterior (AP) axis of the adult organism. It is widely accepted that the sperm-donated centrosome interacts with the posterior cortex to initiate polarity establishment, but the criteria for the timing and duration of such contact are not as well established. In order to study the nature of these centrosome/cortex interactions in living one-celled embryos, we will use fluorescently-tagged proteins to make time-lapsed movies that will allow us to follow the significant factors in this early axis establishment. We investigated these criteria using two worm strains with different centrosome behaviors. Using a strain with fluorescently-tagged histones and β-tubulin, we observed that in wild type (WT) worms the centrosome contacts the posterior cortex for about 4 minutes in the single-cell stage of development. On the other hand, *pam-1* mutants, who lack a puromycin-sensitive aminopeptidase, host shorter centrosome-cortex interactions. In addition, these interactions are earlier in development than WT with relation to pronuclear breakdown (PNB). *pam-1* mutants also fail to properly establish polarity, often culminating in symmetric first divisions. We are currently using fluorescently-tagged PAR proteins to increase our ability to distinguish the onset of polarity establishment and to further separate out the relative importance of timing vs. duration of centrosome/cortical contact in polarity establishment. Additionally, we plan to measure centrosome/cortex contact time in relation to meiotic exit, which has been suggested to be delayed in *pam-1* mutants. We hope our work will further elucidate the centrosome's role in polarity establishment and maintenance in *C. elegans*.

7. JAK-STAT REGULATION OF *DROSOPHILA* GERM CELL SEX DETERMINATION

Andrea Lin, Tigist Tamir, Rebecca Obniski, Matthew Wawersik, College of William and Mary.

Germ cells are the only cells in the body that make sperm and eggs required for sexual reproduction. Proper germ cell development is, therefore, essential for survival of future generations. A critical step in this process is the decision made by germ cells to develop along male or female fates. In flies and mice, both cell autonomous and somatic signals control germline sexual identity. Our previous data shows that Jak-STAT activation in *Drosophila* germ cells by male somatic gonad plays a critical role in establishment of male germ cell fate during embryogenesis (Wawersik et al, 2005). However, the extent to which this pathway promotes germ cell sex determination is not clear. Here, we show that male germline gene expression in adult XX germ cells is induced through hyper-activation of the Jak-STAT pathway during ovary development. Induction of these genes correlates both spatially and temporally with formation of ovarian germ cell tumors, a hallmark of altered germ cell sex determination. We also find that XX germ cells masculinized by mutations in *snf* and *ovo* induce STAT gene expression in a subset of ovarian germ cell tumors. Finally, we show that genetic interactions between *snf* and *jak* result in ovarian germ cell tumor formation; indicating significant cross talk between the Jak-STAT pathway and genes required for female germ cell sex determination. Together, these data suggest that modulation of Jak-STAT signaling plays a critical role the assignment and maintenance of sexual identity in *Drosophila* germ cells.

8. ZEBRAFISH EMBRYOGENESIS

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In many species, including zebrafish, there is little transcription in the mature oocyte, and zygotic transcription does not begin until the blastula stage; therefore maternal mRNAs are the primary source for new gene products during late oogenesis and early embryogenesis. In several species the translation of key maternal transcripts is coupled to their cytoplasmic polyadenylation. Several families of RNA-binding proteins have been implicated in regulating this process, in particular the CPEBs (cytoplasmic polyadenylation element binding proteins), which bind to a specific sequence in the 3'UTRs of regulated messages. Homologs of two CPEBs have been identified in zebrafish; *zorba* (the homolog of *Drosophila orb*) whose protein operates in the oocyte, and *ElrA* (a member of the *elav* family) whose transcripts we have demonstrated are maternally provided, and translated immediately following fertilization. We also have evidence that the translation of *ElrA* at fertilization is itself regulated, at least in part, by cytoplasmic polyadenylation. In addition, there are two *ElrA* transcripts that differ at both the 3' and 5' ends, and both are present as maternal mRNAs; however only the transcript with the shorter 3'UTR is polyadenylated at fertilization. Therefore we are currently investigating whether the transcript with the longer 3'UTR is playing an additional role in regulating *ElrA* translation in the early embryo. Furthermore, since we can demonstrate that the increase in *ElrA* transcript levels seen after the midblastula transition is primarily due to an increase of the longer form, there may be differential regulation of the zygotic transcript at the transcriptional and/or post-transcriptional levels. Taken together, the picture that is emerging regarding the regulation of *ElrA* is a complex one, which is consistent with the fact that the protein product is itself likely to be involved in a cascade of translational control.

9. IDENTIFICATION AND CHARACTERIZATION OF PATERNAL-EFFECT EMBRYONIC LETHAL MUTANTS IN *C. ELEGANS*

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At fertilization the oocyte and the sperm fuse, restoring the somatic chromosome number and initiating zygotic development. The distinct morphologies of the oocyte and sperm, despite both deriving from meiosis, reflect their roles in fertilization and early embryogenesis. Oocytes are large, sedentary cells that provide a haploid genome and large stockpiles of maternal RNA and proteins necessary for early embryogenesis until zygotic transcription is initiated. Sperm are small, motile cells streamlined for fertilization but also play essential roles in early development. The sperm provides a haploid genome, centrosomes, and initiates the embryonic program. There is evidence that sperm contain other factors required for embryogenesis. Absence of these paternally provided components results in paternal-effect embryonic lethality (PEL). In *C. elegans* only a single PEL gene has been characterized, *spe-11*. SPE-11 is a novel protein supplied by the sperm. The absence of functional SPE-11 results in embryonic failure at early stages. We are interested in the further characterization of SPE-11 through structure function analysis as well as identifying and characterizing other potential PEL candidates. Previous genetic screens identified embryonic lethal mutants that could be rescued by wild-type males indicating a sperm defect. We have identified four potential novel PEL mutants. Analysis of these mutants showed that all have >75% rescue of the embryonic lethal phenotype upon mating to wild-type males. Initial analysis of one of these mutants, *mel-15*, confirms that sperm from *mel-15* mutant males produce dead embryos, even when fertilizing wild-type oocytes. In addition, characterization of early embryonic events indicates fertilization is successful and that sperm components (e.g. centrosomes) are transmitted to the embryo, but that *mel-15* male sperm lack DNA. We are currently in the process of determining the molecular identity of the four novel PEL mutants.

10. IDENTIFICATION AND CHARACTERIZATION OF SUPPRESSORS OF *PAM-1* THAT RESCUE ANTERIOR-POSTERIOR AXIS ESTABLISHMENT

Ashley Kimble, Zac Klock, Kevin Kozub, Eva Jaeger, Rebecca Lyczak
Ursinus College

In the model organism, *Caenorhabditis elegans*, the anterior-posterior (AP) axis is established at the one cell stage. Our research suggests that a puromycin-sensitive amino-peptidase, PAM-1, regulates centrosome position during AP axis establishment and is necessary in establishing this polarity. We took advantage of the embryonic lethal phenotype of *pam-1* mutants to conduct suppressor screens to identify strains that rescue this embryonic lethality and may thus be involved in the PAM-1 pathway. We have identified four suppressor strains, which hatch at rates between 28% and 49%. Phenotypes associated with the *pam-1* mutation include weak/absent pseudocleavage, cytokinetic blebbing, and a symmetric first cell division, signs that the AP axis has not polarized. The presence of a suppressor reduces the incidence of these phenotypes towards wild-type levels. After isolating suppressors, **we use SNP mapping** to give preliminary information about the chromosomal location of the suppressors in preparation for Whole Genome Sequencing (WGS), which allows for determination of the positions more rapidly and precisely. Genetic characterization of these suppressors suggests they are all homozygous recessive suppressors, which are ideal for WGS analysis. The aforementioned screens and mapping techniques will help us understand more about the function of PAM-1 and its potential targets.

11. SPATIAL ACTIVATION OF CELL MOTILITY IN *DROSOPHILA* OOGENESIS

Lathiena Manning and Michelle Starz-Gaiano, University of Maryland Baltimore County, Baltimore, MD 21250

Collective cell migration is crucial to an organism's capacity to perform morphogenesis thereby creating body plans and organ systems. Motile cells, which often arise as part of a tightly connected epithelial layer, must disconnect from their neighboring cells to travel the necessary distance for proper development. Differential molecular cues across the epithelium establish which cells leave or reside in the epithelium. We are employing the *Drosophila melanogaster* oocyte development to study cell specification amongst a small population of specialized cells referred to as border cells. Border cells differentiate and detach from the anterior epithelium and migrate posteriorly toward the oocyte while remaining in a cluster. Border cells display the characteristics of collective cell migration as they move. The JAK/STAT signaling pathway is essential in the expression of specific genes needed for coordinating border cell migration. We have found that border cell specification and migration can be genetically separated and that specification depends on proper spatial expression of JAK/STAT signaling. We are using a combination of genetic, immunohistological, and live-imaging approaches to define border cell arrangement prior to their detachment from the anterior epithelium. Conventional views indicate that STAT activity occurs in a gradient across the anterior epithelium. The cells closest to the polar cells have the highest STAT activity and therefore migrate. An atypical view revealed unexpected differences in STAT activity among the follicle cells in contact with the polar cells in the anterior epithelium. These improved characterizations of migratory cell specification prior to epithelial detachment will lead to an enhanced general understanding of cell migration.

12. PSEUDOPHOSPHATASE MK-STYX INDUCES NEURONAL DIFFERENTIATION IN PC12 CELLS

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Psuedophosphatase MK-STYX [MAPK (mitogen-activated protein kinase) phosphoserine/threonine/tyrosine-binding protein] is involved in neuronal cell differentiation. Proteins of the MAPK family are necessary for cell proliferation and differentiation. MK-STYX shares structural homology with these proteins suggesting a direct involvement in neuronal differentiation. To verify the role of MK-STYX in neuronal differentiation, PC12 cells were transfected with pMT2, MK-STYX-FLAG, and pEGFP vectors. 5 days post-transfection the cells were examined and scored. MK-STYX was shown to be endogenously expressed in PC12 cells and over-expression of MK-STYX enhanced neurite outgrowth length. Due to the endogenous presence of MK-STYX, we hypothesized that MK-STYX can trigger neuronal differentiation through modulation of the MAPK pathway and modification of the activity of small G-protein, RhoA. Sustained activation of MAPK induces neuronal differentiation. MAPK activation was blocked with MEK inhibitor PD89059. However in the presence of this inhibitor, MK-STYX could induce neuronal differentiation. Furthermore, inactivation of RhoA induces neuronal differentiation. 24 hours post-transfection serum starved PC12 cells were simulated with NGF for 1 min, 3 min, 5 min, 12 min, 30 min, 24 hr, and 48 hr. Activated RhoA was quantified with a RhoA G-LISA activity assay. MK-STYX had a substantial decrease in the activity of RhoA. These results demonstrate that MK-STYX can induce neuronal differentiation through inactivation of RhoA and independent of the MAPK pathway. This strongly suggests that pseudophosphatase MK-STYX has a substantial role in neuronal differentiation.

13. LOCALIZATION DYNAMICS OF SPE-6, A SPERM-SPECIFIC CK1 IN C. ELEGANS.

Peterson JJ, Waller B, and Shakes DC. College of William and Mary

During spermatogenesis, diploid germ cells develop into small, haploid spermatozoa which only acquire full motility during the final step of sperm activation. In *C. elegans*, sperm activation occurs in response to an external signal which converts sessile round spermatids into bipolar, crawling spermatozoa. Notably, this final conversion occurs in the absence of either transcription or translation, suggesting that these changes are driven solely by post-translational modifications. In fact, since *C. elegans* spermatocytes cease transcription prior to the meiotic divisions and cease translation immediately after the meiotic divisions, many of the key developmental changes occur in the absence of transcription and translation. Therefore, key motility proteins such as the major sperm protein (MSP) must be translated early in spermatogenesis and sequestered within fibrous body membranous organelles (FB-MOs). Here we describe the dynamic localization of a sperm-specific casein kinase 1 (SPE-6). In wild type spermatocytes, SPE-6 is present in both the cytoplasm and in association with FB-MOs. During the post-meiotic budding division, SPE-6 segregates to the spermatids and away from residual bodies, eventually localizing to the sperm chromatin. Then during sperm activation, SPE-6 re-localizes to the pseudopod. In several non-null alleles of *spe-6*, sperm activation occurs in the absence of an activation signal. Our analysis of *spe-6(hc163*) reveals that this precocious activation phenotype is accompanied by dramatic alterations in SPE-6 localization patterns. In developing spermatocytes, the mutant protein is mostly cytosolic. During the budding division, it segregates aberrantly to the residual body; any SPE-6 that does end up in spermatids fails to localize to the chromatin. We present a model of how this aberrant pattern of protein localization may explain the precocious activation phenotype and provide clues regarding the function of SPE-6 in sperm activation.

14. ANALYSIS OF THE SPERMATOGENESIS DEFECTS OF ERI-I CLASS MUTANTS REVEALS NEW INSIGHTS INTO MEIOTIC CHROMOSOME SEGREGATION.

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The regulation and coordination of cellular events is crucial for development. One highly conserved mechanism for regulating development is RNA silencing. In the nematode, *Caenorhabditis elegans*, RNA silencing has become an essential tool for determining gene function. In order to obtain the most severe loss-of-function phenotypes, RNA silencing studies of somatically expressed genes are often carried out in *eri* (Enhanced RNA Interference) strains. However, a subset of these ERI strains exhibit a temperature sensitive sterility phenotype at the elevated temperature of 25^{o}C. Studies of these ERI-I class strains by the Fire and Kennedy labs have linked this sterility phenotype to a defect in spermatogenesis, yet the specific nature of the spermatogenesis defects remains poorly understood. To further investigate the specifics of the spermatogenesis defects, we are using a variety of cytological markers to determine which specific cellular sub-programs are or are not affected. Here we report on our studies of a non-null allele of Dicer, *dcr-1(mg375*). Our preliminary studies of the meiotic chromosome segregation defects suggest that the observed delays in meiotic chromosome segregation are associated with defects in chromosome-microtubule attachments. In addition, our analysis suggest that, during meiosis II, drastic defects in chromosome segregation may be partially suppressed by a polarization event that normally follows anaphase II and facilitates the budding of spermatids from a central residual body. Thus these on-going studies are not only providing new insights regarding how the RNA silencing machinery promotes normal spermatogenesis, but they are also revealing unexpected insights into the fundamental biology of meiotic chromosome segregation and sperm individualization.

15. THE EFFECT OF CALCIUM ACTIVITY PERTURBATION ON GENE EXPRESSION IN THE DEVELOPING NERVOUS SYSTEM OF *XENOPUS*

Brian A. Rabe, Wendy A. Herbst, Margaret S. Saha

Calcium ions serve as ubiquitous secondary messengers in a wide array of cellular processes, particularly during early neural development. Beginning during gastrula stages, neural precursor cells undergo rapid spontaneous calcium transients during which calcium is released from internal stores and then rapidly re-sequestered. While these calcium transients have been implicated in neural specification and differentiation, little is known regarding the mechanisms by which different patterns of calcium activity influence phenotype. In order to address this question, we have investigated the effect of calcium activity perturbation on gene expression during neural development using Xenopus primary cell culture of presumptive neural tissue. The neural plates of early neurula stage *Xenopus laevis* embryos were dissected, dissociated, and plated in either normal physiological calcium (2mM Ca^{++}) or elevated calcium (10mM Ca^{++}) which results in significantly increased spiking activity. After culturing these cells until sibling embryos have reached late neurula, early tailbud, or early swimming tadpole stage, we extracted RNA and performed microarray analysis to investigate differential gene expression on RNA extracted from cell cultured to each of these three stages. We have utilized a number of techniques to analyze the resulting data using a modified t-test, fold change analysis, and a novel mathematical algorithm, developed in collaboration with the William and Mary Math Department to discern differentially expressed genes when the sample size is low. Our preliminary results indicate a wide array of genes exhibit differential expression ranging from genes whose products have known calcium binding activity to genes controlling cell cycle and stress responses. Each time point reveals a unique set of differentially expressed genes, suggesting a dynamic regulation of response to increased calcium. Furthermore, when siblings have reached the early swimming tadpole stage, cells cultured in media with increased calcium concentrations show increased expression of muscle differentiation genes. To complement these approaches we are also using RNAseq of similarly collected samples as well as an in vivo approach using a GCaMP.

16. HOW NERUAL CELLS ACQUIRE AN IDENTITY: THE ROLE OF CALCIUM SIGNALING AND VOLTAGE-GATED CALCIUM CHANNELS IN PHENOTYPE SPECIFICATION

Lindsay M. Schleifer, Vollter Anastas, Brittany Lewis, Margaret S. Saha

There has been a significant amount of research analyzing the role of 'hard-wired' mechanisms (e.g., transcriptional cascades) in neuronal fate acquisition. Little is known regarding the role of the spontaneous calcium transients which play a critical role in neuronal development and phenotype specification. To address this question, we have performed analysis at the single cell level during different developmental stages to correlate a single neuron's spontaneous calcium activity with its neurotransmitter phenotype. We examined whether increased levels of calcium activity lead to increases in inhibitory (GABAergic) phenotypes and decreases in excitatory (Glutamatergic) phenotypes. When compared with cells negative for the above phenotypes, cells with inhibitory or excitatory phenotypes spiked significantly less across the examined stages. This correlation was also found when comparing spiking activity of cells positive or negative for voltage-gated calcium channel α subunits. Interestingly, at a lower threshold of spiking than previously studied, cells that developed an inhibitory phenotype were found to spike significantly less than those that developed an excitatory phenotype. When testing for global similarities in calcium activity across all stages of interest, clear differences in the pattern of activity were found. We noted enhanced calcium activity when cells were cultured in 10mM calcium compared to 2mM. Experiments were then performed with the hypothesis that pharmacologically antagonizing VGCC function would lead to an upregulation of excitatory neurotransmitter phenotype markers and a downregulation of inhibitory markers. Cell cultures exposed to diltiazem, an L-type VGCC antagonist, significantly increased the number of excitatory neurons, and decreased the number of inhibitory neurons. Further imaging of cells exposed to VGCC antagonists and agonists are being performed to elucidate the role of these channels in neurotransmitter specification.

17. DISSECTING THE REGULATION OF A NOVEL GENE *cg11910 IN THE LONGITUDINAL GLIAL CELLS OF DROSOPHILA EMBRYOS*

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The long-term goal of this research project is to understand the molecular mechanisms that regulate the differentiation of glial cells within the central nervous system (CNS) of *Drosophila*. Within the CNS of *Drosophila*, the glial cells are broadly divided into two classes, the midline and lateral glia, based on their cellular origin and location within the CNS. The midline glial cells develop from mesectodermal tissue, while the lateral glial cells arise from the neuroectoderm. The differentiation of the lateral glial cells is regulated by the transient expression of the transcription factor, Glial cells missing (Gcm). Gcm initiates lateral glial cell differentiation by inducing the expression of downstream transcription factors such as Reversed polarity (Repo), Pointed (Pnt) and Tramtrack (Ttk). While the initial events of lateral glial cell determination have been well characterized, the generation of diversity in the sub-types of lateral glial cells is poorly understood. In order to understand how cell fate specification of the different types of lateral glial cells is established, we are investigating the regulation of a novel gene *cg11910* that is expressed in a sub-type of lateral glial cells called the longtidudinal glia (LG). The regulatory region of *cg11910* has predicted binding sites for Repo and Pnt, which suggests that Repo and Pnt might regulate its expression. Consistent with this hypothesis *cg11910 mRNA* expression is drastically reduced in *gcm* mutants and completely abolished in both *repo* and *pnt* mutant embryos. Our hypothesis is that Repo and Pnt function in concert with other spatially restricted transcription factors to regulate the expression of genes in LG cells. Distal-less (Dll) is a promising candidate as it is expressed in all LG cells, but not in other Repo positive lateral glial cells. We will perform *in situ hybridization* to determine if there is a loss or reduction in the expression of *cg19110* mRNA in *dll* homozygous mutant embryos.

18. TISSUE SPECIFIC DEREPRESSION OF TCF/LEF CONTROLS THE ACTIVITY OF THE WNT/BETA-CATENIN PATHWAY

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The Wnt/β-catenin signaling pathway is one of the main signaling pathways that control cell proliferation and cell fate determination during embryonic development and tissue homeostasis. Upon stimulation by Wnt ligands, the Wnt/β-catenin canonical signaling pathway results in the stabilization of β-catenin and its translocation into the nucleus to form transcriptionally active complexes with sequence-specific DNA-binding TCF/LEF transcription factors. In the absence of Wnt signals, β-catenin is degraded in the cytoplasm via the ubiquitin-proteasome pathway and TCF/LEF is maintained in a transcriptionally inactive state by its binding to the corepressor Groucho/TLE.

We report here the discovery of a novel mode of regulation of the canonical Wnt/β-catenin signaling pathway. The molecular analysis of the function of the zebrafish homeobox gene *Ladybird2* (Lbx2) presented in this study reveals that this transcription factor positively controls the Wnt signaling pathway in the non-axial mesoderm at gastrula stage. We found that Lbx2 exerts its action by interacting directly with Groucho/TLE, attenuating its corepressive activity. Therefore, in ventral and lateral mesoderm, the transcriptional activity of the canonical Wnt pathway is promoted by a decrease of interaction between Groucho/TLE and TCF/LEF. In the absence of this derepression, embryos lack all tissues derived from ventral, lateral and paraxial mesoderm, a phenotype identical to the phenotype resulting from a complete loss of Wnt function within this germ layer. This finding explains how, in a tissue dependent manner, the level of response to Wnt signaling can be modulated, not at the level of β-catenin itself, but by promoting the formation of β-catenin-TCF/LEF complexes through the decrease of Groucho/TLE interaction with TCF/LEF.

Neural Development

19. DISCOVERING THE ROLE OF WNTLESS IN HABENULAR DEVELOPMENT

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The paired habenular nuclei are a highly conserved region of the dorsal forebrain whose development is not well understood in any vertebrate model. In the zebrafish larval brain, the dorsal habenulae show prominent left-right (L-R) differences. Wnt signaling is thought to play an essential role in the patterning of the L-R axis in the early embryo and in the formation of brain asymmetry. Wntless (Wls), a protein concurrently discovered by three groups in 2006, is believed to be necessary for the secretion of all Wnts. Surprisingly, homozygous *wls* mutant zebrafish have intact L-R asymmetry of the dorsal habenulae, however, the habenular nuclei are reduced in size. Additionally, *wls* mutants do not exhibit the variety of defects typical of Wnt mutants. It appears that maternal deposition of Wls allows for normal early development. This hypothesis is supported by the expression of a canonical Wnt signaling reporter in homozygous *wls* mutants throughout early development that noticeably decreases in mutants after 35 hpf. Initial loss of the canonical Wnt reporter in mutants begins in the region of the habenulae, and is later followed by a whole body loss of reporter expression. I am examining how the eventual loss of *wls* is responsible for the small habenula phenotype, and which Wnts are involved.

20. BEHAVIORAL ANALYSIS IN TRANSCRIPTIONAL CO-REGULATOR BRD2-KNOCKDOWN ZEBRAFISH LARVAE

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Zebrafish are a prolific model for a wide range of biological assessments. Their conceived simplicity lends itself to formulating and testing complex interactions between genetics, morphology, and behavior. One intriguing and critically positioned gene understood to be involved in the regulation of mitosis, cellular development, and apoptosis is Brd2. In 24 hour old (prim5) Brd2 knockdown zebrafish embryos, the isthmus (midbrain-hindbrain barrier) is ill-defined and the hindbrain is reduced. Does this early phenotype translate to continued morphological defects or behavioral artifacts in further developed embryos? This study is three-fold. The first part of the experiment involves observation of control (WT) and Brd2 knockdown (MO1) methylene blue stained slide-mounted sections of 5 days-post-fertilization (dpf). The second part is immunohistochemistry and fluorescence microscopy of WT and MO1 5dpf embryos tagged for proteins unique to granule and Purkinje cells, respectively. The third and final part of the experiment is a behavioral analysis in the form of cerebellar dependent classical conditioning in 6dpf embryos. Due to the conserved nature of Brd2 as a maternal effect and embryonic developmental regulatory across species, and its status as a candidate gene for juvenile myoclonic epilepsy, these studies could give insight into the transition between maternal and zygotic developmental control, and the differentiation and morphogeneis of the central nervous system in vertebrates. In addition, these results could illuminate other neurobiological diseases including cerebellar cognitive affective disorder due to the largely conserved structural entity of the cerebellum in vertebrates.

21. THE ROLE OF CALCIUM ACTIVITY IN NEURONAL PHENOTYPE SPECIFICATION

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While calcium activity has been implicated in a wide variety of developmental processes, including neurotransmitter phenotype specification, little is known about the underlying mechanisms regulating spontaneous calcium activity in early development. Given that voltage-gated calcium channels (VGCCs) are expressed in early neural development and mediate large influxes of calcium ions into the cell, we hypothesized that these channels regulate spontaneous calcium activity and neurotransmitter phenotype specification. To test this hypothesis, antisense morpholino oligonucleotides (MO) were employed to impede the expression of a specific calcium channel, $Ca_v2.1$. This VGCC was selected for loss-of-function analysis because of its widespread expression in the neural tube of *Xenopus laevis* during the neurula stages of development. The MO-injected embryos were analyzed for differences in calcium activity and gene expression. In situ hybridization experiments revealed reduced expression of glutamic acid decarboxylase (GAD), a marker of inhibitory (GABAergic) neurons, in $Ca_v2.1$-MO embryos. Additionally, confocal microscopy was implemented to image the calcium activity of neural tissue from $Ca_v2.1$-MO embryos. Analysis of calcium spiking indicated that these embryos displayed fewer calcium spikes than controls. In order to better understand the connection between calcium activity and specific neuronal cell types, we are also imaging calcium activity *in vivo* in early neurula *Xenopus* embryos using a genetically encoded calcium indicator, GCaMP6m. Rhodamine lysine dextran (Rldx) is injected at later stages, to serve as a marker for specific cell locations. The neural plate of *Xenopus* embryos is imaged with confocal microscopy and subsequently analyzed using in situ hybridization, probing for calcium-related genes and neural markers. Calcium activity and gene expression can then be correlated on a single cell level.

22. A PTZ ZEBRAFISH KINDLING PARADIGM FOR THE STUDY OF EPILEPSY SUSCEPTIBILITY

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Inducing an epileptic seizure in model organisms can be done through acute exposure or kindling with a convulsing agent. These different types of stimulation represent two different mechanisms of triggering an epileptic seizure. Acute exposure allows one to study the biological response to one exposure of a known convulsive dose. Kindling on the other hand, produces a long lasting sensitization of behavioral convulsing activity through repeated administration of a subconvulsive dose. Unlike acute exposure, kindling has only been performed in rodents and has never attempted in zebrafish. The purpose of my research is to produce a kindling paradigm for wildtype zebrafish in order to study epileptic susceptibility using Pentylenetetrazole (PTZ). Trials consist of 64 embryos exposed to four consecutive exposures of PTZ at subconvulsive concentrations of 0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 2.5mM. Each treatment of eight embryos is exposed once a day for 40 minutes, from seven to ten days post fertilization (dpf). Each exposure is filmed and data are recorded in terms of the time after recording at which an embryo reaches a certain stage of epileptic behavior, defined as the latency to the response. Five replicates of this four day trial have been performed. Initially, I expected a kindled state would be represented by a decreased latency to seizure as the number of exposures increased for a specific embryo. When comparing latency values for a specific embryo, data trends were inconsistent and showed significant variation. From a broader perspective, more embryos were exhibiting seizures as the exposure number increased. This evidence suggests that kindling is something worth studying further, just not in terms of latency values. Modifications were made based on rodent kindling where an embryo is analyzed as to whether or not it seized during each exposure. An embryo will be considered kindled if, within the four exposures, it did not seize at first but did in both the second and third exposures. This kindled state will be verified with one last seizure in a fifth exposure after resting 48 hours. Being able to successfully kindle zebrafish is important because it would provide an alternate approach to the mechanistic study of epilepsy using a model organism that is easy to manipulate genetically and test in large numbers.

23. DEVELOPMENT OF A TRANSGENIC ZEBRAFISH LINE INDUCIBLY OVEREXPRESSING *brd2a*

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Brd2a mediates interactions between chromatin modifiers and the transcriptional machinery. Studies in the DiBenedetto lab suggest that during zebrafish development brd2a regulates egg-to-embryo transition, apoptosis, and central nervous system (CNS) patterning through homeotic gene regulation. Other studies have shown that mammalian Brd2 (ortholog of brd2a) regulates mitosis and tumorigenesis in adult tissues; however, neither mouse knockout nor zebrafish knockdown of Brd2 significantly affects mitosis during embryonic development. One possible explanation is that other mitotic regulators compensate for losing Brd2 during development. If brd2a does regulate mitosis during development, overexpressing brd2a should alter mitotic rate, despite such redundancies. In zebrafish, expression of brd2a restricts primarily to the CNS by segmentation stage, and disappears by five days. I am developing a transgenic line of zebrafish inducibly overexpressing brd2a in the CNS. I plan to produce one line bearing a CNS-specific transcriptional activator (TA) dually inducible by doxycycline and tebufenozide; and a second line, bearing a responsive transgene containing brd2a fused to HA (HA-brd2a). Crossing the lines should produce embryos containing both transgenes, which should inducibly overexpress brd2a in the CNS. I have produced fish bearing each transgene. After identifying TA-bearing F1 embryos, I will inject some of them with the responsive HA-brd2 plasmid, and test for expression of HA-Brd2a by qPCR using primers for HA. The lab will use this line to study effects of CNS-specific brd2a overexpression during development, including possible effects on mitotic rate and tumorigenesis. If a study using this transgenic zebrafish line demonstrates such effects, it would suggest that Brd2 regulates mitotic rate during development and/or is a protooncogene in non-mammalian vertebrates.

24. MATERNAL DETERMINANTS OF NEURAL FATE IN *XENOPUS LAEVIS*

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In many organisms, important developmental events are regulated by mRNAs that are synthesized in the egg and locally stored for later use in embryonic development. In the frog, several maternal mRNAs are highly localized in the animal blastomeres that give rise to the ectoderm. Three transcription factors that are localized to animal blastomeres have important roles in the formation of the neural plate after gastrulation (FoxD4L1, Gem, Zic2). My project is to determine whether they also have a function before gastrulation using a culture system that removes cells from the embryonic environment prior to the onset of embryonic (zygotic) gene transcription and gastrulation. Expressing each of these three mRNAs in non-neural isolated ventral cells causes expression of zygotic neural genes, indicating a maternal function in neural development. My work in the Moody lab is to determine whether the maternal factors are required for zygotic neural gene expression in isolated dorsal animal blastomeres using gain- and loss-of-function experiments. Currently, my experiments have focused on the Zic2 maternal factor and have demonstrated that heightened levels of Zic2 enhance the expression of neural genes in dorsal cells. Understanding how neural stem cells are formed in the embryo will provide critical information about how to create and manipulate neural stem cells for therapeutic uses.

25. FUNCTIONAL ROLE OF THE WNT TARGET ANNEXIN A6 IN TRIGEMINAL GANGLIA FORMATION

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Neural crest cells (NCCs) are derived from the ectoderm during vertebrate embryogenesis. Premigratory NCCs undergo an epithelial-to-mesenchymal transition (EMT) to become migratory, giving rise to derivatives including sensory ganglia, the craniofacial skeleton, and melanocytes. Wnt proteins can induce NCCs from naïve neural tissue, but downstream targets of Wnt are still unknown. We have identified *Annexin A6* as a gene up-regulated by Wnt signaling. Annexins are Ca^{2+}-dependent, membrane-binding proteins with roles in cell migration, cell adhesion and differentiation. Prior work from our lab has shown *Annexin A6* transcripts in chick premigratory and emigrating cranial NCCs. Moreover, knockdown or overexpression of Annexin A6 in developing cranial NCCs reduces or expands the migratory NCC domain, respectively, through effects on cadherins during EMT. The function of Annexin A6 in later stages of NCC migration and differentiation into cranial sensory ganglia, however, has not been determined. Using the trigeminal ganglion (TG) as a model for cranial gangliogenesis, we have evaluated Annexin A6 protein distribution in NCCs and placode cells, the two cell types that form this ganglion. Our preliminary data indicate the presence of Annexin A6 protein in placode cells but not in NCCs during TG assembly. We hypothesize that Annexin A6 may be down-regulated in later migratory NCCs and instead play a critical role in placode cells during TG assembly. To address this, we will 1) document Annexin A6 protein distribution in migratory NCCs and placode cells at later chick developmental stages; 2) determine the function of Annexin A6 through overexpression and knockdown assays in migrating NCCs and placodes independently; and 3) delineate the molecular mechanism by which Wnt regulates *Annexin A6*. Our results will provide insight into the role of Annexin A6 during NCC migration and gangliogenesis.

26. CHARACTERIZATION OF THE SUBCELLULAR LOCALIZATION OF SCRIBBLE DURING ZEBRAFISH DEVELOPMENT

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Scribble (Scrib), a member of the LAP family, plays a critical role in establishing and regulating cell polarization in epithelia and during cell migration. In zebrafish, Scrib mutants have a defect in convergent extension (CE) cellular movements required for elongation and narrowing of the vertebrate body axis. In addition, Scrib is also required the proper caudal of facial branchiomotor neurons (FBMNs) in the developing hindbrain. Using chimeric analysis, we have previously shown that Scrib is required both cell autonomously and cell non-autonomously during FBMN migration. Despite these genetic studies, little is known about the subcellular localization of endogenous Scrib in cells undergoing CE movements or during neuronal migration. We have generated a monoclonal antibody against the C-terminus of zebrafish Scrib and show that this antibody works both for immunocytochemical and immunblot detection of endogenous zebrafish Scrib protein. Western blot analysis indicates that this antibody detects one major band that is absent in lysates generated from maternal-zygotic (MZ) scribble mutant embryos. Whole mount immunostaining of zebrafish embryos at various developmental stages have shown that Scrib distinctly localizes to the membrane throughout development, including mesodermal and neuroectodermal cells undergoing CE movements. In the developing nervous system, we show that Scrib is localized to the basolateral surfaces of neuroepithelial cells with no distinct signal at the apical portions of the cell membrane. We see a similar basolateral localization in other epithelial structures, such as the developing otic vesicle. In migrating FBMNs, we show that Scrib is localized in distinct puncta near cell membranes. Importantly, this subcellular localization of Scrib is lost in MZ-scribble mutant embryos, demonstrating the specificity of our antibody. We aim to further characterize the subcellular localization of Scrib in migrating cells during vertebrate development.

27. INVESTIGATING THE ROLE OF CELL CYCLE DYNAMICS ON NEUROTRANSMITTER PHENOTYPE SPECIFICATION IN XENOPUS LAEVIS

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Cell cycle dynamics and the decision to exit the cell cycle is an important aspect of neuronal cell determination and differentiation, including neurotransmitter phenotype specification. However, investigating cell proliferation in vivo has been hampered by the difficulties associated with the use of the most prevalent method for assaying cell proliferation which is based on the incorporation of the thymidine analogue 5-bromo-2'-deoxyuridine (BrdU). The analogue is incorporated during the S-phase of mitotic cells. A newer, more efficient, 5-ethynyl-2'-deoxyuridine (EdU) assay uses the same incorporation method, but uses a bioorthogonal "click reaction" detection instead of an antibody approach, resulting in more precise proliferative tagging. The detector azide molecule is 1/500 the size of the anti-BrdU antibody, allowing deeper penetration into the whole mount embryo, resulting in more complete visualization of proliferative cells. We have evaluated two different methods of performing the fluorescent click reaction. The first method uses the pre-fluorescent Alexa fluor 488 azide molecule, which conjugates with EdU, allowing visualization but presents the slight possibility of background fluorescence. The second method uses our in-house synthesized 3-azido-7-hydroxycoumarin, which fluoresces upon completion of the click reaction, due to removal of a fluorescent quenching effect upon formation of the triazole product. This removes the presence of background fluorescence. While researchers have used the EdU assay to tag proliferative cells in *E. coli*, whole mount chick embryos, and *Xenopus* egg cell extracts, we have successfully performed this in whole mount *X. laevis* embryos. We are currently using the EdU assay to determine the extent to which cell cycle dynamics, in particular the perturbation of the cell cycle using a cocktail of cell cycle inhibitors Hydroxyurea and Aphidicolin (HUA), impact neurotransmitter phenotype specification and developmental patterning.

28. A ROLE FOR RETINOIC ACID IN CRANIAL NERVE DEVELOPMENT

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The anatomy of the cranial peripheral nervous system has been studied for over a century, yet surprisingly little is known about how the nerves are guided to their targets. The study of the guidance of these nerves has important implications for our understanding of craniofacial anomalies and possible treatments for both injury and genetic disorders of axon guidance such as developmental facial paralysis. We have discovered that retinoic acid (RA) may play a role in the development of the trigeminal nerve. Inhibition of retinoic acid receptor gamma (RARy) results in trigeminal nerves that become defasciculated in the eye region. To further understand how RA is affecting trigeminal development we searched for genes downregulated in response to RARy inhibition and have identified neurotrophin-3 (NT-3) and Semaphorin 4B (Sema4B). We have analyzed the expression patterns of these genes by *in situ* hybridization and have found NT-3 expression in the eye and Sema4B in the embryonic target of the trigeminal nerve and along the boundary of the first and second branchial arches. The pattern of expression of these two genes combined with known expression of NT-3 receptors allows us to suggest a model whereby retinoic acid signaling regulates Sema4B and NT-3, which together support the survival and fasciculation of the trigeminal nerve. This work has the potential to better understanding of the complex nature of cranial nervous system development.

29. INTERACTIONS BETWEEN EARLY NEURAL ECTODERMAL GENES AND THE ORGANIZER GENES THAT REGULATE THEM

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The process of neural induction, which transforms embryonic ectoderm into neural ectoderm, is multi-faceted. It is known to involve Organizer inhibition of factors that repress neural genes, such as BMP, Wnt and Nodal. However, Organizer genes are likely to directly activate early neural ectodermal genes as well. We recently determined the transcriptional relationship between four early neural ectodermal genes: FoxD4L1, Sox11, Gmnn, and Zic2 that comprise a gene regulatory network controlling the progression of neural ectodermal precursors to neural stem/progenitor cells. We next sought to understand the interactions between these neural ectodermal genes, and the Organizer genes in order to provide a more-complete understanding of neural induction. We used an ectopic induction assay in which Organizer genes were expressed in *Xenopus* blastomere precursors of the epidermis and their ability to induce expression of FoxD4L1, Sox11, Gmnn, and Zic2 was assayed by ISH at neural plate stages. We found that the signals and transcription factors leading to the ectopic induction of FoxD4L1/Sox11 and Gmnn/Zic2 are not identical. Induction of FoxD4L1 and Sox11 requires simultaneous blockade of both BMP and Wnt signaling, whereas Gmnn and Zic2 can be induced by blocking only Nodal signaling or only BMP signaling. FoxD4L1 and Sox11 are only ectopically induced by Siam and Twn, whereas Gmnn and Zic2 can be induced by Siam and Twin, which partially requires FoxD4L1 activity, as well as Lim1, FoxA4, Otx2 and Pou2. FGF signaling but not Nodal signaling is required for the Siam-mediated induction of all four early neural ectodermal genes. These findings demonstrate that at least two independent pathways lead to neural ectodermal gene expression. In one pathway, Siam/Twn activate FoxD4L1 transcription, directly and indirectly via FGF signaling and in the absence of both BMP and Wnt signaling, and FoxD4L1 in turn activates Sox11, Gmnn and Zic2. In a second pathway Gmnn and Zic2 are activated by other Organizer transcription factors, both directly and indirectly in the absence of BMP and/or Nodal signaling. These studies provide greater detail of the molecular interactions that regulate the induction of neural ectoderm.

Evo-Devo and Eco-Devo

30. 9TH GRADE BIOLOGY 1A INDEPENDENT RESEARCH PROJECTS

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At Sidwell Friends High School, biology is required for all ninth graders. Students who choose to take Bio 1A, an accelerated class, have the opportunity to perform a long-term experiment on subjects that interest them, called an Independent Research Project (IRP); it is meant to foster creativity as well as expose students to long-term research projects. The only guidelines for this project are that the experiment must relate to biology and be accomplished using the facilities and resources of Sidwell or those made available by external scientists. Working in small groups, this year's Bio 1A performed these IRPs.

Students ran three experiments. One was to test the action potential of *chara corallina*, a green algae composed of nodal and internodal cells. The experiment measured the action potential of individual internodal chara cells while in solutions whose calcium chloride concentrations were either hypertonic or hypotonic to those of the cells. The experiment took place in a Faraday cage to minimize the interference of external variables. The expected results were varied action potentials among the cells depending on the ion concentrations they were placed in. Another prediction was that the action potential would stop cytoplasmic streaming, the controlled movement of cytosol delivering nutrients and other materials to different parts of the cells, within the cell after it rose beyond a given point.

31. TOXICOLOGICAL EFFECTS OF THE FRACKING CHEMICALS BORIC ACID, ETHYLENE GLYCOL AND GLUTERALDEHYDE ON THE DEVELOPING ZEBRAFISH EMBRYO

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Hydraulic Fracturing (also known as Fracking) is increasingly becoming a method that companies are using to harness natural gas as a source of energy. This process involves the use of many chemicals that may leak out or infect various water sources. The effects of these chemicals have not been documented with regards to the wildlife that may be subjected to infected ecosystems or to humans by way of contaminated well water. This study looks at the developmental effects of individual chemicals found within fracking fluid (Boric Acid, Gluteraldehyde, and Ethanol Glycol) on zebrafish embryos. Zebrafish are an excellent model organism to use in completing a toxicological study due to the fact that they can provide up to 200 embryos per female, have a vertebrate body plan with a basic system not much different from a human or mouse, and are small and transparent so the developmental process can be manipulated and monitored. This study includes two types of experiments, a range-finding test and a full early life stage test. Both involve the embryos being placed in a 96-well dish with varying concentrations of one of the chemicals for a five day period. The range-finding test uses the concentrations of 1000mg/L, 100mg/L, 10mg/L, 1mg/L and .1mg/L of the chemical being tested. The endpoints for the range-finding test were malformations, hatching, and mortality. A range-finding test was conducted for all three chemicals and an LC50 value was calculated for Boric Acid and Gluteraldehyde, the two chemicals that were shown to have some effect on the developing process of zebrafish embryos. From the LC50 value calculated for Gluteraldehyde, a full early life stage test was performed with a narrower range of 70mg/L, 45mg/L, 30mg/L, 20mg/L and 15mg/L with a broader set of endpoints that include teratogenic, lethal and sublethal points. The results from the full early life stage will enable a more accurate LC50 value to be calculated. Furthermore, the obtained results indicate that Gluteraldehyde delays or prevents hatching of the embryos and may be teratogenic by causing deformations of the tail. Further exploration of this topic is needed but overall, the study indicates that individual fracking chemicals such as Boric Acid and Gluteraldehyde can be very dangerous to the development of zebrafish embryos.

The other two experiments performed were on *Danio Rerio,* informally known as “zebrafish”. They are a model organism due to easy maintenance, large clutch sizes, and a sequenced genome. One experiment tested the effects of Ultraviolet B radiation, radiation that causes skin damage by causing mutations in DNA, on zebrafish embryo development. The effects were expected to be curvature in their tails, decreased hatch rate, and an increased death rate. The other experiment on *Danio Rerio* tested the effects of light on hair cell regeneration. Hair cells on zebrafish sense movement in the water around them, providing spatial awareness. First, the hair cells of the embryos were destroyed using neomycin sulfate. Certain groups were then subject to different durations of light exposure. It was expected that if the fish were exposed to more light, the hair cells would regenerate more slowly and to a lesser extent.

32. *VITELLOGENIN1* IN EMBRYONIC ZEBRAFISH AS A BIOINDICATOR TO DETECT ESTROGENIC COMPOUNDS WITHIN THE ROANOKE RIVER (VIRGINIA)

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Aquatic ecosystems are susceptible to effluent pollution events, which pose a threat to the wildlife that inhabits these waterways. High numbers of intersex fish are becoming a commonality in waterways, which may be due to agricultural, industrial, and sewage effluent estrogenic contamination. Here, we use zebrafish embryos as a bio-indicator of estrogen levels in the Roanoke River (Virginia). Sample sites along the river include areas upstream and downstream of a sewage treatment plant as well as direct effluent. The activity of the estrogen sensitive gene, *vitellogenin1* (*vtg1*), is quantitatively assessed using qPCR. If estrogenic compounds are present and biologically active, the levels of *vtg1* mRNA should increase relative to a control gene. The increased activity of this gene may indicate that estrogenic chemicals are impacting embryonic and adult wildlife. Further studies will be conducted to determine if seasonal changes and meteorological effects will alter estrogenic levels in the river.

33. CHOICE ANALYSIS IN SHOALING BEHAVIOR OF *DANIO RERIO*

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The BRAIN Club (Biological Research And Investigations in Neuroscience) at Sidwell Friends is an extra-curricular activity that allows students of all grades to develop, maintain, and analyze in-depth experiments on a wide variety of subjects pertaining to biology and, more specifically, neuroscience. Students are able to choose and create experiments based on any subject that interests them and perform them on their own time using the school's facilities. The club also gives students the opportunity to attend various conferences on biology and neuroscience, both local and national. Here is an example of one of our experiments. The purpose of this experiment was to study color-based and numerical shoaling preferences in *Danio rerio*, a species of fish native to the Ganges River and known informally as "zebrafish". Zebrafish are model organisms because they are inexpensive and effective due to their easy maintenance, large clutch sizes, and fully sequenced genome. Shoaling, a behavior found in many species of fish, is a collective action in which fish group together for increased reproductive success and protection from predators. In this experiment, zebrafish were tested to see their preference between different groups of fish based upon color and quantity. We used a shoaling tank, a large tank separated by 2 Plexiglass dividers, creating 3 compartments. Test fish were raised in groups of unanimous coloring, either the same color as the test fish or a different color. The fish were placed in the middle compartment with two groups of fish in the side compartments. The groups of fish in one of the side compartments were the color of fish that the test fish was raised with and the other side compartment contained a group of any other color. The time spent by the test fish near both side compartments was recorded and aggregated with data from other trials. We cycled different colors of fish and change the number of fish in the side compartments. The data showed that fish have a preference toward the color of fish they were raised with and normally choose a larger number of fish, enabling greater reproductive success, and protection from predators. This tells us a great deal about the social capabilities of zebrafish, as well as their awareness and instincts. We are also planning other experiments to be performed in the future.

34. THE EFFECT OF METHYLMERCURY ON NEURAL GENE EXPRESSION IN ZEBRA FINCH DEVELOPMENT

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Mercury is a highly toxic pollutant that adversely affects the nervous system, particularly when exposure occurs early in life. Preliminary data reveal that male juvenile zebra finch (Taeniopygia guttata) learn a less complex and lower pitched song after developmental exposure to methylmercury. Zebra finches are the most commonly used laboratory songbird species, yet their embryological development has been poorly characterized. Most studies to date apply Hamburger and Hamilton stages derived from chicken development, however significant differences exist between development of the two species. Here we provide the first detailed description of embryological development in zebra finch under standard artificial incubation. Once embryology was characterized, expression of key song learning genes in 25 zebra finch embryos whose parents had been raised on a diet containing low, biologically relevant levels of mercury was characterized. The genes examined in this study were FoxP2, a winged-helix transcription factor implicated in language disorders, and CNTNAP2, a downstream target of FoxP2 also associated with language impairment. We used in situ hybridization on histological sections to compare spatiotemporal gene expression patterns between unexposed control embryos and embryos developmentally exposed to 1.2ppm and 2.4ppm methylmercury. Levels of gene expression of FoxP2 and CNTNAP2 were quantified using qRT-PCR. Preliminary results suggest no significant differences between treatment groups, however we also note that embryos had a great deal of biological variation in FoxP2 levels at the stages analyzed. Ongoing experiments are currently investigating potential causes of this variation. To further examine the effects of prenatal methylmercury exposure, we quantified the levels of FoxP2 expressed in juvenile male brains. Results show that 50% of juvenile males prenatally exposed to 2.4ppm MeHg have significantly lower FoxP2 levels compared to controls or to birds exposed to lower Hg levels.

35. OF THE PEA APHID, *ACYRTHOSIPHON PISUM*

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Environmental changes can elicit alterations in the form, behavior and/or physiology of all species and this developmental response to environment is known as phenotypic plasticity. Despite its ubiquity, the molecular basis for phenotypic plasticity is not fully understood. The pea aphid, *Acyrthosiphon pisum*, serves as a model for discontinuous phenotypic plasticity, also known as polyphenism. Changes in photoperiod stimulate a switch in female aphid reproductive mode from asexual to sexual reproduction over the course of one generation without changes in genotype. This reproductive polyphenism results in female aphids with ovaries of one of two types: sexual ovaries (producing haploid oocytes via meiosis), or asexual ovaries (producing identical diploid aphid clones via parthenogenesis). Hormones such as Juvenile hormone are predicted to play crucial roles in the regulation of reproductive polyphenism. To better understand the effects of various hormones on determining reproductive mode, we are developing an *in vitro* culture system. Through the use of a variety of media conditions we are developing a system capable of maintaining functional dissected asexual ovaries in culture. To track germarium and oocyte development at a more detailed level, imaging techniques, such as fluorescent DNA stains and fluorescent antibody staining, are used. Once successful this system will be used to perform hormone experiments on the ovaries and test for gene expression changes. Our research will help verify the influence of hormones on determining reproductive mode in *A. pisum*.

36. USING TRANGENESIS TO ANSWER QUESTIONS ABOUT THE DEVELOPMENTAL ASPECTS OF REPRODUCTIVE POLYPHENISM SEEN IN PEA APHIDS

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Phenotypic plasticity, a biological phenomenon defined as alterations in the physical structure, function, and behavior of organisms induced by environmental changes, is an extremely intriguing area of study. Though phenotypic plasticity is prevalently found among a wide variety of organisms, its molecular mechanism has not been comprehensively revealed. The pea aphid, *Acyrthosiphon pisum,* is a model organism for studying phenotypic plasticity. In response to changes in photoperiod, *A. pisum* changes its mode of reproduction from asexual to sexual over the course of a single generation without any changes to the genotype. Each reproductive morph has a morphologically distinct set of oocytes and this project attempts to gain insight into these developmental differences. During spring and summer seasons, aphids reproduce asexually through parthenogenesis, a form of asexual reproduction in which the embryo develops without fertilization. Parthenogenesis traditionally produces clones of the mother, and evidence suggests that genetic recombination during asexual reproduction does not take place. On the other hand, sexual aphids are generally characterized as conducting recombination. Yet, a detailed overview of these processes in aphids is yet to be elucidated. Secondly, asexual female aphids, characterized by two X chromosomes, produce males, characterized by one X chromosome. How a female loses an X chromosome during parthenogenesis to produce males is unknown. It is precisely these aspects of oocyte development which we will be focusing on. We will observe the chromosomal development in both asexual and sexual aphid ovaries to see whether or not genetic recombination takes place among homologous chromosomes and how asexual females are able to lose an X chromosome during parthenogenesis to produce males. These goals will be met by developing a transgenesis system in aphids using available vectors. The vectors, due to their transposable elements and fluorescent protein tags, will enable us to specifically tag proteins such as histones and tubulin to visualize both chromosomes as well as the mitotic/meiotic spindles. A well-established transgenesis system will allow us to obtain an unprecedented amount of information in regards to the different reproductive processes of *A. pisum.*

37. METHYLMERCURY AND DISRUPTION OF DEVELOPMENT IN THE FROG *XENOPUS LAEVIS*

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Methylmercury is a toxic derivative and the primary biologically available form of mercury in the environment. With a relatively long half life in living biological tissue of nearly two months, methylmercury is known to bioaccumulate. Due to the ability to conjugate with the exposed thiol group of cysteine, it forms a methionine mimic that is actively transported across the blood-brain and placental barriers. Although the direct mechanisms of methylmercury toxicity are still largely unknown, methylmercury in as low as mM quantities has been shown to inhibit $GABA_A$ receptor and Ca^{2+} mediated neuronal currents, aberrantly activate the notch signaling pathway, and increase oxidative stress. Consequently, high profile methylmercury poisoning events in human populations, such as the Ontario-Minamata disease and Basra poisoned grain disaster, have exemplified the danger of methylmercury, particularly to the nervous system of adults and especially the developing fetus. To understand the degree and process by which methylmercury toxicity affects development, we have employed the model system *Xenopus laevis*, due to its large embryo size and well studied developmental stages. Using a range of methylmercury chloride concentrations from 0.05 ppm to 0.2 ppm, we developed a graded 1 to 5 scale to semi-quantify morphological abnormalities due to exposure to methylmercury. By following exposed groups over time, it was found that embryos primarily began to deform during neurulation at stage 13-15 in a dose responsive manner. Several markers of neurodevelopement and differentiation were subsequently assayed via *in situ* hybridization, including Sox2, En2, and Delta; but did not suggest significant pattern differences between control and exposed groups. However, RT-PCR on xGAD and xGAT mRNA suggested a decrease in the transcription level of both genes between the control and exposed, potentially implicating the GABAergic system as a methylmercury target, and requiring further exploration. Thus, while many mechanisms of methylmercury activity remain to be uncovered, our data demonstrates developmental defects primarily manifest at neurulation.

38. 17-B ESTRADIOL CAUSES CRANIOFACIAL CHONDROGENESIS DEFECTS IN ZEBRAFISH (*DANIO RERIO*)

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Estrogen is a steroid hormone that is universal in vertebrate bodies, but its role in cartilage formation has not been extensively studied. Abnormalities of craniofacial cartilage and bone account for a large portion of birth defects in the United States. Zebrafish (*Danio rerio)* have been used as models of human disease, and their transparency in the embryonic period affords additional advantages in studying craniofacial development. In this study, zebrafish embryos were treated with 17-β estradiol (E_2) and observed for defects in craniofacial cartilage. Concentrations of E_2 greater than 2 μM caused major disruptions in cartilage formation. Concentrations of E_2 at or below 1μM generally did not cause significant alterations in cartilage morphology. The angles formed by cartilage elements in fish treated with 1.5μM and 2 μM E_2 were increasingly wide, while the length of the primary anterior-posterior cartilage element in these fish decreased significantly, yielding fish with shorter, flatter faces with increased estrogen. Further investigation of the phenomena described with Aromatase Inhibitor (AI) in this study could lead to a better understanding of the etiology of craniofacial birth defects.

39. INVESTIGATING THE CELLULAR MECHANISMS OF SKEWED SEX RATIOS IN NON-C. ELEGANS NEMATODES

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During the post-meiotic phase of spermatogenesis, various proteins and cellular components are either retained by the sperm or discarded in a cytoplasmic "waste bag" (residual body). In nematodes, this spermatid-residual body partitioning event occurs unusually early; either during or immediately after anaphase II. In addition, since nematode sperm are non-flagellated, one of the discarded components is tubulin, which is essential for meiotic chromosome segregation but (except for the centriole) superfluous for the subsequent function of either the spermatids or crawling spermatozoa. Instead, the motility of nematode spermatozoa is driven by the major sperm protein (MSP) which forms dynamic filaments within the treadmilling pseudopod. Our lab has recently discovered that a dramatic sex skew in the non-*C. elegans* species, *Rhabditis* sp. SB347 (Felix, 2004) can be explained by an asymmetric partitioning of cellular components during anaphase II of spermatogenesis (Shakes et al, 2011) To investigate the evolution of this trait and rule out that it is merely an isolated curiosity of *R.* sp. SB347, we are analyzing spermatogenesis in other members of this clade. Here we report similar partitioning patterns during spermatogenesis in species *R.* sp.*SB372* and *R.* sp.*JU1809.* As in the dividing secondary spermatocytes of SB347, tubulin is partitioned exclusively to the nullo-X sperm while MSP is partitioned exclusively to the X-bearing sperm. We also find that the two chromatin masses exhibit differential compaction. Thus, these meiotic asymmetries serve as a common mechanism for generating skewed sex ratios within this clade and potentially throughout the phylum. Ultimately, these ongoing studies may provide new insights regarding not only the evolution of reproductive modes and sexual plasticity but also the cellular and biochemical mechanisms of cell polarization.

40. USING *IN SITU* HYBRIDIZATION TO INVESTIGATE DIFFERENTIAL GENE EXPRESSION BETWEEN OVIPAROUS AND VIVIPAROUS APHID OVARIES

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Phenotypic plasticity is the ability of certain organisms to change their phenotype in response to environmental cues. *Acyrthosiphon pisum*, the pea aphid, displays phenotypic plasticity in its reproductive mode. In response to shortened photoperiods, asexual females produce a single generation of male and female sexual offspring. In order for this one genome to produce multiple phenotypes, it is likely that certain genes are expressed differently between the oviparous and viviparous developing *A. pisum* ovaries. Several genes have been shown to be differentially expressed between sexual and asexual ovaries. We investigate the roles of several of these genes, including *Piwi 1*, *Wee 1.3*, *Wee 1-like*, and *Apnanos*, by performing *in situ* hybridizations on fixed oviparous and viviparous ovaries. Successful *in situ* hybridizations resulting in clear differential gene expression patterns between the oviparous and viviparous ovaries would help to identify genes that may be involved in the phenotypic plasticity of the pea aphid's reproductive mode.

41. CHARACTERIZATION OF SEXUAL OOGENESIS & MEIOSIS IN *Acyrthosiphon pisum*

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Natural or synthetic changes in the environment have been shown to cause alternate phenotypes, behaviors, and functions in an organism by a process known as phenotypic plasticity. The pea aphid, *Acyrthosiphon pisum*, is an emerging model organism for phenotypic plasticity that displays alternate reproductive modes (reproductive polyphenism) from a single genotype in response to changes in the environment. This reproductive polyphenism in *Acyrthosiphon pisum* leads to different developmental pathways for future progeny depending on the mode of reproduction. In asexual aphids, the embryo develops viviparously contrasted to the sexual embryo, which develops oviparously. Asexual oogenesis in aphids has been well described[1]; however, there is no formal description of sexual aphid oogenesis, leading to an inability to compare sexual and asexual oogenesis.

We tracked the nuclear position and changes in chromatin behavior over the course of sexual oocyte development using nuclear stains and fluorescent in situ hybridization, respectively. Observing a pattern in the nuclear position may indicate possible developmental cues, as seen in other model organisms such as *Drosophila melanogaster*, where close association of the oocyte nucleus with the membrane of the oocyte leads to the establishment of developmental axes in the oocyte. Tracking chromatin behavior during sexual oogenesis would provide a formal description of meiotic events in the sexual oocyte, allowing these meiotic events to be linked to developmental stages in the sexual oocyte. This complete description of sexual oogenesis and meiosis would allow us to understand more precisely how asexual meiosis is modified compared to sexual meiosis.

Morphogenesis and Organogenesis

42. Hsp47, A MOLECULAR CHAPERONE OF PRO-COLLAGEN INVOLVED IN BONE GROWTH

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Our lab is interested in identifying underlying mechanisms that regulate skeletal morphogenesis during zebrafish fin regeneration. The zebrafish caudal fin is composed of 16-18 bony fin rays, each fin ray is comprised of multiple bony segments separated by joints. Our examination of the fin length mutant, *short fin (sof)*, has revealed that the gap junction protein Connexin43 (Cx43) promotes cell proliferation and inhibits joint formation during fin regeneration. Interestingly, in humans, mutations in *CX43* cause occulodentodigital dysplasia, a disease that affects the craniofacial and distal limb skeleton. Therefore, function of Cx43 while unknown, is conserved across species, from zebrafish to human. Previously, a microarray analysis through our lab identified potential downstream regulators of *cx43, serpinh1b* being one of them. *Serpinh1b* codes for a glycoprotein called Hsp47, an ER resident stress protein. Unlike other proteins in ER, it is the only stress protein that is induced by heat shock. Interestingly, it binds specifically to procollagens in ER and helps in its transport to the Golgi. Association of Hsp47 to procollagens prevents local misfolding of procollagen triple helix and aggregate formation. Mutations in type 1 collagen genes (*COL1A1* and *COL1A2*) have been identified as one of the leading causes of Osteogenesis imperfect (OI) or 'brittle bone disease'. Recent studies have identified an autosomal recessive mutation in *serpinh1b* that causes severe OI phenotypes in human. Since both *cx43* and *serpinh1b* genes are involved in causing skeletal diseases, the question of whether these genes function in a common signaling pathway is raised. Morpholino mediated knock down of *serpinh1b* in regenerating fin has been shown to reduce segment length without affecting cell proliferation. Interestingly, these short segments are formed due to formation of premature joints, and not due to 'brittle bone', suggesting the possibility of cross-talk between collagens and genes involved in the joint formation pathway. A detailed analysis of the collagen matrix and the bone structure following Hsp47 knockdown will provide valuable information and will help us in our understanding of bone growth and joint formation during regeneration.

43. ALPHA INTEGRIN CYTOPLASMIC TAILS EXECUTE TISSUE-SPECIFIC SIGNALING DURING *C. ELEGANS* DEVELOPMENT.

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Cell migration is a highly regulated process by which cells move to specific destinations in an organism. Integrin receptors are alpha/beta heterodimeric transmembrane proteins that facilitate cell migration by connecting the surrounding extracellular matrix with the actin cytoskeleton. Integrin-directed cell migration requires the coordination of a multitude of signals in a changing environment. To dissect integrin signaling mechanisms, a cell migration model based in the nematode *C. elegans* was employed. In *C. elegans*, distal tip cell migration during gonadogenesis is dependent upon two alpha integrins, *ina-1* and *pat-2*. During the dorsal stage of distal tip cell migration, both alpha integrins are used simultaneously, but for different purposes. *Ina-1* is critical for cell motility, while *pat-2* contributes to pathfinding. The occurrence of multiple cell behaviors from simultaneous integrin signaling suggests those signals are differentiated in some way. Chimeric proteins consisting of the extracellular domain of one alpha integrin attached to the cytoplasmic domain of the other were used to address this issue. Interestingly, each chimeric protein was able to rescue integrin lethality; however, distal tip cell migration, muscle actin organization and vulva morphogenesis could not be fully restored by an improper cytoplasmic tail. These findings show alpha integrin cytoplasmic tail function varies in a tissue-dependent manner. Further studies are underway to address the signaling partners used by each cytoplasmic tail.

44. WNT5A REGULATES NEPRHRIC DUCT EXTENSION AND ITS EXTENSION AND IS IMPLICATED IN DUPLEX KIDNEY FORMATION

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Duplex kidney formation is a relatively common congenital abnormality affecting about 1% of the population, but the molecular basis for this defect is poorly understood. While investigating the role of Wnt5a in metanephric development, we found that its specific inactivation in mesoderm using *T-Cre* results in bilateral duplex kidney formation. The ureteric buds in mutants emerge as doublets from the nephric duct without any apparent expansion of the *Gdnf* expression domain. *Wnt5a* is normally expressed in a graded manner at the posterior end of the intermediate mesoderm prior to ureteric bud outgrowth, but its expression is down regulated by the time the bud forms. Consistent with its expression pattern, *Wnt5a* ablation during development of the intermediate mesoderm results in duplex kidneys, whereas normal kidneys arise if *Wnt5a* is ablated following nephric duct extension. The duct itself is shortened and broadened at its caudal terminus in mutant embryos prior to ureteric bud outgrowth and prompts the formation of twin epithelial ureters and duplex kidneys. Although Wnt5a can activate β-catenin signaling, TCF-dependent canonical Wnt activity is not decreased in *Wnt5a* mutants in the posterior nephric duct during nephric duct extension, suggesting that a non-canonical mechanism is responsible for nephric duct extension. Taken together, these results demonstrate that Wnt5a plays an essential role in maintaining the tubular integrity of the nephric duct and that the abnormal extension of the nephric duct, following Wnt5a deletion, causes duplex kidney formation.

45. EPITHELIAL BEHAVIORS DURING FOREGUT COMPARTMENTALIZATION

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One of the major morphogenic events during the development of the respiratory and alimentary tracks is the compartmentalization of the common foregut tube into the dorsal esophagus and ventral trachea. A disruption of this process can result in a severe birth defect, esophageal atresia and tracheoesphageal fistula, in which the lumens of the trachea and esophagus remain connected. While the signaling that initiates this separation is well understood, the underlying cell biology required to complete this morphogenic process is unknown. Compartmentalization of the foregut into the esophagus and trachea takes place between embryonic day 10.0 and 11.0 in mouse (E10-E11). We found that at E10.5 the epithelium at the dorso-ventral midline (D/V midline) is a pseudostratified epithelium. Interestingly, earlier at E9.5 the foregut epithelium at the D/V midline is a simple columnar epithelium. The importance of the changes in the epithelium during compartmentalization has never been investigated. We hypothesize that this pseudostratification is required for the compartmentalization of the foregut. We are using a mouse model where β-catenin is removed specifically from the ventral foregut to examine the role of epithelial changes at the D/V midline. The loss of β-catenin results in a loss of pseudostratification and foregut compartmentalization. We are taking advantage of this phenotype to test the hypothesis that changes in epithelial morphology are necessary for the separation of the esophagus and trachea. In addition to this genetic approach, we have established a robust tissue culture system, which allows us to investigate if perturbations to the epithelial layer disrupt compartmentalization. These techniques have allowed us to gain insight into the cellular behaviors important during foregut morphogenesis, which cannot be examined in other mouse models.

46. *emulsion/cax1*, A MATERNAL-EFFECT GENE THAT REGULATES ZEBRAFISH CYTOPLASMIC SEGREGATION, CLEAVAGE DIVISIONS AND AXIS FORMATION

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In zebrafish egg maternal organelles, RNAs and proteins included in the future cytoplasm of the embryo are initially found within the yolk in cytoplasmic domains. Following fertilization and egg activation, these components segregate from the yolk cell to the blastodisc in a process called cytoplasmic segregation. Thus, the blastodisc grows by accummulation of cytoplasm transported to the animal hemisphere from the yolk cell at different speeds along specific pathways or streamers. An understanding of how maternal genes regulate cytoplasmic segregation is the main aim of this work. We have identified a reccesive maternal-effect mutant, *emulsion* (*emn*), in a forward genetic screen for maternal factors involved in zebrafish early development. The *emn* embryo exhibits a smaller blastodisc, reduced cytoplasmic segregation, abnormal cleavage pattern and axis determination. A positional cloning approach indicates that the *emn* gene encodes a CAtion/proton eXchanger 1 protein (CAX1), known to be involved in the maintenance of ion homeostasis. Functional studies, combined with the analysis of zebrafish mutants generated by reverse genetic strategies, are expected to elucidate the mechanisms by which the *emn/cax1* gene regulates cytoplasmic movements, cleavage and axis establishment during early vertebrate development.

47. ESTROGEN REPLACEMENT THERAPY PREVENTS CARDIAC AND VASCULAR FAILURE IN THE 'LISTLESS' ZEBRAFISH (*DANIO RERIO*) DEVELOPMENTAL MODEL

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The presence of a robust estrogen (E2) response system throughout heart and blood vessel tissues of vertebrates, including humans, has led to the speculation that this ubiquitous hormone may play a prominent role in the health and maintenance of the adult cardiovascular system (CVS). We have established an embryonic zebrafish model called 'listless', which results from the inhibition of E2 synthesis by treatment with aromatase inhibitors (AIs). In addition to lacking numerous sensory-motor functions, 'listless' fish developed edema as indicated by the filling of the cardiac sac with fluid and also died from cessation of heart function, as well as the collapse of blood circulation; these symptoms are reminiscent of human congestive heart failure. Our observations led to the hypothesis that treatment of the 'listless' fish with E2 replacement therapy (E2 + AI) would decrease cardiac edema and protect fish from cardiovascular system failure. Indeed, data from the current study demonstrates that E2 + AI treatment: 1) reduced the severity of cardiac sac abnormalities; 2) protected heart function with increased survival; 3) prevented reduction in heart size; and 4) prevented blood vessel bed deterioration or delay in development. In addition, with removal of fluid surrounding the cardiac sac by puncture, heart function and survival were significantly increased. These results strongly indicate the important role played by E2 in the developing CVS of the zebrafish, and offers a potential new model for the study of its role in CVS development, maintenance and disease conditions such as congestive heart failure.

48. ALK5 INHIBITION RESULTS IN ANTERIOR NEUROCRANIUM ABNORMALITIES

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TGF-β signaling is an important regulator of craniofacial development, in particular, through controlling the fate of cranial neural crest cells, but also in later developmental patterning. In humans, TGF-β signaling has been implicated in orofacial clefting. We decided to use Zebrafish as a model to explore the role of TGF-b signaling in craniofacial development, focusing on the anterior neurocranium, pharyngeal and facial musculature. We cultured zebrafish larvae in a small molecule inhibitor of TGF-β family receptor type I (ALK5) larvae at 10 and 20 hours post-fertilization (hpf). Larvae were collected 5 days post-fertilization and we used alcian blue staining to visualize craniofacial cartilages or immunohistochemistry with the 12/101 antibody for skeletal muscle. We found numerous abnormalities to the anterior neurocranium and pharyngeal cartilages. With high dose inhibition at both 10 and 20 hpf, we observed a discontinuous ethmoid plate, and ectopic cartilages joining the trabeculae, as well as a ventral shift of Meckel's cartilage, and a shortened mandibular arch. At lower doses of inhibition and at 10 and 20 hpf, the phenotype was less severe in the ethmoid plate but there was initiation of the ectopic cartilage on the trabeculae. Surprisingly, ALK5 inhibition did not affect the development or patterning of pharyngeal and facial musculature. We conclude TGF-β signaling is required to pattern the ethmoid plate in Zebrafish, and our data shows that Zebrafish are a valuable tool for elucidating genetic pathways contributing to craniofacial anomalies.

49. EXTRACELLULAR ATP REGULATES CONTRACTILE MECHANICAL TONE OF EARLY EMBRYONIC EPITHELIA

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Cell signaling and mechanical forces play vital roles in morphogenesis and organogenesis. However, pathways that trigger the generation of forces shaping embryonic tissues are not well understood. We previously utilized nano-perfusion assays to demonstrate that cell lysate can induce F-actin remodeling and epithelial contractions. This study identifies ATP as the active component in lysate, participating in a G-protein coupled receptor (GPCR) pathway to regulate contractile tone of epithelium during gastrulation. After testing a series of candidate factors, ATP was identified as the primary candidate in lysate causing contractions. ATP was nano-perfused over the animal cap of *Xenopus laevis* embryos. Acute exposure produced spatiotemporally-specific epithelial contractions while chronic exposure caused exogastrulation. Using the ATP hydrolyzing enzyme apyrase, embryos were rescued from the effects of ATP or lysate exposure on blastopore closure. Incubation with apyrase alone resulted in slightly faster blastopore closure, implicating a role of endogenous extracellular ATP regulating gastrulation. Using various inhibitors of F-actin and non-muscle myosin II, we showed actomyosin contractility is a key target in the response to ATP. Of the possible receptors for extracellular ATP, we demonstrated that GPCRs of the P2Y family were likely candidates by expressing Gγ-SaaX mutant mRNA in embryos, which reduced or abolished contractility. Temporal expression of P2Y-family proteins, analyzed using PCR, indicated P2Y2R as the target receptor. Two morpholino oligonucleotides specific to alloalleles A and B (P2Y2R-MO) were injected into epithelial precursors at the 8-cell stage to knock down P2Y2R expression. Nano-perfusion of ATP over P2Y2R-MO injected embryos had diminished or no contractions. This strongly suggests that ATP induces epithelial contractility through the P2Y2R GPCR, offering an example of a robust pathway controlling embryonic ectodermal contraction mechanics.

Stem Cells and Regeneration

50. CHARACTERIZATION OF PROGENITOR CELLS IN ADULT MOUSE PANCREAS

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Pancreatic progenitor cells with the capacity to generate insulin-secreting β-cells offer a potential cure for diabetes. Thus, the identification of multipotent progenitor cells in the adult pancreas is a crucial step towards this goal. In a normal pancreas, the amount of cell turnover is low compared to other organs such as the intestine. This scenario complicates the process of identifying pancreatic progenitor cells *in vivo*. Unlike intestinal and hematopoetic stem cells, no definitive marker has been identified for the identification of adult pancreatic progenitor cells. Recently, various studies have demonstrated that a specific pool of multipotent progenitor cells reside within the ductal cells of adult pancreas. Here, we utilize two mouse lines: Pdx1:eGFP and Prominin1 (CD133):CreERT2 to identify and isolate the Pdx1 and CD133-expressing cells in 6 to 8-week old adult pancreas. Using Fluorescence Activated Cell Sorting (FACS) and Magnetic Activated Cell Sorting (MACS), we sorted cells expressing Pdx1 or CD133 to study their capacity to differentiate into other pancreatic cell types, especially insulin-secreting β-cells, *in vitro*. Our preliminary data show that both Pdx1 and CD133 are expressed in a subset of ductal epithelial cells. When cultured in 3D matrigel, cells sorted for either Pdx1:eGFP expression or CD133:Cre activity formed spheres/round colonies that are 30-100um in size. These colonies also express E-Cadherin, demonstrating an epithelial identity. In future experiments, we will analyze these spheres/colonies using differentiation markers for all pancreatic cell types to assess if Pdx1 and CD133-expressing cells can serve as a multilineage progenitor source in the adult pancreas.

51. BMP REGULATION OF GERMLINE STEM CELL DEVELOPMENT DURING *DROSOPHILA* TESTIS FORMATION

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Stem cells are vital for organogenesis, tissue regeneration, and tissue homeostasis. These asymmetrically dividing cells provide the functional cell types necessary for organogenesis while maintaining a stem cell population that continuously replaces damaged and dying cells. Despite the fundamental importance of stem cells to living systems, the mechanisms regulating stem cell development are not well understood. One of the most thoroughly studied systems for examining stem cell behavior is the adult *Drosophila* testis, which is composed of sperm-producing germline stem cells (GSCs) and somatic cyst stem cells (CySCs) anchored at a discrete niche found at the testis apex. Within this niche, the bone morphogenetic protein (BMP) signaling pathway has been shown to regulate GSC maintenance in the adult organism. Here we examine the role of BMP signaling during the dynamic process of development. Specifically, we determine the pattern of BMP activation in GSC precursors, called primordial germ cells (PGCs), and assess the capacity of BMP signaling to control the initial establishment and maintenance of GSCs in larval testes. We find that BMP signaling expresses a dynamic pattern of activation in developing PGCs during embryogenesis, and that high-level BMP activation becomes restricted to GSCs localized to the larval testis apex after stem cell niche formation. Additionally, we find that BMP signaling is both necessary and sufficient for the maintenance of undifferentiated GSCs within the testis niche just after the onset of asymmetric GSC divisions. Thus, restricted activation of BMP signaling to the newly established GSCs adjacent to the niche appears to promote the maintenance of undifferentiated GSCs within the niche, while also permitting differentiation of more distant GSC daughter cells. Furthermore, this dynamic pattern of BMP activation in early PGCs suggests a possible role for BMP signaling in GSC establishment during testis development.

52. DEFINING THE *CX43*-DEPENDENT JOINT FORMATION PATHWAY IN THE REGENERATING FIN

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Joints are essential for skeletal flexibly, form and strength, yet the process underlying joint morphogenesis is poorly understood. Zebrafish caudal fins are comprised of numerous bony segmented fin rays, and each newly segment or joint is added at the distal end of the fin. Therefore, the least mature joints are always more distal than the more mature joints, providing context for evaluation of joint morphogenesis. Prior studies from our lab strongly indicate that the gap junction protein Cx43 suppresses joint formation. Here we identify a molecular pathway acting downstream of Cx43. First, we show that joint differentiation in regenerating fins can be studied by evaluating joint gene expression along the distal-proximal axis. The earliest joint marker, *evx1*, is expressed in the most distal domain of the fin, directly adjacent to the *cx43*-expressing population of dividing cells. More mature markers, such as *dlx5* and *mmp9*, are expressed more proximally. Remarkably, changes in Cx43 activity shift the expression pattern of these markers. For example, when Cx43 is reduced joint formation occurs prematurely (i.e. we observe short bony segments) and *evx1*-*dlx5*-*mmp9* expression patterns are shifted to more distal positions. In contrast, when Cx43 activity is increased joint formation is lost and *evx1* expression is also lost. Together our results suggest that Cx43 suppresses joint formation by suppressing the expression of *evx1* and the subsequent players of this joint formation pathway. Continued studies will be aimed at defining how Cx43 function influences the expression of *evx1*.

53. ELUCIDATING THE ROLE OF HAPLN1A DURING SKELETAL PATTERNING IN REGENERATING CAUDAL FIN

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Cell-cell communication facilitating the exchange of small metabolites, ions and second messengers takes place via aqueous proteinaceous channels called gap junctions. Connexins are the subunits of a gap junction channel. Mutations in zebrafish *cx43* produces the *short fin* (*sof ^{b123}*) phenotype characterized by short fins due to reduced segment length of the bony fin rays. Previously established results from our lab demonstrates that Cx43 plays a dual role regulating both cell proliferation and joint formation during the process of fin regeneration. In this study we show that Hapln1a, an ECM protein, functions downstream of Cx43. Hapln1a (Hyaluronan and Proteoglycan Link Protein 1a) also called as Cartilage Link Protein belongs to the family of link proteins that play an important role in stabilizing the extracellular matrix by linking the aggregates of hyaluronan and proteoglycans. We validated that *hapln1a* functions downstream of *cx43* by in situ hybridization and quantitative RT-PCR methods. Moreover, in situ hybridization on regenerating fins at different time points following amputation show that *hapln1a* expression peaks at 3dpa and is expressed in different compartments of the fin tissue including distal blastema, basal layer of epidermis and by the lateral skeletal precursor cells. Furthermore, morpholino mediated knockdown of Hapln1a results in reduced fin regenerate length, reduced bony segment length and reduced cell proliferation recapitulating all the phenotypes of Cx43 knockdown. Additionally, qRT-PCR results on Sema3d knockdown and Hapln1a knockdown samples suggest that, these two genes function independent of each other producing the same phenotype but in a Cx43 dependent manner. Collectively our data suggest that Cx43 dependent phenotypes are mediated in part by *hapln1a* and it functions in a common molecular pathway downstream of *cx43*.

54. THE FUNCTION OF SOX11 IN PRIMARY NEUROGENESIS

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Neurogenesis is the fine-tuned process of generating neurons from neural progenitors. During neurogenesis, many proteins, and in particular, the high mobility group Sox transcription factors, function to maintain a balance of proliferating progenitors and differentiating neurons. While a number of studies have demonstrated that SoxB1 proteins are necessary for neural progenitor maintenance, much less is known about the role of the SoxC proteins. Recent studies indicated that Sox11, a SoxC protein, is necessary for neuronal maturation and functions downstream of the proneural bHLH proteins in the chick spinal cord. But how Sox proteins interact with other proteins to balance the number of neural progenitors and mature neurons remains largely unknown. To investigate the role of Sox11 in primary neurogenesis, we analyzed the effect of loss and gain-of-Sox11 function in *Xenopus laevis*. We find that loss of Sox11 function decreases the expression of markers of mature neurons, and slightly increases that of neural progenitor markers. Thus Sox11 is required for neuronal maturation. However, our gain of function data showed that overexpression of *sox11* increases the number of mature neurons, yet also expands the neural progenitor domain. One interpretation is that Sox11 has dual roles; whereas zygotic Sox11 drives the progression of neurogenesis, maternal Sox11 participates in neural fate determination or maintenance of neural progenitors. Based on this, we will investigate the dual roles of Sox11 in neural development what will allow us to have a comprehensive understanding of the role of Sox11 in neurogenesis.

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55. CHARACTERIZATION OF THE Gal-4/UAS GENE EXPRESSION SYSTEM FOR ANALYSIS OF DROSOPHILA TESTIS DEVELOPMENT

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Stem cells are vital for maintenance and generation of healthy tissues. However, our understanding of how stem cells develop during organ formation is limited. Testis of the fruit fly, *Drosophila melanogaster*, are among the most accessible and thoroughly characterized systems for studying stem cell behavior. Little research has looked into how the stem cell populations are first established during testis formation during embryonic and larval development. The Gal4/UAS gene expression system is particularly useful for examining the process of testis development. Gal4 drivers are powerful tools used in *Drosophila* genetics to artificially express foreign genes in specific cells and tissues at different stages of development. Many Gal4 drivers have been studied and used in the adult *Drosophila* testis to induce expression in both the germline and the surrounding somatic cells. However, the expression of these and other Gal4 drivers have not been well characterized during development. To accurately utilize the Gal4/UAS system during embryogenesis and larval testis development, we characterized a number of Gal4 drivers in the developing gonads from stage 12, when gonads first begin to form, through the 2nd larval instar molt, when the testis stem cell niche has formed and spermatogenic differentiation is clearly observed in germ cells. Data show unique temporal-spatial patterns of Gal4 transgene activity in the developing testis. Future experiments can utilize these, and other, Gal4 drivers to induce expression of activators and/or inhibitors of specific genes of interests in order to determine their roles in gonad morphogenesis. This can enhance our knowledge of mechanisms controlling organogenesis that are conserved in other organ systems and organisms, and lead to a better understanding of stem cell maintenance and tissue generation.

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