Program/Abstract # 1

Role of the core promoter in the regulation of gene expression

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The RNA polymerase II core promoter comprises the stretch of DNA that directs the initiation of transcription. This basic definition might suggest that core promoters are simple transcriptional elements that function via common mechanism. Current evidence indicates, however, that there is extraordinary diversity in core promoter structure and activity. For instance, there are a variety of sequence-specific DNA elements in core promoters that include the TATA box, Inr, MTE, DPE, BRE, and TCT motifs. These elements do not act universally – the well-known TATA box is present in only about 15% of human promoters. Instead, specific core promoter elements confer distinct transcriptional activities to core promoters. For example, nearly all of the *Drosophila* Hox genes have DPE-dependent core promoters, and the Caudal protein, a master regulator of the Hox genes, is an enhancer-binding protein that activates transcription specifically through the DPE motif. Hence, the Hox genes are a DPE-based transcriptional network, and Caudal is a DPE-specific activator. In addition, another core promoter element, the TCT motif, is dedicated to the expression of the network of ribosomal protein genes. These and other findings collectively indicate that the core promoter is a diverse transcriptional regulatory element that is employed for the regulation not only of individual genes, but also of gene networks. Moreover, at a more practical level, optimized versions of the core promoter can be used to achieve the desired specificity and level of expression of transgenes. It is thus evident that a strong understanding of the core promoter is essential for the effective analysis of gene expression.

Program/Abstract # 2

Measuring transcriptional dynamics of single cells reveals mechanisms that compensate for the cost of bistability

Boettiger, Alistair, Harvard, United States; Bothma, Jacques; Perry, Michael; Levine, Michael, UC Berkeley, United States

Cell fate decisions during development produce a discrete set of distinct cell types – rather than a continuum of states. One of the most popular models to explain the formation of distinct states without mixed intermediates envisions development to be comprised of a sequence of binary choices for each cell. However, few examples of such binary fate decisions have been worked out in developing systems and little is known about the molecular mechanisms and functional constraints which enable such decisions in the context of a developing embryo. We present evidence that the mesoderm-ectoderm boundary in the early *Drosophila* embryo is formed by just such a bistable interaction. Simultaneous expression of competing transcriptional repressors in the ectoderm forces these cells to make a binary choice between a presumptive muscle, or presumptive nerve fate. Genetic perturbation experiments illustrate that the expression of the competing genes is highly constrained by a need to suppress transcriptional noise. Small degrees of expression noise similar to those observed in other systems lead are amplified by the bistable interaction with catastrophic effects on development. To understand how such noise is repressed at the molecular level, we quantify mRNA expression in single-cells throughout early development. We find that redundant repressors, redundant enhancers, and near-saturating transcription levels all contribute ensure unprecedented low levels of noise in expression of the key regulatory gene, snail. Perturbations to any one of these three mechanisms enhance the molecular variations in snail between sister cells and can lead to dramatically increased variation in boundary placement.

Program/Abstract # 3

The MED12-MED13 module of Mediator regulates multiple developmental phase transitions during the *Arabidopsis* life cycle

Gillmor, Stewart, Langebio, CINVESTAV-IPN, Mexico; Willmann, Matthew (U Pennsylvania, United States); Silva-Ortega, Claudia (Langebio, CINVESTAV-IPN, Mexico); Poethig, Scott (U Pennsylvania, United States)

Temporal coordination of developmental programs is essential for multicellular development, and alteration of developmental timing has been proposed to play an important role in evolution. We have previously shown that the MED12 and MED13 genes CENTER CITY (CCT) and GRAND CENTRAL (GCT) are essential for temporal coordination of pattern formation during *Arabidopsis* embryogenesis (Gillmor et al., Development, 2010). In the current study, we describe a role for GCT and CCT in global coordination of developmental phase transitions. *gct* and *cct* mutations cause embryo-specific genes to be expressed during seedling development, delay vegetative phase change, and delay flowering. Genetic analysis indicates that GCT and CCT regulate the progression of vegetative growth and flowering in parallel with the phytohormone GA, and the chromatin remodeling protein PICKLE. We demonstrate that the delay in the onset of adult vegetative traits is due to upregulation of miR156 expression in *gct* and *cct* mutants, and that the delay in flowering is due in part to upregulation of the floral repressor FLC. Thus, GCT and CCT regulate vegetative and floral transitions by repressing the repressors miR156 and FLC.

Program/Abstract # 4

RA-FGF antagonism during vertebrate body axis extension: feedback signaling from stem cell progeny to niche

Duester, Gregg L.; Cunningham, Thomas; Brade, Thomas, Sanford-Burnham, La Jolla, United States; Trainor, Paul, Stowers, Kansas City, United States; Sandell, Lisa (University of Louisville, United States)

Vertebrate embryos develop in a head to tail fashion from a caudal progenitor zone. During body axis extension, bipotential neuromesodermal stem cells in the caudal progenitor zone generate progeny that contribute to both neuroectoderm (hindbrain-spinal cord) and paraxial mesoderm (somites). Presomitic mesoderm exits the caudal progenitor zone and undergoes segmentation into somites anterior to a wavefront of Fgf8 activity generated in caudal progenitors. A major transition in mouse development occurs at E7.5 when presomitic mesoderm begins generating retinoic acid (RA) via Rdh10 and Raldh2 enzymes that sequentially metabolize retinol to retinaldehyde and then to RA which acts as a diffusible signal. Embryos deficient in RA synthesis exhibit small somites and limb defects that we have hypothesized are the result of expanded Fgf8 expression, but it is unclear how RA regulates mesoderm development. Here, we show that reduction of FGF signaling in Raldh2-/- embryos lacking RA synthesis is sufficient to rescue somitogenesis. Rdh10 mutants initially lack RA synthesis and exhibit expanded Fgf8 expression and small somites as well as stunted forelimbs, but later recover normal caudal somites and hind limbs due to late-appearing RA activity that arises at the neuroectoderm/epiblast junction; a RA-reporter transgene sensitive to 0.25 nM RA demonstrates that RA activity does not act in presomitic mesoderm for somitogenesis and that RA is not required for limb patterning. Our findings demonstrate that progeny of the caudal progenitor zone produce RA that controls axial development by feedback signaling to the border of the stem cell niche designed to repress Fgf8 in neuromesodermal stem cells at the neuroectoderm/epiblast junction.

Program/Abstract # 5

Muscle satellite cells are primed for myogenesis, but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules

Crist, Colin G., McGill University, Montreal, Canada; Montarras, Didier; Buckingham, Margaret (Institut Pasteur, Paris, France)

Regeneration of adult tissues depends on stem cells that are primed to enter a differentiation programme, while remaining quiescent. How these two characteristics can be reconciled is exemplified by skeletal muscle where the majority of quiescent satellite cells transcribe the myogenic determination gene *Myf5*, without activating the myogenic programme. We show that *Myf5* mRNA, together with microRNA-31, which regulates its translation, are sequestered in mRNP granules present in the quiescent satellite cell. In activated satellite cells, mRNP granules are dissociated, relative levels of miR-31 are reduced, and Myf5 protein accumulates, which initially requires translation, but not transcription. Conditions that promote the continued presence of mRNP granules delay the onset of myogenesis. Manipulation of miR-31 levels affects satellite cell differentiation ex vivo and muscle regeneration *in vivo*. We therefore propose a model in which post-transcriptional mechanisms hold quiescent stem cells poised to enter a tissue specific differentiation programme.

Program/Abstract # 6

Predominant role of Hoxa5 gene during mouse lung development

Jeannotte, Lucie; Boucherat, Olivier; Montaron, Séverine; Aubin, Josée (University of Laval, Canada); Philippidou, Polyxeni; Dasen, Jeremy (NYU School of Medicine, United States)

Lung development depends on reciprocal interactions between the epithelium and the surrounding mesenchyme. The mesenchyme can instruct epithelial differentiation but the nature of the mesenchymal factors involved still remains elusive. Hox genes encode transcription factors specifying regional identity along the body axes and in regulating morphogenesis during development. In mammals, 39 Hox genes are organized in 4 clusters and classified in 13 paralog groups. Several Hox genes are expressed in a distinct spatio-temporal fashion during lung ontogeny and their expressionis mainly restricted to lung mesenchyme. Except for Hoxa5, the lack of overt lung phenotype in single mutants suggests that, taken individually, these Hox genes do not play a predominant role in lung ontogeny. Functional redundancy may also mask anomalies. Most Hoxa5-/- mice die at birth from respiratory distress due to tracheal and lung dysmorphogenesis, whereas mutations of the other paralog members Hoxb5 and Hoxc5 do not cause phenotypes that impact on life. The severity of the Hoxa5 lung phenotype indicates a major role for Hoxa5 in lung formation. We have produced Hoxa5;Hoxb5 compound mutants to evaluate the relative importance of these two paralogs in lung development. Hoxa5 gene plays an exclusive role in the specification of epithelial type 1 alveolar cells, in trachea formation and in diaphragm innervation. Branching of the bronchial tree and goblet cell specification involve both Hoxa5 and Hoxb5 genes. Thus, Hoxa5 and Hoxb5 genes share some functions during lung ontogeny, but Hoxa5 appears to play a predominant role. (Supported by a CIHR grant)

Program/Abstract # 7

Forward genetics identifies Tmem107 as a novel gene required for ciliary protein composition and Sonic hedgehog signaling

Basch, Kasey J.; Kong, Yong; Weatherbee, Scott D. (Yale University, New Haven, United States)

Cilia are dynamic organelles that are essential for a vast array of developmental patterning events, including left-right specification, neural development, and organogenesis. Despite recent advances in understanding cilia form and function, many key ciliogenesis factors have yet to be identified. Using a forward genetics approach, we isolated a novel mutant allele (schlei) of the mouse Transmembrane protein 107 (Tmem107) gene, which we show here is critical for cilia formation and embryonic patterning. Tmem107 is required for normal Sonic hedgehog (Shh) signaling in the neural tube and acts in combination with Gli2 and Gli3 to pattern ventral and intermediate neurons. schlei mutants also form extra digits, and we demonstrate that Tmem107 acts to determine digit number, but not identity, by regulating a subset of Shh target genes. Phenotypically, schlei mutants share several features with other cilia mutants; however, spatial restriction of mutant phenotypes in schlei suggests differential requirements for Tmem107 in cilia formation in distinct tissues. Also, in contrast to mutants with complete loss of cilia, schlei mutants retain some function of both Gli activator and repressor forms. Together, these studies provide unique insight into how ciliary factors affect Shh signaling and cilia biogenesis in distinct tissues. We further show that Tmem107 associates with the cis-Golgi, and that it is required for ciliary localization of several key cilia components including polycystin2 and Arl13b. Thus, our analysis of the schlei mutant phenotype not only identifies a previously unknown regulator of ciliogenesis, but also provides key insight into the factors responsible for determining the protein composition of the cilium.

Program/Abstract # 8

Towards a circuit-based understanding of the origins of a morphological novelty

Rebeiz, Mark; Pileggi, Rachel; Elliot, Chas; Glassford, William; Johnson, Winslow, University of Pittsburgh, United States

A major goal of evolutionary developmental biology is to understand how complex morphological structures arise. To obtain a stepwise view of this process, we must (1) map the regulatory network controlling the structure's development, and (2) elucidate the evolutionary history of each node of its network. The posterior lobe is a recently evolved genital formation, unique to the *D. melanogaster* clade that is a well-poised model system to study the genetic origins of morphological structures. This clade includes the model organism *D. melanogaster*, allowing the application of the full array of genetic and molecular tools of this system to elucidate the evolution of this newly derived organ. Here, we define roles for some of the major signaling pathways in posterior lobe development, and demonstrate how a conserved gene regulatory sequence has been co-opted to generate a new crucial role in the generation of this novel structure. These findings provide a foundation to unravel the origins of a recently derived developmental network.

Program/Abstract # 9

Robustness, evolvability and evolution of *Caenorhabditis* vulva development

Barkoulas, Michalis (École Normale Supérieure, CNRS-Inserm-ENS, Paris, France); Braendle, Christian (CNRS, Nice, France); Duveau, Fabien (École Normale Supérieure, CNRS-Inserm-ENS, Paris, France); Marie-Anne Felix, Université de Paris, France

The nematode vulva provides a model system to study the evolution of developmental processes. In *C. elegans*, the vulval precursor cells acquire specific cell fates, due to inductive signaling from the gonadal anchor cell and lateral signaling between vulval precursor cells. We measured the precision of vulval cell fate patterning in different environments and for different wild genotypes. The relative frequency ranges of specific variants correspond to measurable properties of the developmental system, such as sensitivity to the dose of the relevant signaling molecules. A key question regarding phenotypic evolution is the respective role of random genetic variation and natural selection. Natural selection plays an important role, yet a bias may already be provided by the phenotypic spectrum produced by random genetic variation. The relative evolvability (mutational variance) of the different vulval cell fate variants was measured in random mutation accumulation lines. Some vulva traits evolve faster than others upon random mutation, which explains observed evolutionary trends in the *Caenorhabditis* genus and beyond. Mutational effects may thus produce phenotypic trends, in the absence of selection. The buffering of the network output to environmental variation results in buffering to some genetic variation, allowing for evolution in the process without change in its output.We could reveal such cryptic evolution within *C. elegans* and among *Caenorhabditis* species. To explore the network quantitatively, we combined experimental studies with computational modeling. We showed that cryptic quantitative variation in the same network allows distinct modes of cell fate patterning and evolution among closely related species.

Program/Abstract # 10

Evolution of sex determination in animals that produce males, females and hermaphrodites

da Silva, André Pires; Kache, Vikas, University of Texas, Arlington, United States; von Reuss, Stephan (Cornell University, Ithaca, United States); Chaudhuri, Jyotiska; Bateson, Christine (U Texas, Arlington, United States)

The evolution of mating systems has fascinated biologists since the time of Darwin, specifically the causes and consequences of a species transition from one mating system (e.g. dioecy) to another (e.g. hermaphroditism). Theory predicts that these transitions likely involve one or more intermediates. To understand how animals transition from one mating system to another, we are studying the mechanisms by which a yet undescribed nematode generates male, female and hermaphrodite progeny. This is likely to represent a transitory system from the ancestral male/female to a hermaphroditic mode of reproduction.We found that the male /non-male decision in this nematode is chromosomally determined, whereas the hermaphrodite/female decision is epigenetic. We identified the molecular nature of a pheromone that alters the development of female-fated juveniles to develop into hermaphrodites. The study of the molecular mechanisms underlying sex determination in this and other closely species might shed some light in how mating systems evolve.

Program/Abstract # 11

Unraveling a transcriptional network involved in maize domestication

Doebley, John, University of Wisconsin, Madison, United States

Maize is a domesticated form of a wild Mexican grass called teosinte. The domestication of maize from teosinte occurred about 9,000 years ago. As a result of human (artificial) selection during the domestication process, dramatic changes in morphology arose such that maize no longer closely resembles its teosinte ancestor in ear and plant architecture. We have identified and analyzed three of the genes involved in these morphological changes. First, *teosinte branched* (*tb1*) is largely responsible for the difference between the long branches of teosinte versus the short branches of maize. *tb1* encodes a transcriptional regulator that functions as a repressor of branch elongation. Gene expression analysis indicates that the product of the teosinte allele of *tb1* accumulates at about half the level of the maize allele. Fine-mapping experiments show that the differences in phenotype and gene expression are controlled in part by an upstream transposon insertion that acts as an enhancer of gene expression. Second, *teosinte glume architecture* (*tga1*) is largely responsible for the formation of a casing that surrounds teosinte seeds but is lacking in maize. *tga1* also encodes a transcriptional regulator, however in this case a single amino acid change represents the functional difference between maize and teosinte. This single amino acid change appears to convert the maize allele into a transcriptional repressor of target genes. Third, *grassy tillers* (*gt1*) contributes to differences in plant architecture and encodes an HD-ZIP transcription factor. Causative changes at *gt1* appear to be complex, involving multiple changes. *tb1*, *tga1* and *gt1* are members of the same developmental network which regulates shade avoidance. This pathway was a target of human selection during the domestication process.

Program/Abstract # 12

Evolution of obligate heterodimerization among grass B class genes

Whipple, Clinton J.; Bartlett, Madelaine; Williams, Steven, Brigham Young University, Provo, United States

Homeotic regulation of floral organ identity, as described in the ABC model of floral development, is primarily controlled by MADS-box transcription factors. B class genes regulate second (petal) and third (stamen) whorl identities, and are represented by two paralogous gene lineages, DEF/AP3 and GLO/PI. Unlike other ABC MADS box proteins that can bind DNA as homodimers, B class proteins only bind DNA as obligate heterodimers. B class genes also undergo positive autoregulation, and it has been proposed that B class obligate heterodimerization evolved from sequential loss of the ability to homodimerize and that the unique combination of obligate heterodimerization with an autoregulatory feedback mechanism was important in the canalization of eudicot flowers. We have been examining B class function in the monocot model grass species maize (*Zea mays*). We have positionally cloned the *sterile tassel silky ear1* (*sts1*), which has a phenotype similar to other B class mutants in the grass family. *sts1* encodes one of three GLO/PI orthologs in maize, but shows no evidence of functional redundancy as it regulates transcription of the paralogous Zmm18 and Zmm29. Interestingly, maize B class proteins bind DNA as an obligate heterodimer, while B class proteins from close outgroups homodimerize. In order to understand the evolution of obligate heterodimerization in the grasses, we have isolated GLO/PI orthologs from diverse grasses and outgroups and assayed their dimerization specificity. Our results indicate that shifts to and from obligate heterodimerization are frequent, and controlled by a small number of amino acid changes. The possible developmental consequences of this evolution in protein binding will be discussed.

Program/Abstract # 13
Evolution and development in Lycophytes
Ambrose, Barbara A.; Smalls, Tynisha; Vasco, Alejandra, The New York Botanical Garden, New York, United States
Evolutionary developmental biology studies in lycophytes are integral to our understanding of land plant evolution and development as the lycophytes occupy a key phylogenetic position within the land plants as sister to all other vascular plants. In addition, lycophytes have interesting morphological structures, including microphylls, rhizophores and heterospory, whose evolution and development has been debated for more than a century. Compared to the flowering plants, lycophytes have not only simple sporophyte bodies but also simple genomes. Recent genome sequencing of the lycophyte, *Selaginella moellendorffii*, shows that there is no whole genome duplication characterizing this species. We are cloning and analyzing several key transcription factor families across the lycophytes as well as analyzing the expression of known key regulators in leaf and reproductive organ development from angiosperms in *S. moellendorffii*. The Type II MADS-box genes are well known for their role in flower development, and even though there are no orthologs ofthese floral MADS-box genes in the *S.moellendorffii* genome, the Selaginella Type II MADS-box genes are expressed in reproductive structures. Type I MADS box genes play a large role in gametophyte, embryo and seed development and our analyses suggest that these may be important for sporangia development in *S. moellendorffii*. We have also analyzed the leaf development network in *S. moellendorffii* and have found that a large number of the members of this network are present in the *S. moellendorffii* genome and are expressed during microphyll development. Our analyses provide insights into lycophyte evolution and development and highlight the need for a functional model system in the lycophytes.

Program/Abstract # 14
Development of a localized nervous system in a dipleurula-type larva.
Yankura, Kristen; Koechlein, Claire; Hughes, Stephanie; Hinman, Veronica Frances, Carnegie Mellon University, Pittsburgh, United States
The development of centralized nervous systems in many species of arthropods and chordates is extremely well characterized; however, very little is known about the formation of localized systems of neurons that are present in the vast diversity of animals. We provide an explanation for the development of a localized system of neurons that is associated with the ciliary bands of the dipleurula-type larvae of seastars. These larvae have two distinct ciliary bands, positioned above and below the mouth, that extend posteriorly and anteriorly along the dorsal surface and loop back to the ventral surface. Neurons associated with these bands are therefore located at multiple points along the anterior-posterior (AP) and thedorsal-ventral (DV) axes of the larva, suggesting that they cannot be specified by a simple integration of the AP/DV signaling system that is common to arthropods and chordates. Rather, we show that conserved Wnt, Nodal and BMP signaling pathways establish a pro-ciliary band territory that is needed to establish a neurogenic potential “edge” along which ciliary band associated neurons become specified. This constitutes a novel mechanism for neural specification in animals. We discuss how this mechanism for neural patterning contributes to an understanding of the evolution of centralized nervous systems in chordates.

Program/Abstract # 15
Evolution of development in the amniotes: New insights from genomic studies of somitogenesis in the lizard and alligator
Eckalbar, Walter L., Arizona State University, Tempe, United States; Elsey, Ruth (Louisiana Dept of Wildlife and Fisheries); Lasku, Eris (Arizona State U); Allen, April; Corneveaux, Jason (Translational Genomics Res Inst); DeNardo, Dale; Wilson-Rawls, Jeanne (Arizona State U); Huentelman, Matthew (Translational Genomics Res Inst); Rawls, Alan; Kusumi, Kenro (Arizona State U)
The segmented spine arises from the developmental process of somitogenesis, which is regulated through the cycling mechanism termed the ‘segmentation clock’. Major regulatory changes in this developmental clock have been associated with vertebrate evolution, but our ability to refine this analysis has been limited by major gaps, particular the lack of data from reptiles. We have extended this comparative analysis to two key reptilian taxa: a squamate, the green anole *Anolis carolinensis*, and an archosaurian reptile, the American alligator, *Alligator mississippiensis*. The newly sequenced genomes as well as our own deep sequencing of embryonic transcriptomes has provided intriguing data for analysis in the anole and alligator. Further, in situ hybridization was used to localize dynamic expression patterns. This analysis revealed key changes in segmentation clock gene expression, reflecting convergence and divergence of regulatory mechanisms within the amniotes. We found that cyclical genes in the clock were particularly divergent. This includes expression of lunatic fringe, which has cyclical expression in mouse and chicken, but not in the lizard or alligator, as well as delta like 1, which shares cyclical expression in the anole and mouse. Gradient and determination front components of the segmentation clock were generally more conserved, with the exception of hes6, which is expressed in a posterio rgradient in anoles, like

in zebrafish and *Xenopus*, but absent in the chicken or mouse. Data from squamate and archosaurian reptiles has markedly changed our view of the evolution of the segmentation clock inamniotes, pointing to rapid changes in the regulation of cycling genes relative to other genes in somitogenesis.

Program/Abstract # 16
Genomic control of morphogenesis in ciliated epithelia
Wallingford, John B., University of Texas, Austin, United States
Cilia have emerged recently as essential organelles in developmental, but most studies have focused on solitary, non-motile primary cilia. By contrast, multi-ciliated cells (MCC), which harbor many dozens of motile cilia play crucial roles in the development and homeostasis of the airway, brain, and reproductive tracts but remain poorly studied. Here, we will describe a novel model system for the study of mucociliary epithelia and a battery of approaches that integrate genomics, bioinformatics, in vivo time-lapse imaging, and experimental embryology to accelerate our understanding of MCCs. In particular, we will discuss the role of planar cell polarity (PCP) proteins in motile ciliogenesis and the transcriptional control of cilia structure and function. Finally, MCCs derive from basally-located p63+ progenitor cells, and newly born MCCs must migrate apically and insert into the epithelium during homeostasis and following injury. We will discuss genomic and cell biological mechanisms of MCC apical migration and insertion into the epithelium.

Program/Abstract # 17
Zebrafish Placenta-specific 8.1 (Plac8.1) links ubiquitination regulating protein Cops4 to motile cilia morphogenesis and function
Ma, Haiting, Washington University, St. Louis, United States; Li, Cunxi (Vanderbilt University, Nashville); Sepich, Diane (Washington U); Coffey, Robert (Vanderbilt U); Solnica-Krezel, Lilianna (Washington U)
Placenta-specific 8 (Plac8) proteins constitute a family of vertebrate-conserved proteins of elusive function. To investigate the function of Plac8 homologs in vertebrate development, we identified and studied zebrafish Plac8.1, a Plac8 homolog.With an anti-Plac8.1 antibody and immunofluorescence, we found that in multiple tissues. In mesenchymal cells Plac8.1 was located in cytoplasm and at the cell membrane, while in ciliated epithelia, Plac8.1 was concentrated at the apical cell membranes where cilia reside. To investigate the function of Plac8.1, we injected into embryos antisense morpholino oligonucleotides that could efficiently reduce protein levels of Plac8.1 resulting in morphologic features consistent with cilia defects: a ventrally curved body, left-right asymmetry defects, and kidney cysts. In Plac8.1 deficient embryos motile cilia numbers in the Kupffer's Vesicle (KV) and kidney ducts were significantly reduced, and cilia in kidney ducts were abnormally curled with detached membranes around the ciliary axonemes. Moreover, zebrafish embryos with deficient Plac8.1 also showed impaired beating of motile cilia in the KV, kidney, and the nasal pit. Finally, to investigate the molecular mechanism of how Plac8.1 regulates cilia morphogenesis and activity, we found that Plac8.1 bound Cops4, an integral component of the ubiquitination regulating complex COP9 signalosome (CSN). Co-injection of MOs targeting Plac8.1and Cops4 enhanced defects in cilia morphology and function. Collectively, our results identify Plac8.1 and Cops4 as new regulators of motile cilia, and suggest that ubiquitination modification might be involved in motile cilia morphology and function.

Program/Abstract # 18
From neural fate specification to neural plate patterning in Ascidian embryos.
Hudson, Claire, University of Paris, France
One of the defining features of the chordate body plan is the presence of a dorsal hollow central nervous system. We are studying the formation of this structure using embryos of the ascidian *Ciona intestinalis*. Ascidians are members of the urochordates, which forms a sister group to vertebrates. Their larvae exhibit a classical chordate body plan, but develop with very small cell numbers that adopt stereotypical cleavage patterns. We have been focusing on the formation of the posterior part of the larval CNS. I will describe the generation of these CNS lineages step-by-step, from the first division oriented along the animal-vegetal axis that generates the 8-cell stage embryo, to the patterning of the precisely aligned 44 cells of the neural plate. I will describe how at each step, signals of the canonical and non-canonical Wnt pathway, and ephrin-Eph and FGF/ERK pathways play critical roles in the generation of neural precursors. Subsequently, Nodal, Delta/Notch and FGF/ERK signals act sequentially on the neural plate such that each neural plate cell receives a different combination of these three signals and appear to be specified following Cartesian-like grid coordinates. Finally, I will show evidence that, despite their similarity in structure, the mechanisms governing patterning of the ascidian and vertebrate CNS appear to be quite distinct.

Program/Abstract # 19

Lethal giant larvae 2 functions in development of ciliated epithelia

Tay, Hwee Goon, SUNY Upstate Medical U, Albany, United States; Schulze, Sabrina (Max Delbrück Ctr for Molec Med, Germany); Compagnon, Julien (Inst of Science and Technology, Austria); Foley, Fiona (SUNY Upstate Medical U, United States)

Motile cilia perform critical functions during embryonic development. In the zebrafish embryo, lethal giant larvae 2 (lgl2) is expressed prominently in organs with motile cilia. Lgl was first described as atumor suppressor in *Drosophila*, and two homologs (Lgl1 and Lgl2) have been identified in vertebrates. Lgl proteins are involved in establishing cell polarity and have been implicated in vesicle trafficking. However, due to maternal contribution and/or functional redundancy between Lgl1 and Lgl2, functions for Lgl during early vertebrate embryonic development remain largely unknown. Using antisense morpholinos to deplete bothmaternal and zygotic Lgl2 in zebrafish, we have identified a role for Lgl2 in the development of ciliated epithelia in pronephric ducts of the kidney, otic vesicles that give rise to the inner ear and Kupffer's vesicle (KV) that orients left-right patterning of the embryo. Using KV as a model epithelium, we found that depletion of Lgl2 disrupted lumen formation and reduced cilia number and length. Consistent with these defects in KV development, left-right patterning was altered in Lgl2 deficient embryos. Immunostaining of KV cells in Lgl2 deficient embryos revealed a loss of the adherens junction component E-cadherin at lateral membranes. Genetic interaction experiments indicate Lgl2 cooperates with the small GTPase Rab11a to control E-cadherin and mediate lumen formation, potentially by regulating polarized vesicle trafficking. These results uncover new roles and interactions for Lgl2 that are critical for lumenogenesis and ciliogenesis.

Program/Abstract # 20

Genetic and genomic approaches demonstrate that multiple signaling pathways shape epithelial tubes

Boyle, Michael; Peters, Nathaniel; Zimmerman, Sandra; Altaras, Ariel; Thayer, Nathaniel; Tompa, Martin; Berg, Celeste, University of Washington, Seattle, United States

Tube formation is a highly conserved process that mediates the development of organs such as the heart, neural tube, and gut. Similar shape changes and rearrangements take place during ovary development in the fruit fly, *Drosophila*, when follicle cells that surround the oocyte reorganize from a flat sheet into a pair of tubes that produce the dorsal appendages (DAs), eggshell structures that facilitate air exchange for the future embryo. Using this exceedingly tractable model, we are investigating the mechanisms that control tubulogenesis. We identified mutants through genetic screens and characterized cell behaviors through fixed-tissue and live-image analyses. Mosaic studies demonstrate that Bullwinkle, a SOX transcription factor, regulates signals in the germ line, activating Shark and Src42A, non-receptor tyrosine kinases in the overlying "stretch" follicle cells. The stretch cells project cytoplasmic extensions toward the DA-forming cells, potentially guiding tube elongation. Surprisingly, trimeric G proteins are required only in the leading rows of DA-forming cells to enable migration over the stretch cells and to shape the entire tube. Tramtrack69, a zinc-finger repressor, also acts in these cells to regulate tube expansion. To identify the missing links in these three signaling pathways, we are using micro-array, mass spectrometry, RNA-seq, RNAi, and computational approaches. By studying conserved genes that regulate tube formation, our work illuminates the events that control organ formation in all animals, gives insight into common birth defects that occur when tube formation fails, such as spina bifida, and suggests molecular mechanisms that could transform stationary tumor cells into invasive cells.

Program/Abstract # 21

Phenotypic and molecular analyses of different vangl2 mutants demonstrates dominant effects of the Looptail mutation during hair cell development

Deans, Michael R.; Yin, Haifeng; Copley, Catherine, Johns Hopkins University, Baltimore, United States; Goodrich, Lisa (Harvard, Boston, United States)

Experiments utilizing the Looptail mutant mouse, which have craniorachischisis due to a missense mutation in vangl2, have been essential for developmental studies of planar polarity. As the name indicates, Looptail heterozygotes have distinctive looped tails suggesting that the mutation has a semi-dominant phenotype. However it is unclear how mutant Vangl2 protein exerts this effect because the mutant protein is unstable and is not delivered to the cell surface. We addressed this by comparing inner ear hair cell development of Looptail mice and vangl2 KO mice missing a large portion of the vangl2 gene, and by assaying molecular interactions between mutant Vangl2 and normal proteins in vitro and in vivo. Overall the vangl2 KO phenotype is milder than compound mutants with both the Looptail and vangl2 KO alleles. In addition, the protein encoded by the Looptail mutation (Vangl2S464N) disrupts Vangl1 and Vangl2 delivery to the surface of cells in vitro due to oligomer formation between Vangl1 and Vangl2 coupled with the intracellular retention of Vangl2S464N. As a result, Vangl1 protein is missing from the apical cell surface of vestibular hair cells in Looptail

mutants, but is retained at the cell surface in vangl2 KOs. Similarly the distribution of Prickle-like2, a putative Vangl2 interacting protein, is differentially affected in the two lines. We propose that altered Vangl2S464N trafficking prevents the delivery of multiple polarity proteins to the cell surface and that this net effect underlies the dominant phenotypic traits associated with the Looptail mutation. One interpretation is that prior genetic interactions with Looptail may be indirect and reflect a permissive enhancement of this semi-dominant phenotype.

Program/Abstract # 22
miRNA-mediated regulation of shoot maturation in plants
Yang, Li; Willmann, Matthew; Park, Mee Yeon; Wu, Gang; Poethig, Scott, University of Pennsylvania, Philadelphia, United States

A plant shoot changes with time. Some changes occur quickly and are associated with major alterations in shoot architecture, whereas others occur gradually and may have no obvious morphological manifestation. Microarray analysis of gene expression in shoot apices, leaf primordia, and fully-expanded leaves of early (FRI flc-3) and late-flowering (FRI FLC) genotypes of Arabidopsis revealed six major temporal programs. Three of these programs are features of leaf development (leaf maturation, leaf aging, leaf senescence), and three involve changes in the character of the entire shoot (vegetative phase change, flower induction,aging). We are particularly interested in the mechanism of vegetative phase change—the transition from a juvenile toan adult phase of shoot development. This process is regulated by a decrease in the expression of two related microRNAs miR156 and miR157, which act to promote the expression of the juvenile phase. Ablation studies reveal that this decline is mediated by a factor(s) produced by existing leaf primordia, which acts on newly formed primordia. Evidence indicating that this factor is a sugar will be presented.

Program/Abstract # 24
The recruitment of poised Pol II is regulated over developmental time
Gaertner, Bjoern; Chen, Kai; Shao, Wanqing; Meier, Sam; Johnston, Jeff ; Zeitlinger, Julia, Stowers Inst for Medical Research, Kansas City, United States

Poised RNA polymerase II (Pol II) is predominantly found at developmental control genes and is thought to allow their rapid and synchronous induction in response to extracellular signals. However, whether the recruitment of poised RNA Pol II is itself regulated during development has not been explored. We have analyzed the genome-wide pattern of poised Pol II during the activation of zygotic transcription in early *Drosophila* embryos, as well as by isolating muscle tissue at five stages of differentiation. We show that the recruitment of poised Pol II is predominantly established during the second wave of zygotic genome activation, also known as midblastula transition. During differentiation, many more genes acquire poised Pol II over developmental time and this recruitment is associated with changes in chromatin. Furthermore, the genome-wide pattern of poised Pol II helps predict future gene activation in a stage-specific but not tissue-specific fashion. We conclude that the recruitment of poised Pol II is a checkpoint for developmental timing.

Program/Abstract # 25
Understanding and predicting cis-regulatory activity
Furlong, Eileen, EMBL-Heidelberg, Germany

The precise regulation of gene expression is crucial for almost all biological processes. In development, spatio-temporal patterns of gene expression are controlled by extensive regulatory networks, where the activity of transcription factors converge on cis-regulatory modules (CRMs) or enhancer elements. The location and even combinatorial occupancy of CRMs can be experimentally measured using ChIP-seq at specific stages of development, at high-resolution. A current major challenge however, is how to interpret these transcription factor's binding data in terms of the resulting spatio-temporal enhancer activity. Using the integration of a machine learning approach with enhancers of known activity, we recently demonstrated that transcription factor occupancy alone is sufficient to predict enhancer spatio-temporal activity during development. We have now complemented this by generating cell-type specific information on chromatin state within the context of a developing embryo using a new method that we developed called Batch Tissue Specific ChIP(BiTS-ChIP). The data reveal heterogeneous combinations of chromatin marks linked to active enhancers. Using a Bayesian network, we show that chromatin state is sufficient to predict, not just the location, but activity state of regulatory elements, accurately distinguishing between enhancers in an active versus inactive state. The model revealed that Pol II occupancy is highly predictive for the precise timing of enhancer activity and is tightly correlated with both the timing and location of transcription factor occupancy. Taken together, this approach provides a systematic and high-resolution view of dynamic enhancer usage during development, and essential step toward deciphering developmental networks.

Program/Abstract # 26
On the combinatorial function of multiple cis-regulatory modules
Nam, Jongmin, CALTECH, Pasadena, United States
Cis-regulatory modules (CRMs) for gene expression control are modular in the sense that they can activate basal promoters from other genes in small reporter constructs. We have learned from decades of studies that, although only a small fraction of CRMs have been identified in the metazoan genomes, CRMs outnumber genes in the genome and that multiple CRMs control expression of a gene. Two alternative models for how multiple CRMs control gene expression prevail, each with supporting experimental data .However, little is known about the relative prevalence of the two models. In the first model different CRMs control gene expression differentially in time and space, and there is no overlapping activity between CRMs; any changes in CRM activity will result in coherent gene expression changes. In the second model multiple CRMs function in a combinatorial manner. In this model, a group of activator CRMs and/or repressor CRMs function together, and overlapping activities between CRMs may happen; changes in CRM activity may not result in coherent gene expression changes. Using high-resolution temporal activity profiles of 47 genes and their 126 CRMs in developing and perturbed sea urchin embryos, I tested the two alternative models of CRM function. The results predominantly supported the second model: Overlapping activities between CRMs located around/within the same gene were common and incoherent responses between genes and their CRMs to the same perturbation were prevalent. Functional and evolutionary significance of this finding will be discussed.

Program/Abstract # 27
Identifying regulators of early differentiation and primary germ layer induction
Oron, Efrat, Yale University, New Haven, United States; Wu, Jiaqian; Snyder, Michael (Stanford University, United States); Ivanova, Natalia (Yale University, New Haven, United States)
Embryonic stem (ES) cell research lies at the interface between developmental biology and medicine. Fundamental questions such as how do cells become restricted in their developmental potential and differentiate giving rise to all the cells and tissues that make up an organism will ultimately help us learn how to efficiently manipulate ES cells grown in culture for therapeutic use. Among the first processes of differentiation is specification of the primary germ layers: Endoderm, Mesoderm and Ectoderm. Using an in vitro ES cell differentiation system combined with a functional genomics approach we identified genes required for ES cell differentiation and build a simplistic model of their transcription regulation hierarchy during germ layer induction. To identify genes that are important for early differentiation we generated RNAseq mRNA time course profiles from mouse embryos sampled at five developmental timepoints, and ES cells differentiated as embryoid bodies (EBs) for eleven days. Comparative analysis of expression from embryo vs. cell lines was used to identify matching developmental time windows between mouse embryos and ES cell lines and to further select a panel of 160 candidate transcription factors with expression patterns suggestive of an involvement in differentiation. Candidate genes were individually inactivated in ES cells by lentiviral shRNAs. shRNA-expressing cells were differentiated as EBs and evaluated for primary germ layers induction using a panel of markers. 44 genes were found to affect differentiation to at least one of the three primary germ layers and a simplistic model of transcription regulation hierarchy during germ layer differentiation is suggested based on germlayer-specific markers.

Program/Abstract # 28
Good at being bad: Counterintuitive genomic responses to developmental signals via low-affinity transcription factor binding sites.
Barolo, Scott, U Michigan Med Sch, Ann Arbor, United States
Signaling pathways such as Hedgehog, RTK/MAPK, Notch, BMP, and Wnt relay patterning information to transcription factors (TFs), which in turn control developmental cell fate by regulating gene expression. Because these pathways are extremely pleiotropic— that is, they are active in many cell types during development and adult life—we are particularly interested in how cis-regulatory DNA sequences interpret these "generic" signals in a tissue-specific manner. By altering the affinity of signal response elements in vivo, we have discovered important, sometimes surprising roles for low-affinity, non-consensus binding sites for signal-regulated TFs in the regulation of wingless, dpp (a BMP ligand), Pax2, and patched. In certain enhancers of these genes, weak binding is specifically required for proper responses to Hedgehog or Notch signaling; improving binding affinity can either cause ectopic responses to a signal or, unexpectedly, switch the response from activation to direct repression. The regulation of the patched gene is particularly interesting: what first appeared to be a simple constitutive response to Hedgehog in all tissues is in fact mediated by a large number of enhancers, all of which respond to the same signal, but in different developing tissues and adult stem cell systems—including large numbers of "shadow" enhancers in multiple tissue types. Our preliminary data suggest fascinating new mechanisms by which the patched locus has solved the patterning problem of responding to a single generic signal across diverse tissues. I will

address the implications of our findings for genomics-based and computational approaches to understanding transcriptional networks.

Program/Abstract # 29
Maternal mRNA retention as a mechanism for maintaining totipotency in primordial germ cells
Swartz, S. Zachary, Brown University, Providence, United States; Raz, Tal; Milos, Patrice (Helicos Biosciences); Hamdoun, Amro (Scripps Inst of Oceanography); Wessel, Gary (Brown U)
The germ line contains the stem cells responsible for transmitting all heritable information between generations. Unlike the soma, which lacks reproductive potential and will ultimately die, germ line stem cells (GSCs) retain the capacity for totipotency and create the sperm and eggs necessary for developing a new organism. A widely conserved strategy for segregating primordial germ cells (PGCs) involves maternally loaded and spatially localized determinants, often called a "germ plasm," which direct the cells that inherit it toward GSC fate. Our investigations have not revealed a localized germ plasm in the early embryo of the sea urchin *S. purpuratus*. However, several mRNAs commonly found in germ plasm are maternally loaded and ubiquitously distributed in the early embryo, followed by later restriction to the PGCs. To better understand the initial segregation of PGCs in the sea urchin, we performed deep sequencing and differential expression analysis of FACS isolated PGCs. We identified a set of genes whose mRNAs are ubiquitous in eggs and early embryos, but are later restricted to the PGCs. This suggests a general post-transcriptional mechanism that retains mRNAs in the PGCs, and degrades them in somatic cells. The 3'UTRs of these mRNAs contain shared sequence motifs which may accelerate their destruction in somatic cells. In addition, we found that the CCR4-NOT deadenylase complex member Cnot6, a poly-A nuclease, is absent in the PGCs but present in all other cells of the embryo. This observation suggests a mechanism for the stabilization of germ line mRNAs in the PGCs but turnover elsewhere. Finally, the 3'UTR of Cnot6 contains three putative Nanos response elements (NREs), which may be targeted for destruction by Pumilio and its partner Nanos, which is selectively expressed in the PGCs. Through this work we are uncovering a new mechanism by which PGCs are protected from differentiation by the retention of mRNAs of the early totipotent embryo.

Program/Abstract # 30
Specification of the proximodistal axis by Irx3 and Irx5 homeobox genes prior to limb bud initiation
Hui, Chi-Chung (Hosp Sick Children, Toronto, Canada)
Pattern formation requires coordination of growth in three dimensions. The anteroposterior (AP) and proximodistal (PD) axes of the developing limb are linked when Shh from the zone of polarizing activity (ZPA) and Fgfs from the apical ectodermal ridge (AER) form a positive feedback loop in the limb bud. It is unknown whether the AP and PD axes are coordinated before limb bud formation. In this study, we show that both axes are regulated by Iroquois homeobox (Irx) genes Irx3 and Irx5 prior to the establishment of Shh and Fgf8 signaling centers. Through interactions with Gli3, Irx3 and Irx5 regulate AP prepattern as well as PD specification of Shh-independent progenitors in the hindlimb. These findings suggest that AP and PD limb axes are coordinated among limb progenitors prior to outgrowth. Our data also provide the first genetic evidence in support of the early specification of PD pattern.

Program/Abstract # 31
Identifying Hox targets by transcriptional profiling of the mouse hindbrain
Yurieva, Marina; De Kumar, Bony; Krumlauf, Robb, Stowers Institute for Medical Research, Kansas City, United States
The vertebrate hindbrain forms the medulla, pons and cerebellum which play a crucial role in regulating functions such as sleep, respiration and heart rate. During development regional diversity in hindbrain is established through the segmentation of the neural tube into seven morphological discrete domains termed rhombomeres. A network of transcription factors including Hoxa1, Hoxa2, Hoxb1 and Krox20 are involved in establishing the segmental cellular organization critical for hindbrain function. Genetics studies in a number of vertebrates have shown that mutations in these regulatory genes cause dramatic changes in segmentation and rhombomere identity, resulting in severe neuronal defects and lethality in adult animals. Our research has utilized transcriptional profiling and functional validation to identify and characterize rhombomeric-specific patterns of gene expression regulated by these key transcription factors. Using laser capture microscopy to isolate individual rhombomeres from 9.5 dpc mouse embryos, we performed transcriptional profiling on either single rhombomeres from the hindbrains of wild type embryos or whole hindbrains of wild type and Hoxa1, Hoxa2, Hoxb1 and Krox20 mutant embryos. Computational analyses and validation of these results have uncovered several novel downstream targets and pathways associated with their roles in hindbrain specification. For example, Hox genes which are induced by retinoids in turn modulate multiple aspects of retinoid metabolism and catabolism. These feedback loops are important for hindbrain patterning.

Program/Abstract # 32

Imaging *in-vivo* nerve-Schwann cell interactions during peripheral nerve regeneration

Rosenberg, Ali; Granato, Michael, University of Pennsylvania, Philadelphia, United States

Following injury, the distal portion of a peripheral nerve undergoes an active process of self destruction, followed by axonal regrowth. During axon regrowth Schwann cells at the injury site are thought to act as a physical bridge for regrowing axons, secrete extracellular matrix components that serve as a substrate for regrowing axons, and secrete trophic factors and attractive signals that guide injured axons across the injury site. Despite the significance of these proposed roles, when and how Schwann cells interact with the injured and regenerating nerve remains elusive, mainly due to the difficulties of continuously imaging the process of nerve regeneration in live, intact vertebrate animals. We have established a zebrafish model to define the cellular interactions between injured peripheral nerves and Schwann cells at minute level resolution, in vivo (Rosenberg et al 2012, J Neuroscience). Previous studies have suggested an initial Schwann cell independent phase of axonal sprouting followed by secondary, Schwann cell dependent phase of axonal growth. We find that upon genetic ablation of Schwann cells axons extend from the proximal stump. However, these axons extend along aberrant trajectories and fail to navigate towards their original targets. Thus, Schwann cells are dispensable for axonal sprouting but are essential for target-directed axonal regrowth. We are now using cell ablation and genetic mutants to determine if Schwann cells provide cell contact dependent support and/or contact independent, diffusible signals to guide motor axons. Ongoing analysis of these experiments to characterize the precise role of Schwann cells in axon regeneration will be presented.

Program/Abstract # 33

Dynamic filopodia transmit long-range Shh signaling during tissue patterning

Barna, Maria; Martin, Esther Llagostera; Sanders, Timothy, UC San Francisco, United States

The movement of key signaling proteins within tissues and organs is a central feature of metazoan development that must be exquisitely spatially controlled. How this is achieved at a cellular level remains poorly understood. Here we constructed a robust, state-of-the-art imaging system that allows for visualization of signaling at single cell resolution under endogenous spatial and temporal control within living vertebrate embryos. Through this imaging approach, we identified that genetically defined populations of mesenchymal cells involved in Sonic Hedgehog (Shh) signaling within the vertebrate limb bud possess a novel specialized class of actin-based filopodia spanning several cell diameters that have not been previously described. By imaging Shh responding cells in real time, we visualized an exquisite distribution and co-localization of the Shh co-receptors to discrete micro-domains along the membrane of these filopodia extensions, suggesting a functional role in facilitating long-range signaling. We therefore developed a tightly regulated expression system directed by the Shh limb-specific enhancer element to visualize Shh production in vivo. Remarkably, imaging Shh ligand itself revealed that it is normally produced in the form of a particle that dynamically moves along filopodia extensions and accumulates at their tips, which make stabilized contacts with responding cells that contain Shh co-receptors. Strikingly, these stabilized interactions are associated with a dramatic accumulation of Shh to the primary cilium of responding cells and activation of the pathway. To our knowledge, this is the first in vivo demonstration of Shh ligand production and movement. These findings strongly suggest that contact mediated release propagated by specialized filopodia contributes to the delivery and activation of Shh signaling at a distance. Together, these studies identify a new mode of communication between cells that extends our understanding of long-range signaling during vertebrate tissue patterning.

Program/Abstract # 34

mef2cb regulates late myocardial cell addition from a second heart field-like population of progenitors in zebrafish

Lazic, Savo ; Scott, Ian, University of Toronto, Canada)

Two populations of cells, termed the first and second heart field, drive heart growth during chick and mouse development. The zebrafish has become a powerful model for vertebrate heart development, partly due to the evolutionary conservation of heart development pathways. Here we provide evidence that the zebrafish possesses an evolutionary conserved homolog to the murine second heart field. We developed a photoconversion assay to quantify the dynamic and gradual late addition of SHF cells to the zebrafish arterial pole. We further show that SHF cells are derived from the same pre-gastrula embryonic region as early myocardial cells. We identify an extra-cardiac region immediately posterior to the arterial pole as the zebrafish SHF. The zebrafish SHF has cardiogenic properties, expressing myocardial markers such as vmhc and nkx2.5, but does not express a full complement of differentiated cardiomyocyte markers, lacking myl7 expression. We show that mef2cb, a zebrafish homolog of the mouse second heart field marker Mef2c, is expressed in the zebrafish SHF, and is necessary for late myocardial addition to the arterial pole. Ongoing studies aim to characterize the SHF further and to investigate its role in atrium growth. Our study demonstrates that zebrafish heart growth shows more similarities to

murine heart growth than previously thought. Further, as congenital heart disease is often associated with defects in second heart field development, the embryological and genetic advantages of the zebrafish model can be applied to study the vertebrate second heart field.

Program/Abstract # 36

Live imaging of hematopoietic niche colonization reveals distinct endothelial and stem cell interactions

Tamplin, Owen J.; Durand, Ellen; Lawson, Katy; Li, Pulin; Zon, Leonard, Children's Hospital/ Harvad, Boston, United States

Hematopoietic stem and progenitor cells (HSPCs) self-renew and give rise to all blood cell types throughout adulthood. Definitive HSPCs arise from the hemogenic endothelium of the dorsal aorta, are released into circulation, and then seed an intermediate hematopoietic tissue before colonizing the adult marrow. In mammals this intermediate tissue is the fetal liver and in the zebrafish it is the caudal hematopoietic tissue (CHT), a vascular plexus in the ventral tail of the embryo. We created an HSPC-specific transgenic reporter line using the previously described mouse Runx1 +23 kb intronic enhancer. Together with an endothelial reporter (flk1:dsRed), we could use time-lapse live imaging to follow HSPCs as they migrate to the CHT. Upon arrival, HSPCs underwent a number of distinct steps to engraftment, including adherence to the vessel wall, extravasation, and triggering of niche formation—endothelial cells actually remodel around the HSPC to create a niche. To determine if this endothelial niche formation is conserved in mammals, we performed live imaging of mouse fetal liver explants at embryonic day 11.5, the earliest stage of seeding by HSPCs. Strikingly, we observed CD31+ endothelial cells adhere to and form a rosette around single c-kit+ HSPCs, similar to the cellular behaviors observed in zebrafish. To find clues to the molecular mechanisms that regulate these distinct cellular behaviors during hematopoietic niche colonization we performed a chemical genetic screen. We found compounds that both increased and decreased CHT hematopoiesis. We are now applying these chemical hits during live imaging of CHT colonization and are gaining important insights into stem cell engraftment and hematopoietic niche formation.

Program/Abstract # 37

Neuronal guidance cues direct early blood vessel formation

Meadows, Stryder M.; Fletcher, Peter, UT Southwestern Med Ctr, Dallas, United States; Moran, Carlos (U Arizona, Tucson, United States); Ratliff, Lyndsay; Xu, Ke (UT Southwestern Med Ctr, Dallas, United States); Neufeld, Gera (Haifa, Israel); Chauvet, Sophie; Mann, Fanny (Marseille, France); Krieg, Paul (U Arizona, Tucson, United States); Cleaver, Ondine (UT Southwestern Med Ctr, Dallas, United States)

Neuronal guidance molecules are known to influence endothelial cell (EC) behavior. These molecules shape the vasculature by acting as attractive or repulsive signals; however, it is unclear if these molecules affect patterning of the initial blood vessel network. Our analysis of neural guidance cues during embryonic mouse development indicates that multiple repulsive cues are present in the notochord. We hypothesize that overlapping sets of repulsive guidance cues expressed in the notochord direct EC migration and patterning of the first blood vessels that form in the mammalian embryo, the paired dorsal aortae (DA). We have analyzed mutant mice embryos lacking a notochord and similar to previous studies in avian embryos, we observed dramatic vascular abnormalities characterized by scattered aortic ECs that fail to form vessels. In these embryos, all repulsive guidance cues are lost at the midline and ECs crossed the normally avascular midline. Furthermore, we identify a single repulsive guidance cue, Semaphorin 3E (Sema3E), expressed from the lateral plate mesoderm that creates avascular zones which define the lateral edges of the DA. Rather than a single smooth vessel, Sema3E null embryos display a tree-like plexus of aortic vessels with ‘branches’extending into the lateral avascular spaces. Interestingly, despite such a severe phenotype, Sema3E-/-mice survive throughout adulthood. We find that these defective vessels resolve back into normal DA during subsequent development, likely due to additional, undetermined repulsive guidance cues. Overall, these studies demonstrate how multiple, functionally redundant and non-redundant repulsive cues work coordinately to shape the early embryonic blood vessels.

Program/Abstract # 38

Novel role for an Aquaporin gene in neural tube closure

Van Antwerp, Daniel; Weber, Mackenzie; Merzdorf, Christa, Montana State University, Bozeman, United States

As a result of a micro array screen for genes regulated by the Zic family of transcription factors in *Xenopus laevis*, we identified a membrane water channel, or aquaporin protein, which we named aquaporin 3b (aqp3b). Characterizing aqp3b expression through embryogenesis showed that it localized very specifically to a narrow column of cells at the edges of the closing neural plate. As zic genes play a role in neural tube closure, we tested whether aqp3b is involved as a downstream effector of this process. Microinjection of aqp3b morpholino oligos suggested that this gene is important for proper neural tube closure. Further analysis of the cellular identity of aqp3b expression has been aided by detailed, yet decades old, light

and electron microscopy studies of *Xenopus* neural tube closure. In these studies, Thomas Schroeder identified a cell positioned at the interface between the converging epidermal layer and invaginating neural epithelium. Termed the "IS cell", for intermediate superficial cell, it has characteristics of both epidermal and neural epithelial cells, allowing a transitional zone between these diverse cell types. We hypothesize that aqp3b is specifically expressed in IS cells and suggest that IS cells and their expression of aqp3b are required for proper closure of the neural tube. IS cells do not themselves undergo apical constriction, but, upon aqp3b inhibition, we observe a failure of apical constriction in cells that normally form the dorsal-lateral hingepoints. The possibility of intercellular action of IS cells on the neighboring hinge point cells has not been previously predicted. We are attempting to broaden these results to other species and to define the mechanism by which IS cells and aqp3b participate in neural tube morphogenesis.

Program/Abstract # 39
Maintenance of axial patterning in the sea urchin embryo: A role of Wnt1 signaling
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The initial patterning along the anterior-posterior (AP) and dorsal-ventral (DV) axes of sea urchin embryos depends on Wnt and Nodal signaling, respectively, and occurs before gastrulation. The secondary DV axial patterning relies on a Wnt-dependent process that removes a suppressor of nodal expression from non-anterior ectoderm. Here we report that unexpectedly, later, when gastrulation begins, Wnt signaling continues to affect Nodal signaling, not by supporting it, but rather by preventing nodal expression in the ventral-posterior region of the embryo. This region normally gives rise to the posterior-transverse ciliary band, the supra-anal ectoderm and ventral endoderm. When Wnt1 is knocked down, expression of nodal and its target genes, gsc and bra, extends ectopically on the ventral side toward the blastopore. As a consequence, initial fates of cells in this region are changed to oral ectoderm, as shown by lineage tracing. Strikingly, the ciliary band, which forms adjacent to the nodal expression domain, is shifted significantly in the dorsal direction, toward and sometimes beyond the position of the blastopore. This results in the blastopore and stomodeal regions being positioned in the same ventral plane instead of approximately at a 90° angle. Before gastrulation, wnt1 expression is radial in posterior blastomeres. But when gastrulation begins, it is lost from the dorsal side by a Nodal-dependent process and maintained only on the ventral side, where it suppresses nodal. Thus, Wnt- and Nodal-dependent processes mutually antagonize each other to maintain the body plan established at earlier stages by these same pathways.

Program/Abstract # 40
Membrane morphogenesis during tracheal tube development in *Drosophila*
Jayan Nandanan, N; Mathew, Renjith (EMBL, Heidelberg, Germany); Leptin, Maria, EMBO, Germany

The terminal cells of the *Drosophila* respiratory (tracheal) network contain seamless, membrane bounded intracellular tubes through which air travels. The tube-containing extensions of these cells are elaborated during chemotactic growth directed by developmental cues in embryos and subsequently ramify in response to signals from hypoxic tissues in larvae. Extensive membrane traffic occurs during growth, as the cell rapidly and concomitantly elaborates two membrane domains of opposite characteristics. The outer (basal) membrane migrates and grows with highly dynamic filopodial extensions, while at the same time the inner tube of apical characteristics is constructed from as yet unknown membrane sources. We find that the membranes forming the intracellular tubules contain lipids and proteins typical of apical plasma membranes in polarized epithelial cells. The *Drosophila* synaptotagmin-like protein Bitesize (Btsz) and the activated form of its interaction partner Moesin are also located at the growing luminal membrane. Our functional studies indicate that the actin cytoskeleton, through its interaction with Btsz via Moesin directs apical membrane morphogenesis to create and maintain distinct intracellular tubules. Using real-time in vivo imaging we have analysed the assembly of the intracellular tube. We find that ER and Golgi rapidly distribute into the developing cellular extensions prior to the assembly of the membrane bounded tube within. Redistribution of secretory machinery ahead of the growing tube seems to be a prerequisite for proper tube extension.

Program/Abstract # 41
Asymmetric division of luminal cells produces low-polarity high-motility cells that collectively migrate to form mammary ducts
Huebner, Robert J., Johns Hopkins Sch of Med, Baltimore, United States; Lechler, Terry (Duke, Durham, United States); Ewald, Andrew (Johns Hopkins Sch of Med, Baltimore, United States)

We seek to understand the cellular and molecular mechanisms that underlie branching morphogenesis. To overcome the limitations of optical imaging in the intact mouse, we rely on organotypic cultures of primary mammary epithelium. Using 3D time-lapse imaging and transgenic fluorescent reporters, we have resolved cell migration, proliferation, and tight junction dynamics throughout branching morphogenesis. The initial stage of morphogenesis involves a growth factor

dependent transition from a bilayered to a stratified epithelium. Using inducible genetic systems, we have shown that activation of Rac was sufficient for stratification but not branching. However, constitutively active MEK was sufficient to induce initiation of new ducts in the absence of growth factors. We have shown that stratification involves the creation of unpolarized, motile interior cells between the apical and basal cell layers. We observe generation of interior cells through polarity breaking divisions of luminal epithelial cells. New duct initiation involves coordinated migration of these interior cells, but can occur in the absence of proliferation. A major conceptual challenge for this collective migration is the presence of intercellular junctions. Imaging of ZO1-GFP revealed the lumen is maintained throughout morphogenesis, but that this luminal space is dynamic. We observed fusion and fission of ZO1-GFP domains along with de novo formation of secondary lumens. Our data suggest that mammary morphogenesis begins with a proliferation-driven establishment of unpolarized migratory cells, which then migrate to elaborate the epithelial network, and repolarize through the de novo formation of tight junction lined luminal spaces.

Program/Abstract # 42
Regulation of early lineages in the mouse embryo
Manzanares, Miguel, Centro Nacional de Investigaciones Cardiovasculares, Spain

To better understand how gene regulatory networks act in the mammalian blastocyst to define the first embryonic lineages, we are searching for cis-regulatory sequences that control the spatial and temporal expression of genes in the network. In this way we aim to identify the mechanisms by which different stem cell populations at the blastocyst stage are specified and maintained, and which is the regulatory link between early stochastic expression and late maintenance of core transcription factors in the network. To do so, we are using a comparative genomic approach combined with transient transgenesis to find regulatory elements important in the transcriptional network responsible for lineage determination. Once putative elements are identified, their capacities are tested in vivo in mouse embryos by examining their ability to drive lineage-restricted expression of a reporter gene. We are also taking advantage of blastocyst derived stem cells –both trophoblast (TS) and embryonic (ES) stem cells– to find the specific signaling inputs and roles of the regulatory elements characterized in a tissue culture assay. Using this approach, we are studying the regulation of the key trophectoderm transcription factor Cdx2, and have found that specific cis-regulatory elements are involved in different aspects of its expression in the trophectoderm of the blastocyst and in trophoblast-derived stem cells.

Program/Abstract # 43
Investigating body axis extension in the mouse embryo using a single-cell resolution fluorescent Wnt reporter
Ferrer Vaquer, Anna; Tian, Guangnan; Hadjantonakis, Anna-Katerina, Sloan-Kettering Institute, New York, United States

Elongation of the mouse anterior-posterior axis depends on the sequential addition of cells to the caudal end of the embryo. Progenitors residing within the tailbud region fuel the production of cells contributing to the developing somites, neural tube and notochord. Detailed lineage analysis and fate-mapping studies have identified several subdomains of progenitors residing within the node, primitive streak and caudal lateral epiblast (CLE) each of which exhibit different developmental potentials. Notably, progenitors residing at the node-streak border (NSB) represent true multipotent stem cells, since they are able to give rise to neural ectoderm as well as axial and paraxial mesoderm. Interestingly, this NSB subdomain displays high levels of TCF/Lef:H2B-GFP, a fluorescent canonical Wnt signaling reporter we recently generated. Taking advantage of the brightness and single-cell resolution afforded by this reporter we microdissected the various regions of the caudal part of the embryo at E8.5 (8-10 somite stage). Including the ventral part of the NSB, which has been proposed to act as a niche for neuromesodermal progenitors located at and around the dorsal NSB. Gene expression profiling of the ventral NSB region and comparison with the neighboring CLE and paraxial mesoderm will help decipher the mechanisms that regulate behavior of these multipotent axial progenitors. We have also generated a TCF/Lef:CreERT2 strain, representing a new inducible Cre recombinase driver line under the regulation of canonical Wnt-responsive elements. The TCF/Lef:CreERT2 strain should allow us to fate map and genetically ablate the NSB region, as well as conditionally modulate the levels of ß-Catenin to further characterize the role of the NSB in axial elongation.

Program/Abstract # 44
Claudin-10 functions on the right side of Hensen's node to direct left-right patterning
Collins, Michelle M.; Ryan, Aimee (McGill Unversity, Montreal, Canada

In vertebrates, organs must be asymmetrically positioned within the body cavity to allow for normal physiological function. The origin of this asymmetry is initiated during gastrulation in an evolutionarily conserved molecular cascade. We have identified a novel molecule, Claudin-10, that plays a role in directing asymmetric organ positioning in the chick. Claudins are integral components of tight junctions, which act to restrict movement of ions and small molecular within the

paracellular space, and interact with the actin cytoskeleton via adaptor and scaffolding proteins. Here, we report that Claudin-10 mRNA is asymmetrically expressed on the right side of Hensen's node, the site where the left-right patterning cascade is initiated. We demonstrate that overexpression of Claudin-10 on the left side of the node, or knockdown of endogenous Claudin-10 on the right side of the node, randomizes the direction of heart-looping, the earliest morphological sign of disrupted left-right patterning. Furthermore, expression of classic left-right patterning genes Pitx2c and cSnR show altered expressed in manipulated embryos. Our data also show that the PDZ-binding domain of Claudin-10 is required for its function at the node. These data suggest that asymmetric expression of Claudin-10 at Hensen's node is required for normal patterning of the left-right axis.

Program/Abstract # 45
The Development and Evolution of Animal Epithelial Barriers
Juarez, Michelle; Kim, Myungjin; Pare, Adam; Patterson, Rachel; McGinnis, Bill, UC San Diego, United States
The Grainy head (GRH) family of transcription factors are crucial for epidermal-barrier development and regeneration in most or all animals. An important question in regeneration is how transcription factors that program the normal development and differentiation of tissues are functionally reactivated after body parts are lost or wounded. We found that GRH is modified by Extracellular signal-Regulated Kinase (ERK) phosphorylation, and this modification is required for GRH function in the regeneration of an epidermal barrier. However, GRH with mutant ERK phosphorylation sites can still promote barrier formation during embryonic epidermal development, suggesting that ERK sites are dispensable for the GRH function in normal development of epidermal barriers. These results provide mechanistic insight into how epidermal regeneration can be initiated by post-translational modificationof a key transcription factor that normally mediates the developmental generation of that tissue. Interestingly, proteins in the GRH family are also found in many species of fungi, organisms that lack epidermal tissues. We show that the Neurospora GRH-like proteinhas a DNA-binding specificity similar to the animal GRH family proteins. Analysis of the phenotype of Neurospora grhl mutants and the transcriptome of *Drosophila* grh and Neurospora grhl mutants suggest the fascinating possibility that the apical extracellular barriers of some animals share an evolutionary connection with the cell wall of the animal-fungal ancestor, and that the formation of this ancestral physical barrier was under the control of a transcriptional code that included GRH-like proteins.

Program/Abstract # 46
Evolution of Dact gene family
Sobreira, Debora R., Univ Estadual de Campinas, Brazil; Dietrich, Susanne (Portsmouth, UK); Janousek, Ricardo (Univ Estadual de Campinas, Brazil); Schubert, Frank (Portsmouth, UK); Alvares, Lucia (Univ Estadual de Campinas, Brazil)
Dact genes form a small gene family of adaptor proteins important to several processes of vertebrates development. Three Dact genes have been identified in human and mouse, two in chicken, one in frog and two in zebrafish. These proteins play a wide variety of functions during embryonic development and adulthood by modulating the Wnt and TGF-ß signaling pathways. However, while the Wnt and TGF-ß signal transduction engines are ancient, being present throughout the animal kingdom, Dact is one of the very few embryogenesis-coordinator gene families which are restricted to vertebrates and its origin remains unknown. Moreover, it seems that different vertebrates had recruited a particular set of Dact genes in order to regulate and possibly integrate differentially Wnt and TGF-ß signals. In order to understand the origin and evolution of the Dact gene family, in this study we used database mining, phylogenetic, synteny analyses and in situ hybridization assays. Our phylogenetics analysis revealed an ancestral Dact gene in Branchiostoma floridae's genome and two new Dact paralogs (Dact 3 and 4), meaning that a repertoire of four Dact genes is found in vertebrates. The full set of four Dact genes is present in teleosts, lizards and snakes but not in amphibians, mammals and birds. The Dact loci synteny analyses corroborate the phylogenetic data and reinforce the hypothesis that the four Dact genes arose from a common ancestral after successive whole genome duplications invertebrates. Zebrafish in situ hybridization assays were conduced and the results supported the in silico data.

Program/Abstract # 47
Rapid evolution of cis-regulatory architecture in the *Drosophila* yellow gene
Kalay, Gizem; Lusk, Richard; Dome, Mackenzie, University of Michigan, Ann Arbor, United States; Deplancke, Bart (École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland); Wittkopp, Patricia (U Michigan, Ann Arbor, US))
Enhancers control when, where, and how much of a gene is expressed, and evolutionary changes in these sequences are an important source of phenotypic diversity. To better understand how enhancer sequence and function evolves, we have been studying the evolution of cis-regulatory architecture in the *Drosophila* yellow gene. Using reporter genes to functionally test for tissue-specific enhancer activity in the intronic and 5' intergenic sequences of the yellow gene from six different *Drosophila* species has shown that the genomic locations of tissue-specific enhancers have changed multiple times in the

evolutionary history of these species. DNA sequence analysis suggests that these enhancers have changed their genomic position as the result of the gain and loss of individual transcription factor binding sites rather than large duplications or translocations. To better understand the molecular mechanisms responsible for the observed changes in cis-regulatory architecture, we performed a yeast one-hybrid screen testing ~700 transcription factors individually for their ability to bind to each enhancer sequence. Results from this work will be presented and discussed.

Program/Abstract # 48

The mutations, molecular mechanisms, and constraints directing the evolution of a *Drosophila* cis-regulatory element

Rogers, William; Salomone, Joseph; Tacy, David; Williams, Thomas, University of Dayton, United States

A major goal of evolutionary developmental biology research is to illuminate how evolution acts on development to cause phenotypic change. A wealth of data implicates changes in gene expression as the predominant means by which morphological traits evolve, and likely via mutations in cis-regulatory elements (CREs) that specify gene expression patterns. Each expression pattern is encoded in a CRE as a regulatory logic comprised of a collection and organization of binding sites for certain transcription factor (TF) proteins. While several case studies have identified instances of CRE evolution, how encoded regulatory logics evolve remains poorly understood. An intraspecific comparison of *Drosophila melanogaster* sexually dimorphic abdominal pigmentation patterns presents an opportune situation to reveal how regulatory logics evolve. The degree of female pigmentation varies between populations and this variation stems from genetic variation at the bric-à-brac (bab) locus, which encodes the Bab TF proteins that act as repressors of pigmentation development. Bab expression in females is controlled by a CRE known as the dimorphic element. We identified four dimorphic element alleles that possess different gene regulatory capabilities. By determining the sequence and function of the CRE possessed by the most recent common ancestor of these extant populations we were able demonstrate how few mutations were necessary and sufficient to alter the function of the derived alleles. Ongoing studies seek to reveal how these few mutations of a relatively large effect modify an ancestral regulatory logic.

Program/Abstract # 49

A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals

Pearson, Bret; Labbe, Roselyne (Hosp for Sick Children/U Toronto, Canada); Irimia, Manuel; Blencowe, Ben (Donnelly Centre, Canada)

Many long-lived species of animals maintain and require the function of adult stem cells. However, the transcriptomes of stem cells in invertebrates and vertebrates have not been compared, and consequently ancestral regulatory circuits that control stem cell populations are poorly defined. In this study, we have used data from high-throughput RNA sequencing (RNA-Seq) to compare the transcriptomes of highly purified populations of adult pluripotent stem cells from planarians with the transcriptomes of human and mouse embryonic stem cells. From a stringently-defined set of 4,432, orthologs shared between planarians, mice and humans, we identified 123 conserved genes that are specifically up-regulated in stem cells from all three species, and many of which have not been previously implicated in stem cell biology. Guided by this gene set, we used RNAi screening in planarians to discover novel stem cell regulators,including THADA, PSD12, RAN, and CBX3, which affected stem cell-associated functions including tissue homeostasis, regeneration, and stem cell maintenance. Our analysis demonstrates that comparing stem cell transcriptomes from diverse species represents a powerful approach for identifying conserved genes that function in stem cell biology. These results provide insight into which genes and associated functions may represent part of the ancestral circuitry underlying the control of stem cell self-renewal and pluripotency.

Program/Abstract # 50

Coordinated programs of cell growth and transcriptional regulation in lizard tail regeneration

Hutchins, Elizabeth D.; George, Rajani; Markov, Glenn; Eckalbar, Walter; Geiger, Lauren; DeNardo, Dale (Arizona State U, Tempe, United States); Fisher, Rebecca (U Arizona College of Medicine, Tempe, United States); Rawls, Alan (Arizona State U, Tempe, United States); Huentelman, Matthew (Translational Genomics Res Inst); Wilson-Rawls, Jeanne; Kusumi, Kenro (Arizona State U, Tempe, United States)

Uniquely among amniote vertebrates, lizards can lose their tails and regrow a functional replacement. These regenerated tails contain newly formed hyaline cartilage, spinal cord, muscle, and skin. Progress in studying the cellular and molecular mechanisms of lizard regeneration has been limited by lack of genomic resources. However, with the release of the genome of the green anole, *Anolis carolinensis*, we have a unique opportunity to identify the cells and pathways activated in lizard regeneration. Building on our new RNA-Seq based gene annotation, we have quantified the gene expression levels along

the proximal to distal axis of the regenerating tail at early and mid growth stages. Genes with elevated expression at the growing tail tip include members of key developmental regulatory pathways, including the MAPK signaling and Wnt/β-catenin pathways, cell proliferation/migration markers such as sdc1, and transcriptional modulators such as grhl3 and hdac10. Cells progressively show differentiation towards the proximal region of the regenerating tail, and elevated levels of myogenic genes were identified such as the regulators myod1, pax3, and mstn, together with structural genes such as acta1 and tnnt3. In the mouse, satellite cells are a source for adult skeletal muscle repair; thus, we have isolated green anole cells from skeletal muscle with the spindle-like morphology, capacity to differentiate into skeletal muscle, and expression of markers such as pax7 and cd34. Thus, these studies have identified components of a genetic program for regeneration in the lizard that includes both developmental and adult repair mechanisms. Acknowledgements: NIH NCRR/ORIP Grant R21RR031305 and Arizona Biomedical Research Commission.

Program/Abstract # 51
Functional analyses of APETALA1/FRUITFULL genes in basal eudicots
Pabon Mora, Natalia, NY Botanical Garden, New York, United States; Sharma, Bharti; Kramer, Elena (Harvard, Cambridge, United States); Ambrose, Barbara; Litt, Amy (NY Botanical Garden, New York, United States)
The APETALA1/ FRUITFULL (AP1/FUL) MADS-box transcription factors are best known for the role of AP1 in sepal and petal identity, as it fulfills the canonical A-function of the ABC model of flower development. However, like other MADS-box genes, this gene lineage underwent several duplication events during angiosperm evolution providing different taxa with unique gene complements. One such duplication correlates with the origin of the core eudicots, resulting in the euAP1 and the euFUL clades. Whereas euAP1 genes functions in floral meristem and sepal identity, euFUL genes control phase transition, cauline leaf growth, and fruit development. In order to investigate the functional diversification of this gene lineage within the context of these duplications, we are studying the role of the pre-duplication FUL-like genes in poppies (Papaveraceae) and columbines (Ranunculaceae). Our results show that in both poppy and columbine FUL-like genes are expressed in leaves, flowers and fruits. However, functional analyses show divergent roles in each taxon. FUL-like genes in poppies play roles in phase transition, cauline leaf, flower and fruit development suggesting that the core-eudicot AP1/FUL genes underwent sub-functionalization relative to this ancestor. In contrast, the FUL-like homolog in columbine appears to be decoupled from the floral genetic program and instead has been co-opted for leaf morphogenesis. Altogether, these data highlight considerable variation of pre-duplication gene function. We analyze the results in the context of the core-eudicot duplication and we postulate FUL-like protein interactions and local gene duplications and losses within Ranunculales as possible sources of functional variation.

Program/Abstract # 52
Conservation, divergence, and epistasis in evolution of gene regulation
Ruvinsky, Ilya, Universiy of Chicago, Chicago, United States
We dissect cis-regulatory elements from closely related nematodes to infer general rules that govern evolution of gene regulation. This effort is required to utilize the vast amounts of comparative genomic data for inferences of function. I will highlight several recent results. In particular, one reveals how different functional constraints can contribute to different evolutionary rates within a regulatory element. I will also discuss several lines of evidence that suggest that functional divergence and coevolution of gene regulatory mechanisms are ubiquitous even between closely related species and overt sequence conservation may be less informative than is generally thought. A complex picture of regulatory evolution emerges in which numerous, subtle, lineage-specific, and compensatory modifications of interacting cis- and trans-regulators together maintain conserved gene expression patterns.

Program/Abstract # 53
iBeetle: A genomewide RNAi-screen reveals new patterning genes involved in embryogenesis and metamorphosis
Schmitt-Engel, Christian (iBeetle Consortium, Germany); Klingler, Martin (U Erlangen, Germany); Bucher, Gregor (U Göttingen, Germany)
Most of what we know about the genetic control of arthropod development is based on studies in *Drosophila melanogaster*. Even many studies in other insect species rely on candidate genes known from the fly. However, several features of the higher Dipterans are evolutionary derived, impeding the uncovering of ancestral mechanisms. Moreover, the fly-based candidate gene approach is biased towards conserved gene functions, leaving open questions even for well-studied processes. Hence there is a need to identify novel gene functions by unbiased approaches in alternative insect model systems. To meet this demand, we chose the red flour beetle *Tribolium castaneum* and initiated “iBeetle”, the first genomewide RNAi screen in a non-drosophilid insect. Through iBeetle we want to identify missing components of well-

studied processes, open new fields of insect research to functional analysis and finally are developing *Tribolium* into a complementary screening platform. We established a screening procedure for two parallel screens, which allows the rapid, efficient identification of genes involved in embryonic or postembryonic development. After having screened about half of the *Tribolium* genome, we can conclude that more than half of the genes show an effect in our assays. We identified novel genes required for segmentation, axis formation, head development and oogenesis. New factors involved in muscle development are being further analyzed in *Drosophila* and newly identified genes essential for survival, metamorphosis and odoriferous gland biology will be of great interest for many scientists beyond the *Tribolium* community. Thus we show that *Tribolium* truly developed into a powerful model for unbiased screening approaches.

Program/Abstract # 54
A blueprint for heart regeneration
Poss, Ken, Duke University,Durham, United States

By contrast with adult mammals, zebrafish regenerate cardiac muscle after major injury. In recent studies, we used genetic fate-mapping to reveal that this regeneration occurs through activating proliferation of pre-existing cardiomyocytes at sites of injury. Yet, the molecular mechanisms by which injury activates cardiomyocyte proliferation remain elusive. Here, we have used new technologies to identify cardiac cell type-specific gene expression profiles during heart regeneration. Our findings indicate key injury responses that enable cardiomyocyte proliferation and new muscle regeneration.

Program/Abstract # 55
Imparting regenerative capacity to limbs by progenitor cell transplantation
Lin, Gufa; Chen, Ying; Slack, Jonathan (U Minnesota, Minneapolis, United States

Some vertebrate animals, mostly urodele amphibians, can regenerate limbs after amputation while others cannot. Until now it has not been possible to impart regenerative capacity to animals that cannot do it. The frog *Xenopus* can normally regenerate its limbs at early developmental stages but loses the ability in the late tadpole such that postmetamorphic frogs can only produce an unsegmented cartilaginous spike after amputation. This behavior provides a potential gain-of-function model for measures that can enhance limb regeneration. However, all previous attempts to stimulate frog limb regeneration have been unsuccessful or proved irreproducible. Here we show that frog limbs can be caused to form multi-digit regenerates after receiving transplants of larval limb bud cells supplemented with suitable factors. We show that limb bud cells can promote frog limb regeneration, but that success requires the activation of Wnt/beta-catenin signaling in the cells, plus the provision of the exogenous factors Shh, FGF10 and thymosin beta 4. These factors promote survival and growth of the grafted cells and also provide pattern information for the forming limb structures. The eventual regenerates are not composed solely of donor tissue; the host cells also make a substantial contribution despite their lack of regeneration-competence. Cells from adult frog legs or from regenerating tadpole tails do not promote limb regeneration, demonstrating the necessity for limb bud cells. These findings have obvious implications for the development of a technology to promote limb regeneration in mammals. (G. L. and Y. C. contribute equally to this work)

Program/Abstract # 56
Mitotic neurons: failure to withdraw from the cell cycle produces anterograde transport of nuclei and nonautonomous neuronal toxicity
Baker, Nicholas E., Albert Einstein College of Medicine, Bronx, United States

Neurons are such a firmly postmitotic cell type, so rarely forced back into the cell cycle even experimentally that little is known about the mechanisms of their cell cycle withdrawal. We have discovered mutations in three *Drosophila* genes of related function that cause a class of retinal photoreceptor neurons to continue dividing even after their specification and differentiation has begun. These cells execute a remarkable cell cycle in which they lack well-formed cleavage furrows and cytokinesis is replaced by transport of one daughter nucleus into the axons and towards the central brain. The transport depends on kinesin, resembling the anterograde axonal transport of vesicles. Many of these abnormal neurons degenerate before the adult fly emerges. Unexpectedly, genetic mosaic analysis shows that they also cause the loss of many of the neighboring neurons whose cell cycle has usually been normal. This novel syndrome of mitotic neurons suggests connections between cell cycle defects, axonal trafficking, and syndromes of progressive neuronal loss. It will be interesting to discover whether it is the development of an axon that is incompatible with cytokinesis in neurons, and whether such cell cycle defects cause neuronal cell loss in any neurodegenerative diseases.

Program/Abstract # 57
Growing organs communicate and adapt their growth programs and maturation to ensure final correct size via a novel *Drosophila* Insulin-like peptide

Dominguez-Castellano, Maria; Garelli, Andres; Gontijo, Alisson; Miguela, Veronica; Caparros, Esther (Inst Neuroci Alicante, Spain)

Animal size is amazingly constant within species and this constancy is even more striking when we consider the coincidence in size of the left and right sides of bilaterian organisms. To attain such precision, growing organs must be capable to sense and communicate their growth to other organs in the organism and to have flexibility to adjust their growth programmes and the timing of maturation to repair disturbances during ontogeny. How they do so remains a mystery. We have addressed this issue in the imaginal discs of the fruit fly *Drosophila melanogaster*, which are known to have a remarkable flexibility to regulate their size, particularly when they suffer lesions. I will present the findings that growing imaginal discs produce, and secrete to the hemolymph, a novel insulin/relaxin-like peptide that mediates the plasticity of growth and maturation that ensures the proper final size,proportions, and the symmetry between the two sides.

Program/Abstract # 58

!): Trying to fathom the mechanisms of planar cell polarity.

Lawrence, Peter, University of Cambridge, United Kingdom

In the past 100 years or more of developmental biology, the significance of vectorial information has often been overlooked, yet an individual cell cannot contribute properly to building an animal without information both of its position (that relates to its identity) but also its orientation within the whole. For example, it needs to know which orientation to divide, which route to move, which direction to send an axon or outgrowth etc. These properties of polarity have lately become known as planar cell polarity (PCP). I first came across PCP as a graduate student exactly 50 years ago. After having done some work on this problem for a few years, I then dropped it until about 1995 when I returned to it 100%. Since then I have been collaborating with José Casal (Cambridge, UK) and Gary Struhl (Columbia, NY) to research the mechanism of PCP using mostly genetic methods (particularly mosaics) with *Drosophila*. We are unconventional in that we are all 3 doing only research and all of us are aged over 50. I will tell the story of what we have discovered and how we have approached this deep and fascinating problem. Our main findings have been concerned with the functions of three cadherin proteins, Flamingo (aka Starry Night), Dachsous, and Fat. The former protein works with Frizzled and Van Gogh within one pathway and the latter two work together with Four-jointed and Dachs in a second independent pathway. Both pathways help determine the polarity of cells in the epidermis. In both these pathways, polarity information is transferred from cell to cell via cadherin proteins that constitute molecular bridges. These processes appear to be conserved to vertebrates.

Program/Abstract # 59

Tissue specific analysis of chromatin identifies temporal enhancer activity in *Drosophila* mesoderm development

Zinzen, Robert P.; Bonn, Stefan; Girardot, Charles; Perez-Gonzalez, Alexis; Delhomme, Nicolas; Wilczynski, Bartek; Riddell, Andrew; Furlong, Eileen E.E., EMBL, Heidelberg, Germany

Epigenetic histone modifications and genome-wide binding profiles of general transcription factors can serve as reliable, global read-outs for the regulatory state of genes and enhancers. A challenge in a developmental context, however, has been that such features are generally employed throughout the developing embryo. Therefore, whole-embryo studies can only yield non-specific data from multiple tissues and cell types where any informative signatures are diluted and contradicting data from diverse tissues is superimposed. We have developed a new method to batch-isolate tissue-specific chromatin followed by immunoprecipitation (BiTS-ChIP) and have applied this method to the developing *Drosophila* embryo by extracting mesoderm-specific signatures for histone modifications and Pol II positioning. The tissue-specific data is of high sensitivity and specificity and reveals that enhancers exhibit heterogeneous chromatin/Pol II states and that specific states are highly correlated with spatio-temporal enhancer activity. Though H3K4me1 enrichment generally marks enhancers, it provides no information on the activity state of regulatory regions; however, other features such as H3K27ac, H3K79me3 and especially Pol II enrichment clearly mark active enhancers with temporal precision. In a machine-learning approach based on the uncovered enhancer signatures, we were able to faithfully identify new enhancers that direct spatio-temporal activity as predicted. Such cell type-specific data can therefore identify enhancers in active use during development, which will be instrumental in deciphering cis-regulatory networks. Furthermore, our BiTS method should be widely applicable and easily adaptable toother tissues and animals.

Program/Abstract # 60

Regulatory Genomics in *Drosophila*

Stark, Alex, IMP-Vienna, Austria

During animal development, the transcription of genes is tightly controlled in a temporal and spatial fashion by cis-regulatory modules (CRMs) or enhancers. Enhancers are DNA elements that contain sequence motifs for specific

combinations of transcription factors (TFs) and activate or repress the expression of a target gene. Despite detailed analyses of a small number of enhancers, we lack a systematic picture of enhancers and their functions in animal genomes and an understanding of the sequence basis for their activity. To map the regulatory landscape of *Drosophila melanogaster*, we are performing an in vivo screen for enhancer activity during embryogenesis. We screen a large library of several thousand transgenic flies that carry transcriptional reporter constructs with defined ~2kb candidate fragments inserted at a defined genomic position ("VT-library", currently being established in collaboration with the Dickson group [IMP]). We assayed more than 4000 enhancer candidates, which represent around 10% of the entire non-coding *Drosophila* genome. Interestingly, about half of all candidates functionas enhancers during embryogenesis, with diverse temporal spatial patterns and a bias towards late embryonic stages. We apply bioinformatics and machine learning to extract predictive signatures from regulatory DNA sequences. We identified the TAG team motif of Zelda as an important feature of TF binding and enhancer function in the early embryo and the GAGA motif as a predictive feature of *Drosophila* HOT regions. Taken together, we are systematically characterizing thousands of defined DNA sequence fragments to create an unprecedented view on transcriptional regulation and the 'regulatory code' that governs it.

Program/Abstract # 61

Six1 expression is regulated by evolutionarily conserved enhancers

Sato, Shigeru; Ikeda, Keiko, Jichi Med Univ Ctr for Molecular Medicine, Japan; Shioi, Go; Nakao, Kazuki (Kobe, Japan); Yajima, Hiroshi; Kawakami, Kiyoshi (Jichi Med Univ Ctr for Molecular Medicine, Japan)

Six1 homeobox gene plays critical roles in vertebrate organogenesis. Six1 knock-out mice show severe defects in organs such as skeletal muscle, kidney, thymus, sensory organs and ganglia derived from cranial placodes, and mutations in human SIX1 cause branchio-oto-renal syndrome, an autosomal dominant developmental disorder characterized by hearing loss, branchial and kidney defects. In this study, we showed that seven conserved non-coding sequences retained in tetrapod Six1 loci possessed distinct enhancer activities. Their activities were detected in all cranial placodes (excluding the lens placode), dorsal root ganglia, somites, nephrogenic cord, notochord and cranial mesoderm. The major Six1-expression domains were covered by the sum of the activities of these enhancers together with the previously identified enhancer for the pre-placodal region and for egutendoderm. Thus, eight CNSs identified in a series of our study represent major evolutionarily conserved enhancers responsible for the expression of Six1 in tetrapods. Mutational analysis of the most conserved placode-specific enhancer, Six1-21, revealed that the enhancer integrates a variety of inputs from Sox, Pax, Fox, Six, Wnt/Lef1 and basic helix-loop-helix proteins. The involvement of Six protein-binding sites in Six1 regulation suggests the molecular basis of positive autoregulation. Analysis of Six1-21 enhancer and detailed expression analysis of chick Six1 suggest that the function of Six1 may not be conserved during olfactory development between chick and mouse, and raises the possibility of evolutionary changes in the olfactory developmental program.

Program/Abstract # 62

A conserved requirement of MED14 for the maintenance of stem cell populations.

Burrows, Jeffrey T., University of Toronto, Canada; Pearson, Bret (Hospital for Sick Children, Toronto, Canada); Scott, Ian (University of Toronto, Canada)

The mediator complex links the RNA polymerase II transcriptional machinery to the enhancer bound regulatory factors that in turn establish and maintain cell fate. However, the in vivo consequences of loss of function of many mediator subunits are largely unknown. Through positional cloning analysis we have identified med14, a tail component of the mediator complex, as the gene affected in a mutant isolated from an earlier ENU screen. A slight developmental delay is noticeable by 1.5 days post fertilization (dpf) in med14 mutants and the gap in development compared to their wild-type siblings continues to widen thereafter. Ultimately, med14-/- embryos progress only as far as the long-pec stage (normally 2.0 dpf) by 3.0 dpf and expire before the hallmark events of the hatching period commence (i.e. pectoral fine longation and formation of the semi-circular canals). Interestingly, transcription is not broadly affected in mutants with only ~2% (764/34858 with $\geq$ 2 fold difference) of genes assayed by mircoarray being differentially expressed relative to their wild-type siblings at 2.25 dpf. More striking still, there is little difference in cell death or proliferation despite the observed "arrest" in development. To gain further insight, RNAi knockdown of med14 in planarians was pursued. Homeostasis and regeneration assays implicate med14 specifically in the maintenance of the planarian stem cell population. RNA insitu analysis of zebrafish "stem cell" populations suggests that this could be an evolutionarily conserved role.

Program/Abstract # 63

Essential role of the chromatin remodeler Chd1 in mouse embryonic and placental development

Ramalho-Santos, Miguel; Guzman, Marcela; Koh, Fong Ming; Sachs, Michael; Lin, Chih-Jen, UC San Francisco, United States

We are interested in the role of chromatin structure regulation during early mouse development, a topic about which little is known. We have previously shown that the chromatin remodeler Chd1 regulates the decondensed chromatin state of mouse Embryonic Stem (ES) cells in vitro (Gaspar-Maia et al, Nature 2009). To determine the role of Chd1 in vivo, we recently generated Chd1 mutant mice. Chd1-/- embryos arrest around E5.5-6.0, prior to gastrulation. Mutant embryos induce but fail to maintain the pluripotent epiblast and the extraembryonic ectoderm, and do not establish the anterior-posterior axis. This phenotype appears to be due to apoptosis specifically of the epiblast. To gain insight into the mechanism of action of Chd1, we have preliminarily uncovered evidence for a remarkable level of physiological (un-induced) DNA repair in the mouse epiblast. We found that foci of gammaH2AX, a marker and regulator of DNA break repair, are detected at high levels in control 5.5-6.5 mouse embryos specifically in the epiblast, and these foci are absent in Chd1 mutants. We are testing the hypothesis that Chd1 is required for chromatin decondensation events that are essential for DNA repair in the mouse epiblast. We will discuss the potential implications of these findings, including recent evidence suggestive of a role for Chd1 in different types of cancer. We have also generated a conditional (floxed) allele of Chd1 and are using it to delete Chd1 in specific developmental contexts. Serendipitously, we found a surprising and essential role for Chd1 in the fetal component of the mid-gestation mouse placenta, and are dissecting this role further. Our most recent findings will be presented.

Program/Abstract # 64
Educational Activities of the Society for Developmental Biology
SDB Professional Development and Education Committee, Bethesda, United States

Volunteer. Engage. Learn. Share. Educate. Participate with the Society for Developmental Biology (SDB). The primary mission of SDB is to advance understanding of developmental biology at all levels. SDB provides many opportunities for its membership to engage in a variety of educational endeavors. Since 2006, the Professional Development and Education Committee (PDEC) has organized Boot Camps for new faculty and advanced postdocs to improve their leadership skills and learn about teaching, mentoring and lab management. In 2009, the first Re-boot Camp was offered for mid-career faculty to reinvigorate their teaching approaches and to discover strategies for shifting their career focus. SDB has co-organized short courses with the Latin American Society for Developmental Biology since 2005 for advanced graduate students, postdocs and junior faculty. These courses have fostered both scientific and cultural exchanges, as well as research collaborations. SDB Collaborative Resources (CoRe), an online collection of visuals for learning and teaching developmental biology, is an opportunity for all developmental biologists to contribute to greater understanding of their specialties. Course instructors can also submit developmental biology teaching resources at all levels to the APS/SDB Partner Archive (formerly LEADER). SDB volunteers engage the public through outreach activities including the USA Science & Engineering Festival, educational workshops at regional meetings, and public talks on development. Come by the SDB poster and booth to learn how you can contribute, volunteer and be part of an energetic team to promote developmental biology.

Program/Abstract # 65
Exploring Developmental Biology in the Kindergarten and First Grade Classroom
Glickman Holtzman, Nathalia, Queens College, CUNY Biology; The Graduate Center, CUNY, Flushing, United States; Miller, Vanessa; Wilson, Christopher (Central Park East II (P.S. M964), New York, United States)

The value of using developmental biology as a teaching tool for fostering scientific inquiry, teaching general biological concepts and developing observation and documentation skills is clear for students of all ages. We have developed a program that brings these concepts into the classrooms of young children in an age appropriate yet scientifically robust manner. Within this 2 year curriculum students gain an understanding of animal Classifications and Life-Cycles (CaLC). One of the key learning goals in elementary education is the concept of sorting and object classification. For example, a typical task might be: sort the buttons by color or/and by the number of button holes. Students in the CaLC curriculum learn to sort living things, first into their kingdom, then into vertebrates and invertebrates and finally into their key classes (mammal, fish, reptile, amphibian or insect, arachnid, crustacean). The CaLC curriculum also includes 4 additional developmental biology modules (two vertebrate and two invertebrate).With support from the school and SDB, three of the four modules have been initiated this semester. Ms.Miller's kindergarten class has explored the development of chick and frogs while Mr. Wilson has carried out an invertebrate study with his 1st grade class. These studies are designed to meet the many of the Science and Math Core Curriculum content and skills. We will present a summary of the program as well as assessments of meeting our learning objectives.

Program/Abstract # 66
Determination of bisphenol A (BPA) levels in animal cages following different cleaning regimens
Freeman, Edward; Chichester, Kimberly, St. John Fisher College, Rochester, NY, United States
Bisphenol A (BPA) is the monomeric building block used in polycarbonate plastics. The addition of BPA to plastic provides shatter resistance and thermostability. As such, polycarbonate plastics are a prominent feature in numerous products, including animal caging. Because BPA can leach from polycarbonate plastics we sought to determine BPA levels in new cages and in cages that had undergone a cleaning regimen simulating one year of use. Five different cage cleaning methods were tested to determine if any would cause BPA to leach into water stored in cages following treatment cycles. Our research was conducted within an Analytical Chemistry course with students assisting in the determination of BPA levels. Each group of two students was responsible for the evaluation of samples from a single treatment method. An undergraduate research student had previously developed the HPLC method for BPA detection to ensure results were comparable between groups and treatments. Specifically, BPA levels were determined utilizing reverse phase high performance liquid chromatography (HPLC) equipped with a diode array UV-VIS detector. The HPLC method utilized a Zorbax Extend C-18 column, a water: acetonitrile (50:50) mobile phase with a flow rate of 1.25 mL/min, a 20 µL sample injection volume and BPA detection at 224 nm after 2.8 minutes. BPA calibration curves (x=10) resulted in a correlation coefficient of 0.9999 with an LOD of 8.61 nM. Students and course instructors were kept blind to the treatment groups until after all analyses were complete. At the end of the experiment, each student prepared a paper discussing the impact of cage cleaning strategy on BPA levels.

Program/Abstract # 67
About meiosis concept
Sanz, Ana; Diosdado Salces, Esther, Universidad de La Habana, Havana, Cuba
Meiosis is a process hard to understand for students. In our experience, meiosis is often understood as a cytological or cell biology process and not as a process of forming gametes and haploid spores. In Biology major at the University of Havana, meiosis is taught first in the Cell Biology course, in the sixth semester. Later the concept is completed in Developmental Biology course as a process forming male and female gametes during the first stage of ontogenetic development. Students have to achieve the comprehensiveness of the concept of meiosis when Genetic course addressed it from the standpoint of character segregation during genetic exchange or crossing over. Genetics is on the curriculum in the same semester as Developmental Biology. All courses referred in this paper are mandatory for Biology students. A survey with questions about meiosis was applied to 40 people from fifth year and postgraduates. The results were statistically processed and indicate that students have the necessity to receive the same topics from different points of view in order to get a more comprehensive and holistic idea. Fifteen of them said they never realized the concept of meiosis during their studies in Biology And the rest reflected that they completed the concept of meiosis and increased it gradually from Cell Biology, Molecular Genetics, and Developmental Biology and with the contributions of Genetics. Based on these results it was decided among the professors of Developmental Biology and Genetics to impart a seminar with an integrative approach using group teaching techniques to improve the knowledge.

Program/Abstract # 68
C.R.E.A.T.E. Cornerstone: Adapting the C.R.E.A.T.E. strategy for freshmen, to encourage their persistence in STEM and participation in undergraduate research experiences
Hoskins, Sally G., City College of New York Dept of Biology, New York, United States
The C.R.E.A.T.E. (Consider, Read, Elucidate hypotheses, Analyze and interpret data, and Think of the next Experiment) strategy uses intensive analysis of primary literature to demystify and humanize science. In previous studies, third and fourth-year students read sequential articles from single labs, used novel pedagogical tools to analyze individual experiments, designed follow-up studies and email-surveyed authors with a panel of their own questions. Assessments showed significant gains incritical thinking ability, understanding of the nature of science (NOS), interest in research/researchers, and epistemological beliefs about science (Hoskins et al., 2007, Genetics 176,1381;2011, CBE-LSE 10, 368). In interviews,students noted that C.R.E.A.T.E. skills helped them in other courses, suggesting that a “Freshman C.R.E.A.T.E.” could aid student learning while encouraging participation in research projects. C.R.E.A.T.E. Cornerstone, a new elective for entering STEM majors, uses C.R.E.A.T.E. strategies adapted for newspaper or popular-press science as well as analysis of journal articles. First-year students made gains in self-assessed reading/analysis ability, interest in research/researchers, NOS understanding, and attitudes and epistemological beliefs about science. Cornerstone students also gained significantly in experimental design ability, assayed by EDAT. Thus C.R.E.A.T.E. methods can be successfully adapted for entering STEM students. Ongoing tracking of Cornerstone alumni will test the hypotheses that they will be

more likely than students who did not take the course to (1) persist in STEM majors and (2) participate in undergraduate research projects. Thanks to NSFTUES for support (DUE-094279).

Program/Abstract # 69
A thematic integration of development into an introductory organismal biology course
Savage, Rob, Williams College Dept of Biology, Williamstown, United States
Undergraduate students interested in Biology enroll in a sequence of introductory courses that vary in topic, enrollment size, number and order depending upon the institution. Typically, there is a single organismal course that introduces students to evolution, ecology, behavior, and a second course that introduces students to molecular and cell biology. In the introductory sequence, developmental biology tends to be associated conceptually with organismal biology because it is considered a formal component of the study of evolution, but the actual integration of molecular developmental mechanisms into an organismal biology course has been difficult to achieve for a number of reasons. At Williams, the organismal biology course integrates developmental biology into the study of evolution by focusing on how form is created in the context of the studying the mechanisms of evolution. In this course, students examine the sources of genetic variation, the developmental processes that translate the genome into form, and how the environmental filter acts on variation in a population to generate biodiversity. The integration of development into a standard organismal biology curriculum provides a mechanistic understanding of how genomic change drives morphological change and adaptation in evolution. The poster will present the thematic course structure including a syllabus, primary and secondary sources and the laboratory program that includes two multi-week,inquiry-based labs (one in urchin development and the other in wood frog population structure) that correspond directly to lecture.

Program/Abstract # 71
Inquiry-based laboratory exercises in the Biology of Stem Cells
Meyers, Jason, Colgate University Department of Biology, Hamilton, United States
As part of an upper-level seminar course on the Biology of Stem Cells, I developed an associated set of laboratory exercises to introduce students hands-on to several key techniques in stem cell biology and to provide the opportunity to begin learning about experimental design. The laboratory was divided into three units: planaria regeneration, mouse ES cells, and vertebrate regeneration. For each unit, students were introduced to the model system with a basic experimental demonstration, then asked to design a limited experiment to answer some question about the properties of the cells we were working with, the students then learn a given technique for each unit to analyze their experiments and draw conclusions (e.g. microscopy, immunocytochemistry, RT-PCR, etc.). After the three units, the students are asked to design and carry out a final experimental series over 4 weeks using any of the model systems and techniques that we used, basing their experiment on research in the primary literature, and then presenting their findings to their peers. The laboratory units complement the major themes of the seminar course, and by integrating the primary literature, help students connect the literature with the process of doing scientific investigation.

Program/Abstract # 72
Development of a First Year Biology Lab Containing a Strong Research Element
Olena, Abigail; Talley, Jennell M.; Bairley, Robin; Sissom, Charles Brian; Baskauf, Steven J., Vanderbilt University, Nashville, United States
The number of students choosing biological sciences majors and research careers varies significantly, with large numbers of students who major in the biological sciences pursuing non-research careers. The best predictor of pursuit of a career in research is early exposure to research. The beginning biological sciences sequence at Vanderbilt University consists of two semesters of lecture-based seminars that are usually taken in conjunction with introductory biological sciences laboratories. While every student enrolls an identical lab in the first semester, two lab choices are offered in the second semester. The first choice (111b) is the course the majority of students take, and is very like the first semester of lab with generally one experiment per three hour lab period, resulting in a problem set or lab report to be turned in the following class period. The second choice (111c) is worth two credits, as compared to one for 111b, and, in addition to selected pre-designed experiments, devotes significant class time to student development of an independent research project. Students work in groups of four or eight, with one teaching assistant taking responsibility for no more than eight students. At the end of the semester, student groups design and present a poster about their research to the department of biological sciences. Here we describe the development and function of the course, as well as compare student attitudes toward doing undergraduate research and pursuing research careers before and after taking 111b or 111c.

Program/Abstract # 74
A developmental biologist's foray into science policy
Grant, Kelly A., Gannon University Biology, Erie, United States
The American Association for the Advancement of Science (AAAS) offers science policy fellowships to scientists of all disciplines. The fellowship promotes mutually beneficial relationships where scientists share their expertise and analytic skills with Congressional offices and executive branch agencies while scientist learn about policy. As a developmental biologist my policy interests focused on the effects of environmental pollutants on development, especially exposure to endocrine disruptors. Based on these interests, I selected an office at the EPA that offered me the opportunity to evaluate toxicological profiles of chemicals and devise plans to reduce the use of chemicals of concern. In my two years at the EPA, I developed the strategy to reduce human and environmental exposure to bisphenol A (BPA), which included an initiative to find safer replacements to BPA in thermal paper receipts. I also worked on programs to reduce the release of nonylphenol to the environment. Nonylphenol (aka Triton X-100) is roughly 1000xs more estrogenic than BPA and is recalcitrant to biodegradation. Reducing the environmental releases of these chemicals will hopefully ameliorate conditions leading to the feminization of fish, occurring in many lakes and rivers. During my fellowship, I learned about policy making, gained experience communicating to non-biologists, and applied knowledge of developmental biology to environmental policies. I interfaced with toxicologist to develop more meaningful assays to better capture critical stages in development and/or disruption of signal transduction pathways. Finally, the work was exceptionally rewarding because it created concrete, real-world benefits. The poster will discuss my projects in more detail and I will have information about the fellowship.

Program/Abstract #75
Dynamic filopodia transmit lont-range Shh signaling during tissue patterning.
Barna, Maria; Martin, Esther Llagostera; Sanders, Timothy, UC San Francisco, United States
The movement of key signaling proteins within tissues and organs is a central feature of metazoan development that must be exquisitely spatially controlled. How this is achieved at a cellular level remains poorly understood. Here we constructed a robust, state-of-the-art imaging system that allows for visualization of signaling at single cell resolution under endogenous spatial and temporal control within living vertebrate embryos. Through this imaging approach, we identified that genetically defined populations of mesenchymal cells involved in Sonic Hedgehog (Shh) signaling within the vertebrate limb bud possess a novel specialized class of actin-based filopodia spanning several cell diameters that have not been previously described. By imaging Shh responding cells in real time, we visualized an exquisite distribution and co-localization of the Shh co-receptors to discrete micro-domains along the membrane of these filopodia extensions, suggesting a functional role in facilitating long-range signaling. We therefore developed a tightly regulated expression system directed by the Shh limb-specific enhancer element to visualize Shh production in vivo. Remarkably, imaging Shh ligand itself revealed that it is normally produced in the form ofa particle that dynamically moves along filopodia extensions and accumulates at their tips, which make stabilized contacts with responding cells that contain Shh co-receptors. Strikingly, these stabilized interactions are associated with a dramatic accumulation of Shh to the primary cilium of responding cells and activation of the pathway. To our knowledge, this is the first in vivo demonstration of Shh ligand production and movement. These findings strongly suggest that contact mediated release propagated by specialized filopodia contributes to the delivery and activation of Shh signaling at a distance. Together, these studies identify a new mode of communication between cells that extends our understanding of long-range signaling during vertebrate tissue patterning.

Program/Abstract # 76
Characterization of a Wls knockdown in the developing chick spinal chord
Allen, Sean, San Francisco State University, United States
The Wnt signaling pathway contributes to the regulation of important developmental and cellular events, such as cell survival, proliferation, and specification. Within vertebrates, one of the best-characterized examples of a Wnt gradient is found in the developing spinal cord. There, dorsally expressed Wnts form a dorsal to ventral gradient of proliferation and neuronal specification. For Wnts to function properly, they must first be secreted from Wnt-producing cells. Wntless (Wls), a transmembrane protein, is necessary for the secretion of all known Wnt family members invertebrates. Knockout of Wls in the mouse model system causes early embryonic lethality that coincides with the timing of the first known requirement for Wnt signaling. The goal of this project was to determine the role of Wls in Wnt gradient formation in the chick spinal cord. Specifically, we hypothesized that loss of Wls would disrupt the Wnt gradient and thereby cause defects in survival, proliferation, and patterning. To test this hypothesis, electroporation was used to achieve a transient siRNA-mediated knockdown of Wls. Transverse sections were analyzed for morphological changes, the most noticeable of which

was a significant loss of cells on the electroporated side of the spinal cord accompanied by a significant delamination of cells. Current data suggests that this loss of cells is due to inappropriate apoptosis - as assayed by TUNEL. Though knockdown of Wls also decreased the total number of proliferative cells- as assayed by phosphohistone H3, the percentage of proliferative cells was not significantly diminished. Thus, this study suggests that Wls-dependent Wnt signaling is necessary for cell survival in the developing neural tube.

Program/Abstract # 77
RA and ROS act in similar signaling pathways during extraembryonic endoderm formation
Hwang, Jason TK, University of Western Ontario Biology, London, Canada; Wen, Jason (University of Toronto, Toronto, Canada); Kelly, Gregory (University of Western Ontario, London, Canada)

Mouse F9 cells are used to recapitulate the epithelial-to-mesenchymal transition (EMT) associated with extraembryonic endoderm formation. F9 cells treated with retinoic acid (RA) form primitive endoderm and this is accompanied by an increase in reactive oxygen species (ROS). Treating cells with H2O2 is sufficient to induce differentiation. Furthermore, differentiation is blocked when either RA- or H2O2-treated cells are treated with antioxidants or DPI, a NADPH oxidase inhibitor. Together, these results suggest that ROS are sufficient and necessary for primitive endoderm differentiation, but how H2O2 affects the gene regulatory networks responsible for this EMT is not known. To address this, we treated F9 cells with RA or H2O2 and employed a PCR array to profile the expression of 84 genes differentially regulated during EMT. We found that the overall expression profile in RA-treated cells paralleled that seen following H2O2 treatment. Furthermore, many of the genes encoding proteins involved in cell migration and restructuring the extracellular matrix were regulated in a similar manner under both regimens. For example, the expression of b-catenin was up-regulated following either treatment, which is significant as this is an indicator of EMT. b-catenin is also known to signal downstream of Wnt6 to induce extraembryonic endoderm. The Fzd7 gene encoding a Wnt receptor was also up-regulated in response to RA or ROS, but whether this serves to transduce the Wnt6 signal is not known. Together these results provide evidence that ROS and RA act in a similar manner on many gene pathways and in the case of Wnt-b-catenin signaling, ROS impact at different levels of the pathway required for extraembryonic endoderm formation.

Program/Abstract # 78
The role of Notch signaling during cell fate determination in the postnatal mouse retina
Ronellenfitch, Kara; Chow, Robert, University of Victoria, Victoria, Canada

The mammalian retina is easily accessible for experimental manipulation and provides an excellent model for studying cell fate determination. Its layered structure and highly stereotyped organization facilitates the analysis of phenotypic differences caused by experimental manipulation. This study focuses on how cell-cell signaling regulates fate determination during retinal development. Specifically we are exploring the role of Notch signaling during the cell fate decisions of late-born neurons, which differentiate into either photoreceptors or bipolar interneurons. Notch signaling has been shown to promote bipolar interneuron specification at the expense of photoreceptor specification. We investigated whether cell fate specification remains plastic at certain times during development and predicted that Notch inhibition will reverse photoreceptor specification and re-direct cells towards a bipolar cell fate. Late born cell types were tracked and quantified throughout development via in vitro electroporation of plasmid DNAs, which mark late born progenitors early in differentiation, into live retinal tissue. Pharmacological treatments of a Notch inhibitor were applied at various points in postnatal development to target Notch signaling specifically in newly born bipolar and photoreceptor cells. By quantifying the final cell fate of these early progenitor cells we can determine the temporal effect of Notch signaling on the fate determination of these late born retinal cell types.

Program/Abstract # 79
Uif, a large transmembrane protein with EGF-like repeats, antagonizes the Notch signaling pathway in *Drosophila*
Jiao, Renjie, Institute of Biophysics, CAS, Beijing, China; Xie, Gengqiang; Zhang, Hongtao (Beijing, China); Ma, Jun (Cincinnati, United States)

Notch signaling is a highly conserved pathway in multi-cellular organisms ranging from flies to humans. It controls a variety of cellular and developmental processes by stimulating the expression of its target genes in a highly specific manner both spatially and temporally. The diversity and specificity of the Notch signaling output are regulated at distinct levels, particularly at the level of ligand-receptor interactions. Here, we report that the *Drosophila* gene uninflatable (uif), which encodes a large transmembrane protein with eighteen EGF-like repeats in its extracellular domain, can antagonize the canonical Notch signaling pathway. Overexpression of Uif causes Notch signaling defects in both the wing and the sensory organ precursors. Null mutants of uif are early larval lethal while depletion of uif by RNAi can partially rescue defects caused by compromised Notch signaling in the wing. Further experiments suggest that Uif inhibits Notch signaling in cis

and acts at a step that is dependent on the extracellular domain of Notch. Our results are consistent with the possibility that Uif regulates the accessibility of the Notch extracellular domain to its ligands during Notch activation. Our study thus identifies a new modulator that can fine tune Notch activity, further illustrating the importance of a delicate regulation of this signaling pathway for normal patterning.

Program/Abstract # 80
Tenascin is a correlative marker in uterine fibroid
Choi, YunJeong; Park, HyoSang; Lee, Seulkina; Park, YoungHoon; Kang, Sua; Kim, DaeYoung; Hwang, YouJin (Gachon University of Medicine and Science, Incheon, Republic of Korea)

Uterine fibroids are the most common benign tumors in the female reproductive system. However, the contributing factors to the growth of fibroids are hardly known, and there are only few effective methods to treat uterine fibroid. The extracelluar martrix (ECM) plays a fundamental roles in the regulation of diverse cellular events. Tenascin is an ECM glycoprotein and it takes part in cell differentiation, proliferation, and migration. It is also evident in association with the processes linked to embryogenesis during tissue development. In addition, tenascin is conserved as an important marker of tissue regeneration. It is possible that uterine fibroid may be associated with aberrantly regulated cell-ECM interactions. To assess this possibility, we determined the expression of tenascin by Western blot analysis in each human uterine fibroid and normal tissue. Using endometerial stromal cell cultured with and without tenascin, we investigated the expression patterns of collagen by immunocytochemistry, respectively. Mostly, the data showed that not only tenascin expression in uterine fibroids was higher than in normal tissues but also tenascin related to the production of collagen. These results suggest that tenascin contributes to uterine fibroid in endometrium. Therefore, we conclude that tenascin is one of the most important candidate markers in uterine fibroid.

Program/Abstract # 81
Correlation of progressing human gastric intestinal metaplasia and fibrogenesis.
Lee, Seulkina; Park, Younghun; Choi, Yunjeong; Park, Hyosang; Kim, Daeyoung; Hwang, Youjin (Incheon, Republic of Korea)

Human gastric intestinal metaplasia (IM) is known for a disease progressed toward gastric cancer. IM is associated loss of E-cadherin and infection of *H.pylori*. Several types of IM is divided into complete IM (type 1 IM) and incomplete type II and III. But mechanism of IM is not well known. IM is presented by loss of activity in normal gastric epithelial cells caused by chronic gastritis that repeats damaging and recovering of cells. We studied correlation IM and fibrogenesis of human intestinal cells that similarly lose cells' activities. We used several types of gastric IM patients' stomach tissue samples and inflamed human intestinal cells. After that, using western blot analysis, we measured expression of cdx1, cdx2 gene that are realated IM regulation, TGF-β1 and IL-1β that are important factor of intestinal fibrogenesis in inflamed organs. And we calculated relevance of progress of IM to gastric cancer and fibrogenesis. Through this research, we confirmed correlation of change of morphology in intestinal metaplasia and fibrogenesis in intestine. It helps understanding embryologic development as well as in cancer and fibrosis.

Program/Abstract # 82
Forward genetics reveals Xylt1 as a key, conserved regulator of bone development
Mis, Emily K., Yale University Genetics, New HavenUnited States; Kong, Yong (Yale University, New Haven, United States); Liem, Karel (Yale University Pediatrics, New Haven, United States); Domowicz, Miriam; Schwartz, Nancy (Chicago, United States); Weatherbee, Scott (Yale University, New Haven, United States)

Long bones form through the differentiation of mesenchymal cells into chondrocytes that form a cartilage template for the bone. Despite recent advances in understanding chondrocyte proliferation and maturation, many of the factors that regulate these steps are still unknown. Using massively parallel sequencing on a dwarf mouse mutant (pug), we identified Xylosyltransferase 1 (Xytl1) as a key regulator of chondrocyte maturation. Xylt1 is one of two xylosyl transferases found in vertebrates, which catalyzethe initial step in glycosylaminoglycan (GAG) synthesis. pug mutant limbs are shorter than normal limbs by birth, and those that survive display progressive shortening of their long bones throughout adulthood. Histological analyses revealed that pug mutants have reduced zones of differentiating chondrocytes, suggesting that proliferation or maturation could underlie the pug phenotype. We discovered that mutant skeletal elements displayed premature ossification, suggesting that early chondrocyte maturation contributes to the pug phenotype. The pug allele shows reduced xylosyl transferase activity, and consistent with the molecular function of Xylt1, we observed reduced GAG levels in pug mutants. Addition of GAG chains to proteoglycans is essential for normal signaling through multiple regulatory pathways. In pug mutants, reduced GAG levels result in increased short-range Ihh signaling, and expanded

Fgfr3 expression in the limb chondrocytes. Characterizing the pug mutant and defining the in vivo function of Xylt1 will greatly enhance our understanding of the signaling pathways and factors that coordinate skeletogenesis.

Program/Abstract # 83
BMP heterodimer signaling in the developing vertebrate embryo
Mullins, Mary; Dutko, James A. Perelman Sch of Med At Univ of Penn Cell & Developmental Biology, Philadelphia, United States

The vertebrate embryonic dorsoventral (DV) axis is patterned by a bone morphogenetic protein (BMP) activity gradient. The BMP morphogen gradient is shaped primarily by the movement of BMP antagonists emanating from dorsal regions to ventral regions of the embryo. High BMP signaling levels arise ventrally whereas BMP antagonists attenuate signaling dorsally. In the zebrafish embryo, BMP signaling requires two ligands, Bmp2b and Bmp7a, functioning exclusively as a heterodimer, and their corresponding type I receptors, Alk3/6 and Alk8 (Alk2 paralog). Why BMP heterodimers function as the obligate ligand, while BMP homodimers, although present, fail to signal is an unanswered question and fundamental to the BMP signaling mechanism. Here we test one model for the obligatory function of BMP heterodimers in DV patterning: BMP antagonists preferentially block signaling by BMP homodimers. To test this model, we depleted Chordin, Noggin1, and Follistatin-like1b by injecting translation blocking morpholinos into bmp7a or bmp2b mutant embryos, i.e.,embryos devoid of BMP heterodimers. If BMP antagonists preferentially block signaling by BMP homodimers over heterodimers in vivo then we expect BMP homodimers to restore signaling when BMP antagonists are removed. Interestingly, no rescue was observed in embryos lacking BMP antagonists and BMP heterodimers. Thus, BMP heterodimers prevail during DV patterning by a mechanism other than a preferential block to BMP homodimers by BMP antagonists. We are currently investigating alternative hypotheses for the exclusive function of BMP heterodimers in DV patterning, including that BMP heterodimers bind to the receptor complex with a lower overall dissociation constant compared to BMP homodimers.

Program/Abstract # 84
acal is a novel negative regulator of *Drosophila* JNK signaling during embryonic dorsal closure.
Rios-Barrera, L. Daniel, Universidad Nacional Autonoma de Mexico (UNAM), Juriquilla, Mexico; Riesgo-Escovar, Juan R. (UNAM, Queretaro, Mexico)

Dorsal closure is one of the last major morphogenetic rearrangements taking place during *Drosophila embryogenesis*. It consists of the stretching of the lateral epidermis towards the dorsal midline, and is controlled by the JNK signaling pathway. The only *Drosophila* JNK (encoded by basket) is activated at the most dorsal row of epidermal cells, from where it coordinates cell remodeling by inducing cytoskeleton modifications and signaling to adjacent cells. Mutants for JNK pathway genes die harboring a ‘dorsal open’ phenotype in cuticle preparations. In this work, we characterize a novel ‘dorsal open’ group gene, which we named acal. We generated an allelic series of acal mutants and mapped them to a non-annotated transcript with a low in silico protein coding capacity. We hypothesize that this transcript is a microRNA precursor. This contention is supported by high throughput RNA-sequencing by the modENCODE project. We also found that the transcript is enriched in nuclear preparations as expected for a nucleus-processed RNA. To get insight into the role of acal in JNK signaling, we analyzed genetic interactions of acal with basket mutants, and observed that basket mutations rescue acal defects. Similarly, JNK signaling target genes are ectopically expressed in acal mutants. These results show that Acal counteracts JNK signaling. acal is expressed in the epidermis, and its expression pattern is very similar to that of raw, another negative regulator of JNK signaling. Interestingly, acal expression is reduced in raw mutants, suggesting that Raw may act at least partially through Acal to downregulate JNK signaling during dorsal closure.

Program/Abstract # 85
Notch controls daughter cell proliferation in *Drosophila* neural lineages
Bivik, Caroline, Linkoping University, Linköping, Sweden

While tremendous progress has been made with respect to the mechanisms controlling neural diversification, less is known regarding how the precise cell number of each neural sub-type is generated. The *Drosophila* embryonic CNS has been a valuable model for addressing these issues. The fly CNS is generated by a set of 1,000 progenitor cells, denoted neuroblasts, which form in the early embryo. Neuroblasts undergo repetitive rounds of asymmetric divisions, budding off daughter cells, before exiting the cell cycle after a distinct number of divisions. In most, if not all, early stages of lineage development, daughter cells divide once to generate two neurons/glia (Type I behavior). Recently, it has been demonstrated that some lineages display a proliferation switch, such that late-born daughters differentiate directly into neurons/glia (Type 0 behavior). As a consequence of these precise proliferation decisions each particular neuroblast generates a stereotyped lineage tree and lineage size. Thus, development of a lineage depends upon two fundamental

decisions: How many times should each neuroblast and each daughter cell divide? To address these questions we are studying several lineages. We find that the first proliferation decision, Type I, is under the control of the homeodomain protein Prospero. In contrast, the second decision, Type 0, is under control of Notch. Neither Pros nor Notch is involved in the neuroblast cell cycle exit. Global analysis of proliferation control in the CNS reveal that these mechanisms are broadly used. To address the pathway by which Notch controls the daughter proliferation switch we have finally analyzed a number of HES genes, as well as the expression of several key G1 cell cycle genes.

Program/Abstract # 86

A global genomic survey of genes that mediate PAR-4/LKB1-dependent germline stem cell quiescence in *C. elegans*

Chaouni, Rita; Roy, Richard, McGill University, Montreal, Canada

LKB1 is aserine/threonine protein kinase that is implicated in the rare, autosomal dominant disease Peutz-Jeghers syndrome (PJS). In addition to its causative role in PJS, it is also often mutated in sporadic cancers, suggesting that LKB1 acts as a bona fide tumor suppressor. Understanding how LKB1 exerts its tumor suppression is therefore of prime significance. LKB1 affects numerous developmental processes including cell growth and cell polarity. When *Caenorhabditis elegans* larvae encounter harsh environmental conditions, they can alter their developmental course and enter dauer diapause, where the germline stem cells are consequently rendered quiescent. Previous work has revealed that PAR-4, the homologue of LKB1, is required for this arrest and PAR-4 deficient dauer larvae display hyperplasia of the germline at this stage. Although LKB1 is known to activate AMPK (aak-1/aak-2 in *C. elegans*), it is unlikely that AMPK is the sole mediator of germline stem cell quiescence downstream of LKB1/PAR-4. To better understand the tumor suppressor function of PAR-4, we continued to dissect its role in regulating cellular quiescence in developmentally arrested larvae. A genome-wide RNA interference-based screen was performed to identify suppressors of PAR-4-mediated germline hyperplasia. We have identified several genes whose loss of function was found to rescue the germline hyperplasia observed in par-4 dauer larvae. Future endeavors entail the characterization of key candidates, many of which impinge on the actin cytoskeleton and its regulation. Further understanding of the function of these genes will provide additional insight as to how LKB1/PAR-4 blocks tumorous growth by regulating cell cycle quiescence.

Program/Abstract # 87

LKB1 dependent and independent roles in the establishment and maintenance of germline stem cell quiescence in *C. elegans*

Kadekar, Pratik; Navidzadeh, Nathan; Wendland, Emily; Roy, Richard, McGill Univesity, Montreal, Canada

C. elegans execute a diapause like state called ‘dauer’ when they encounter harsh environmental conditions. This stage is associated with global developmental arrest that facilitates their survival in unfavorable growth conditions. Curiously the signals that normally drive germline stem cell (GSC) proliferation are present and active in dauers, therefore we became interested in dissecting the pathways that mediate quiescence in the GSC during this diapause stage. It was previously shown that the *C. elegans* orthologs of LKB1, STRAD, AMPK and PTEN are required in establishing quiescence in the dauer germline. LKB1 acts through AMPK in order to regulate the cell cycle arrest in GSCs. Interestedly, LKB1 knockdown in AMPK null mutants show an additive effect suggesting anAMPK-independent role for LKB1 in the regulation of this quiescence. To identify novel LKB1 targets that may be involved in process, a genome-wide RNAi screen was performed in order to isolate genes that caused germline hyperplasia upon their knockdown during the dauer stage. The screen allowed us to identify 39 candidate genes and 7 of them are involved in regulating cell polarity and cytoskeletal regulation. LKB1 regulates early embryonic polarity and therefore may affect cell polarity in the GSC independently of AMPK to affect quiescence.We have categorized the candidates from this screen to show which candidates function dependently and independently of both LKB1 and AMPK. Our candidates fall into at least two classes: 1) Those that affect early phases of quiescence (establishment) i.e. par-3. 2) Those that show a phenotype in late dauer (maintenance) i.e. cdc-42 and rho-1. How these effectors regulate GSC quiescence is currently under investigation.

Program/Abstract # 88

Centrosome elimination during *C. elegans* development

Lu, Yu; Roy, Richard, McGill University, Montreal, Canada

Centriole duplication is coupled with cell division to ensure that the centriole is duplicated only once per cell cycle. However, this coupling can be altered in specific developmental contexts, although how this uncoupling occurs remains misunderstood. In *C.elegans*, the larval intestinal cell will undergo one nuclear division followed by four endocycles. We use this model to understand how the centriole is coupled to the cell cycle and the mechanisms though which they can be uncoupled during the alternative cell cycles that occur throughout development. By monitoring the levels of SPD-2, a protein critical for centriole duplication in *C. elegans*, we found that the centriole duplicates normally at the L1 nuclear

division, but rapidly loses its pericentriolar material and does not reduplicate during the S-phase of the first endocycle. SPD-2 then translocates to the nucleus where it is eventually eliminated. We observed that modification of a highly conserved CDK-phosphorylation site to non-phosphorylable residue on SPD-2 resulted in the failure of the centriole to duplicate before the intestinal nuclear division. Alternatively, mimicking PLK-mediated phosphorylation of SPD-2 or reducing the activity of ubiquitination pathway leads to its nuclear accumulation likely through stabilization. Overall our study reveals that phosporylation of SPD-2 by key cell cycle kinases may regulate centriole/cell cycle uncoupling and elimination during *C. elegans* development. We are currently investigating whether these modifications are conserved in various developmental contexts and/or in other species.

Program/Abstract # 89
Profiling expression of cell cycle regulators during zebrafish development.
Dobbs-McAuliffe, Betsy L., Central Connecticut State Univ Biomolecular Sciences, New Britain, United States
Embryonic development depends upon cellular proliferation and terminal cell differentiation. These processes can be co-regulated, as cells that enter terminal differentiation stop dividing. We believe that many of the factors that promote terminal differentiation couple these events by altering expression of cell cycle regulators. We wanted to explore this hypothesis while providing independent research projects for undergraduate students. Students were trained in one technique, in situ hybridization, and then executed segments of the study. To this end, we have begun a detailed analysis of expression of cell cycle regulators in the zebrafish embryo. Since we are particularly interested in the timing of cell cycle exit we have initially focused on expression of the cell cycle inhibitors cdkn1b (p27 kip1) and cdkn1c (p57 kip2). We are detailing normal gene expression through 24h of development. Additionally we are monitoring expression of cell cycle regulators in embryos where specific signaling pathways have been altered. To start we investigated expression of cell cycle inhibitors in embryos that lack Hedgehog signaling. We have found that cdkn1c, which is normally expressed both in slow muscle and in primary neurons, requires Hedgehog signaling in the slow muscle, but not in the primary neurons. Previous research has shown that slow muscle precursors switch fate to fast muscle in the absence of Hedgehog signaling. We suspect that slow muscle precursors will show a concomitant switch to expression of cdk1nb, the cell cycle inhibitor expressed in fast muscle. Another target of our studies is the retinoic acid (RA) signaling pathway. Preliminary results suggest that RA down regulates cdkn1c expression in the somites.

Program/Abstract # 90
Barhl2 contribute to a cell-intrinsic mechanism that limits the proliferative response of neural progenitors to their mitogen.
Durand, Béatrice; Juraver-Geslin, Hugo; Duval, Nathalie, CNRS UMR, Paris, France
The gene networks that underpin the overall, and differential, growth of our future brain are still poorly understood. We showed that, in *Xenopus* embryos, Barhl2 limits the proliferation of diencephalic neural progenitors, via the regulation of a CASPASE-3 unconventional activity that inhibits the activation of the major effector of the Wnt canonical pathway, ß-catenin. We showed that Barhl2 expression domains are highly similar in the developing mouse and *Xenopus* CNS: in both, it is expressed in association with Wnt3A and Shh which both promote neuroepithelial proliferation. Barhl2 is coexpressed with Wnt3A in the cortical hem, the cerebellar primordium and in asubset of granule cell progenitors (GCP), a cell population characterized by aprolonged proliferation period. At postnatal stages, Barhl2 is expressed in the cerebellar sulci where the SHH pathwayis most active. We examined whether Barhl2 contributes to intrinsic mechanisms that locally attenuates the GCP proliferative response to their most potent mitogen SHH. To perform loss of function experiments we generated lentiviral vectors that deliver shRNA which silence Barhl2 expression with high efficiency. These vectors allow for efficient and stable Barhl2 silencing in neurons, their progenitors and other neural cell types with no toxicity. We demonstrated that Barhl2 cell-autonomously limits SHH-stimulated GCP proliferation and decreases the dependence of GCP proliferation on SHH. Our results indicate that Barhl2 expression pattern is conserved across species and associated with « mitogeniccenters », and argue that Barhl2 plays a conserved role in the local control of neuroepithelial growth.

Program/Abstract # 91
Identification and expression analysis of two homologs from *Xenopus laevis* of the Tumorhead putative binding protein, FBXO30
Traverso, Edwin, University of Puerto Rico at Humacao, United States; Zbinden, Theodor (Univ of Puerto Rico, Rio Piedra, PR, United States); Flores, Noelia; Núñez, Dariana; Ayala, Jesús (University of Puerto Rico at Humacao, Humacao, PR, United States)
Tumorhead (TH) is a maternal factor that regulates cell proliferation during early embryogenesis in *Xenopus laevis*. To understand how TH functions at the molecular level, we have been studying its relationship with the novel F-Box

containing protein FBXO30, found in a two-hybrid screen for TH binding proteins. Using primers based on the sequence we obtained, along with primers based on the 5' and 3' UTRs of the *Xenopus tropicalis* FBXO3O mRNA, we obtained RT-PCR products with total RNA samples from eggs and embryos at early developmental stages. Using this approach, we have uncovered the presence of two FBXO30 homolog genes in *X. laevis*, FBXO30-A and FBXO30-B. The predicted FBXO30-A full length protein sequence is 91% and 63% identical to its counterparts from *Xenopus tropicalis* and *Homo sapiens*, respectively. These proteins contain very conserved Traf-like zinc finger-containing domains at their N-terminus, and F-Box domains at their C-terminus, while the internal part of the proteins diverge extensively. By RT-PCR, we have found that FBXO30-A and FBXO30-B are maternal factors as their messages are present in the unfertilized egg. The FBXO30-A mRNA persists during the cleavage stages, but decreases after the mid-blastula transition and is barely detected once gastrulation starts. Our studies show the presence of two homologs of FBXO30 in *X. laevis* that are maternally expressed, which could be key regulators of early development working with TH to promote cell proliferation.

Program/Abstract # 92

Transition between two types of oscillators during *Xenopus laevis* early embryonic cell cycle

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Before mid-blastula transition, the *Xenopus laevis* embryonic cell cycle is driven by an autonomous biochemical oscillator based on Cdk1 activation and inactivation. Cycle 2 to 12 have a period of 25 minutes and are highly accurate while the 1st cycle takes ~85 minutes, raising the question of how an autonomous oscillator can be initially tunable yet precise afterwards. We reconstruct the temporal dynamicsof cell cycle oscillation in vivo using individual *Xenopus laevis* embryos collected in fine temporal resolution. We observe a higher threshold for cyclin to trigger mitotic entry in the first cycle due to stronger inhibitory phosphorylation of Cdk1. A positive feedback involving the cyclinB1-Cdk1 complex, their inhibitory kinases Wee1 and Myt1, as well as their activating phosphatase Cdc25, is shown invitro to be important for the robustness of the cell cycle oscillations. Surprisingly,bypassing the positive feedback created a significant phenotype in the first cell cycle, but minimal impact on the subsequent cycles. This implies a transition from a strong positive-feedback oscillator to a weak positive-feedback oscillator. Several mechanisms contributed to this transition, such as the increase of phosphatase abundances and the decrease of kinase activities. We identified that the negative feedback is highly ultrasensitive and could improve the robustness of the oscillator in the absence of the positive feedback. We demonstrated computationally that the presence of positive feedback in the first cycle allows the oscillator to be tunable, and turning down the positive feedback in the subsequent cycles help increase the precision of the oscillatory period. The *Xenopus laevis* may turn down a subset of the regulatory circuit during early embryonic development to match changing developmental objectives.

Program/Abstract # 94

Dynamic cell shape changes are required for mesenchymal condensation

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The physical mechanism of mesenchymal condensation during skeletal development is not well understood. Here, we show that dynamic cell shape changes are required for mesenchymal condensation during chick middle ear morphogenesis. The chick contains a single middle earbone – the columella. The chick columella arises from two separate condensations; the cartilaginous extracolumella and an osseous columella. We demonstrate that the respective condensations arise at distinct timepoints. Our modeling results show that the extracolumella undergoes condensation earlier than the columella. In contrast, overt differentiation of chondrocytes occurs first in the columella condensation. The cellular characteristics between the columella and the extracolumella condensation differ substantially. The extracolumella condensation resembles the classical definition of condensation with tightly packed cells. Conversely, the columella condensation has a novel appearance with a loosely organized web-like network of cells, with elongated cell-to-cell connections. However, dynamic cytoskeletal reorganization is observed in both condensations over several days, indicating that cell shape changes are important. Using Cytochalasin D, an inhibitor of actin polymerization, we disrupted the ability of the mesenchyme cells to reorganize their cytoskeleton. Our results show that inhibition of cell shape changes disrupts mesenchymal condensation during chick middle ear morphogenesis. Overall, our experiments will be helpful in understanding the general principles of self-assembly of multi-potent progenitor cells to form a cartilage template of correct shape and size.

Program/Abstract # 95

Mechanism of cranial neural crest cell migration.

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Cranial Neural Crest (CNC) are pluripotent cells induced at the lateral edge of the neural plate. In *Xenopus laevis*, CNC migrate as a cohesive sheet of cells initially and then as individual cells to produce the face of the embryo. We have previously shown that cell surface metalloproteases from the ADAM family are essential for CNC induction (ADAM19)

and migration (ADAM13). They act both by cleaving the extracellular domain of Cadherin-11 to release an active extracellular domain promoting cell migration and by regulating gene expression via their cytoplasmic domain. While ADAMs are critical for CNC migration in vivo they are not essential for CNC migration in vitro. Here we analyze how the in vivo environment restricts CNC migration in the absence of ADAM, and how the cytoplasmic domain controls gene expression and ADAM function. Our results show that cells within the CNC explants are able to modulate their adhesion to migrate through 20 µm "tunnels" in vitro whether they express ADAM13 or not. Their ability to invade a collagen gel is also not affected. In contrast, mechanically opening the CNC pathways in vivo is sufficient to rescue migration in embryos lacking ADAM13. We further show that ADAM13 function in the CNC depends on a series of phosphorylation by GSK3 and polo like kinase and that this is independent of ADAM13 proteolytic activity. We propose a unified model describing how ADAMs metalloproteases can control cell migration during early embryogenesis and possibly in cancer cell metastasis.

Program/Abstract # 96

Elucidating the role of Stat3 signaling in development of early cranial neural crest stem cells, cranial NC cell derived tissue and coronal suture formation

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Neural crest stem cells (NCSCs) are the transient population of multipotent stem cells that arising in gastrulating vertebrate embryos at the future neural plate and non-neural ectoderm junction, that traverse to various parts of the embryo producing mainly Ectodermal derivatives like neurons, glial cells and melanocytes, along with Mesodermal derivatives like bone, cartilage or smooth muscle (only in cranial neural crest cells). Microarray studies performed on clonally cultured Cranial neural crest cell lines in the lab revealed high levels of expression of members of the Jak2-Stat3 signaling cascade, prompting us to investigate a possible role the Stat3 signaling in this lineage. Subsequently, gross morphological defects in neural crest derived tissue was observed in mice with conditional knockout of Stat3 in neural crest specific cell lineage (under the Wnt-1 Crepromoter) beginning early during neural crest derived tissue development, along with premature lethality. Surprisingly these mice (Wnt1Cre;Stat3 flox/flox) also developed partial bilateral Coronal suture synostosis with a 100% penetrance by 3 weeks, supporting recently published findings that HIES (Hyper –IgE Syndrome)/Job's syndrome, a rare immunological disorder with patients displaying characteristic facial deformities including Craniosynostosis, is genetically linked to a mutation in the DNA binding domain of the Stat3 gene resulting in non-functional Stat3 proteins (loss of DNA binding capacity). Currently we are trying to elucidate how the Neural crest specific loss of the Stat3 gene leads to the loss of defined Coronal suture boundary and mixing of osteoblastic cells early during Suture formation in Wnt1Cre; Stat3 flox/flox mice.

Program/Abstract # 97

Fat-Dachsous signaling coordinates polarity and differentiation of the craniofacial skeleton in zebrafish

Le Pabic, Pierre; Schilling, Thomas, University of California, Irvine, United States

Little is known about the mechanisms of cell-cell communication necessary to assemble skeletal elements of appropriate size and shape. Skeletal progenitors may behave as coherent units by communicating via the planar cell polarity (PCP) pathway. In *Drosophila*, two sets of factors control PCP independently: the Fat and the non-canonical Wnt signaling systems. While a requirement for components of the non-canonical Wnt system was recently demonstrated in regulating the oriented divisions and intercalations of chondrocytes in the growth plates of long bones, a role for the Fat system in skeletal development has not been reported. We find that loss of Fat, Dachsous, Four-jointed or Atrophin-orthologues in zebrafish results in similar skeletal abnormalities, including the shortening of some cartilages, fused joints and chondrocyte stacking defects. Confocal imaging of Fat- or Dachsous-deficient prechondrocyte condensations reveals loss of stacking and polarity – two PCP-regulated behaviors in other contexts such as gastrulation, as well as delays in differentiation. In addition, our chimaeric analysis demonstrates that Fat is both necessary and sufficient to coordinate polarity and differentiation of cartilage in a non-cell autonomous manner. These results provide genetic evidence that skeletal morphogenesis and differentiation are controlled through a conserved Fat signaling pathway, a process that has not previously been associated with defects in skeletal tissuepolarity.

Program/Abstract # 98

Response genes regulate the severity of craniofacial defects

Sheehan-Rooney, Kelly; Seritrakul, Pawat; Eberhart, Johann, Univ of Texas at Austin, United States

Craniofacial malformations are highly variable birth defects, yet we understand little about the pathways regulating this phenotypic variability. To identify and characterize these pathways, we examined the variable craniofacial defects in zebrafish gata3 mutants. In human, mutation of GATA3 causes the highly variable HDR syndrome (Hypoparathyroidism,

Deafness and Renal disease), which can include craniofacial defects. Our zebrafish gata3 mutants display the range of HDR symptoms, the severity of which is genetically modulated. Mutants in one inbred genetic background have nearly wild-type phenotypes, while a second background has profound craniofacial defects. We used a microarray approach to identify pathways that regulate this variability. Twenty-two zebrafish genes with clear human homologues were differentially expressed across mutant, but not wild-type, embryos from the “mild” and “severe” backgrounds. We found 13 “protective”genes, those upregulated in mild mutants and/or downregulated in severe mutants and 9 “deleterious” genes, upregulated in severe mutants and/or downregulated in mild mutants. We show that insulin receptor a (insra) is a protective gene both necessary and sufficient for mild phenotypes and activator of Hsp90 ATPase homolog 1 (ahsa1) is a deleterious gene, necessary and sufficient for severe phenotypes. These response genes tend to be broadly expressed suggesting that they may be general regulators of craniofacial defects. Indeed, altering insra or ahsa1 levels changes the phenotypic severityof both platelet-derived growth factor aand collagen type 11 a2 mutants. Collectively, our results implicate a response gene network in regulating the phenotypic severity of craniofacial defects.

Program/Abstract # 99
The molecular mechanisms of SP8 activity during craniofacial development
Kasberg, Abi; Brunskill, Eric; Potter, Steve, Cincinnati Children's Hospital Research Foundation, Cincinnati, United States

Craniofacial abnormalities such as cleft palate affect 1 in 700 live births. Despite recent advances, the pathways responsible for causing craniofacial disorders remain largely unknown. We have shown that the mutation of the Sp8 gene, which encodes a zinc finger transcription factor, resulted in a dramatic absence of most facial structures. Sp8 mutants exhibited a failure of fusion along the midline, excencephaly, hyperterlorism, and cleft palate. In fact, careful skeletal analysis showed a dramatic loss of many neural crest and paraxial mesoderm derived cranial bones. Analysis of SOX9 expression revealed that Sp8 mutant neural crest located adjacent to the forebrain fail to differentiate and instead remain in a multipotent state. Immunofluorescent experiments showed high SP8 expression within the neuroepithelium including the anterior neural ridge, as well as the epidermal ectoderm. Specific loss of Sp8 in the anterior neuroepithelium produced mice with severe craniofacialmal formations most similar to the global Sp8 mutants, suggesting that anterior neuroepithelial Sp8 expression is critical during craniofacial development. Despite studies that have identified the roles of SP8 during limb outgrowth, the molecular targets of SP8 during craniofacial development remain unknown. Expression analysis at E9.5 indicated that Gli2, Gli3, Fgf8 and Fgf17 are reduced in the mutant anterior neural ridge signaling center. Gene expression analysis indicated a large reduction in the expression of Fgf17 in the olfactory pit of E10.5 mutants, but no apparent change in Fgf8. Of interest, the results also showed increased Smoothened in E10.5 mutants. The gene expression data coupled with the hypertelorism phenotype suggested an upregulation of SHH signaling in Sp8 mutants. Remarkably, embryonic exposure to cyclopamine, a SHH inhibitor, resulted in significant rescue of the craniofacial phenotype in Sp8 mutants. This suggests that elevated SHH signaling in the Sp8 mutants is a key effector of the mutant phenotype. It is interesting to note that FGF signaling has previously been reported to be able to repress the SHH pathway. Therefore, the data suggests that during craniofacial development SP8 might normally activate FGF signaling, which in turn represses SHH signaling.

Program/Abstract # 100
Regulation of jaw development by LAR receptor protein tyrosine phosphatases
Stewart, Katherine, McGill University, Montreal, Canada

Malformations of the lower jaw are associated with many developmental syndromes, and may additionally result in secondary defects of the oral cavity, including cleft palate. As such, understanding the normal patterning and specification of the mandible, as well as the etiology of secondary defects, may provide important therapeutic opportunities to children born within this spectrum of disorders. Recently we have demonstrated a requirement for the leukocyte antigen related (LAR)- family of receptor protein tyrosine phosphatases (RPTPs) in craniofacial development. Embryos lacking both RPTPσ and LAR exhibit micrognathia (small lower jaw), cleft palate, exencephaly (open neural tube) and open eyes at birth. We have currently extended that to include abnormalities in cranial bones and soft tissues derived from the first branchial arch, including defects in bones of the palate and mandible, as well as abnormal tongue shape. We have determined that cleft palate occurs secondary to abnormal mandible formation as initial palatal shelf outgrowth appears normal at E12.5, whereas misspecification of cartilage and bone in the anterior mandible is already apparent. However, the initial stages of craniofacial development, including the population of the first branchial arch by cranial neural crest cells, appear normal. Interestingly, concomitant loss of RPTPσ and LAR within the first branchial arch results in aberrant FGF signaling activity, potentially altering the patterning and subsequent differentiation of the mandibular arch tissue.

Program/Abstract # 101
The influence of novel FGF inhibitors on craniofacial and limb development
Horakova, Dana; Cela, Petra; Buchtova, Marcela, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
The family of fibroblast growth factors (FGFs) regulates many developmental processes including brain patterning, branching morphogenesis and limb development. The FGF ligands carry out their functions by binding and activating the fibroblast growth factor receptors (FGFR). The aim of our recent project is to compare the effect of three FGF inhibitors (NF449, PD161570, PD173074) on limb and craniofacial development. NF449 is inhibitor of FGFR3, PD161570 antagonizes all of the FGF receptors and PD173074 is selective inhibitor of FGFR1 and FGFR3. As chicken limb development is initiated at stage HH17 of embryonic period, we injected inhibitors into stages HH20–22 when the limb anlagen already protruded as the bud. NF449 (200uM) induced shortening and deformation of the wing with reduced autopodium. These malformed embryos mostly died at stage HH25–29. Embryos surviving until stage HH37–39 had normal phenotype and there were no skeletal changes in the wing. PD173074 (1mM) exhibited smaller autopodium, shorter and thinner wing bud. Higher concentrations (25mM and 50mM) resulted in development of small wing rudiment or the total absence of right wing without phenotypic changes on the left side and limbs. Occasionally, cleft lip was present. In comparison to NF449, embryos well survived even high concentrations of this inhibitor. Skeletal analysis displayed the absence of radius, the deformation of metacarpal bones or reduction of digits. PD161570 (1mM) treated wings were also shorter, smaller and deformed. Skeletal morphology resembled PD173074. Any of used inhibitors did not increased the number of apoptotic cells. This work is supported by the GA CR (304/09/0725) and IRP IPAG No. AVOZ 5045015.

Program/Abstract # 102
Ectodermal cell rearrangements in the early limb bud
Lau, Kimberly; Sorfazlian, Natalie; Sturgeon, Kendra; Tao, Hirotaka; Hopyan, Sevan, Hospital for Sick Children, Toronto, Canada
Two tissue types contribute to the initial limb bud. Having previously shown that limb field mesoderm exhibits oriented cell behaviours during initiation, we wished to determine whether the ectoderm contributes to early morphogenesis. We show by immunostaining that lateral plate ectodermal cells exhibit anisotropy that quantitatively changes in the limb field during initiation. Live imaging of mouse embryos harbouring a membrane-specific reporter reveals that dynamic cell rearrangements, including postmitotic daughter cell intercalation and multicellular rosette remodelling, are common in limb field ectoderm. Intriguingly, the orientation of these rearrangements is consistent with the axis of tissue growth. During limb initiation, mesodermal Fgf10 is received by Fgfr2 in ectoderm where it activates the canonical Wnt pathway. Using a live reporter, we find that canonical Wnt-responsive cells converge at the dorsoventral boundary, which might restrain the dorsoventral axis. By generating ectoderm-specific Fgfr2 and ß-catenin mutants, we confirm that initial limb buds fail to progress. Static and dynamic assessment of cell behaviour suggests these pathways influence planar anisotropy and are required to reorient ectodermal remodelling. These data strengthen the possibility that early morphogenesis and pattern specification are regulated by the same pathways. They also provide insight into the mechanisms underlying some human congenital anomalies.

Program/Abstract # 103
The role of Cad99C, the *Drosophila* orthologue of human Usher cadherin PCDH15, in apical membrane dynamics
Chung, SeYeon; Andrew, Deborah, Johns Hopkins Univ Sch of Med, Baltimore, United States
Usher Syndrome (USH) is the most frequent cause of hereditary deaf-blindness in humans. Nine USH disease genes have been identified, most of which are conserved from flies to humans. Cadherin99C (Cad99C), the fly orthologue of human Usher Cadherin PCDH15, regulates microvillar length in ovarian follicle cells and is also expressed in embryonic tubular organs including the salivary gland (SG). Analysis of Cad99C mutants at the light microscope level revealed subtle SG apical membrane defects, whereas analysis by electron microscopy revealed irregular apical membrane attachment to the extracellular matrix of the SG lumen. Over expression of Cad99C, on the other hand, resulted in profound changes in SG epithelial cells, including mislocalization of apical-basal markers, changes in cell shape from columnar to round, as well as loss of the SG lumen. Interestingly, a localized domain of high-level Cad99C staining, which co-localized with Cysteine String Protein (CSP), a secretory vesicle marker, and E-Cadherin, an adherens junction marker, was observed within Cad99C overexpressing SG cells. Electron microscopy of these SGs revealed large vesicle-like structures, which are likely to correspond to the domain of Cad99C staining. Overexpression of the extracellular region of Cad99C resulted in a subset of the phenotypes observed with the full-length protein, suggesting that the functions of Cad99C are separable. We propose a role for Cad99C in attachment of the apical epithelial surface to lumenal proteins. Too much attachment through Cad99C

overexpression is proposed to result in the engulfment and internalization of the lumenal matrix along with the apical membrane domain, resulting in a profound loss of cell polarity.

Program/Abstract # 104
The role of tbc-1 in *Drosophila* salivary gland development
Johnson, Dorothy M.; Andrew, Deborah Johns Hopkins School of Medicine, Baltimore, United States
Rabs are small GTPases involved in vesicle targeting, tethering, and fusion. Rabs' GTPase activity is accelerated by Rab-GAPs (GTPase activating proteins). Recently, a highly conserved *Drosophila* Rab-GAP, known as tbc-1,was discovered to be expressed in the embryonic salivary gland under control of the FoxA transcription factor Fork head (Fkh). An analysis of deficiencies and RNAi of tbc-1 revealed irregular apical membranes in embryos in which tbc-1 was knocked down in the salivary gland, suggesting that tbc-1has a role in salivary gland development. Based on these preliminary findings, tbc-1 knockout lines were generated by homologous recombination and verified by PCR analysis. Future plans include a full characterization of the null salivary gland phenotypes as well as studies to learn which Rab and which membrane fusion events are normally modulated by this GTPase.

Program/Abstract # 105
Wnt/ß-catenin has progressive, spatially-restricted roles in taste epithelium development.
Barlow, Linda; Thirumangalathu, Shoba, University of Colorado AMC, Aurora, United States
Taste buds, located in epithelial-mesenchymal papillae on the tongue, are neuroepithelial cells associated with epithelial appendages early in development. Postnatally, taste bud cells acquire neuronal properties, while papillae maintain a non-taste epithelium. In mice, Wnt governs specification and differentiation of buds and papillae, but its precise role in taste vs non-taste development have not been defined. Because taste precursors express Shh early on and can be distinguished from cells destined to form papillae, we can now test the cell type-specific function(s) of Wnt at precise stages of development. In this study, to distinguish taste precursor from papilla specific functions of Wnt, we activated ß-catenin exclusively within Shh expressing taste precursors during (E12.5) or after (E15.5) their specification. We demonstrate that β-catenin function within Shh expressing taste domains is stage specific with early activation resulting in expanded taste precursors with precocious differentiation of Type I taste cells, and these enlarged buds are situated in enlarged papillae. Importantly, Wnt activity is only reported within taste precursors, indicating that the effect of ß-catenin on papillae is indirect. Late ß-catenin activation causes accelerated differentiation of Type I cells, without taste bud or papilla expansion. Thus, Wnt functions autonomously within taste precursors to define the size of this population early, and regulates differentiation of Type I taste cells later. The impact of ß-catenin on papillae is indirect, but likewise temporally specific. In sum, our data support a model where Wnt/ß-catenin has progressive, spatially-restricted roles in taste epithelium development. Supported by DC008373 to LB

Program/Abstract # 106
Shh is required for development of the circumvallate taste papilla complex
Thirumangalathu, Shoba, Univ of Colorado Health Sci Ctr, United States; Barlow, Linda (UC Denver Anschutz Medical Campus, Aurora, United States)
The tongue is composed of anterior taste field housing rows of fungiform taste papillae each with a single taste bud, and posteriorly with bilateral foliate and a single midline circumvallate papilla (CVP). The CVP has deep epithelial trenches embedded with taste buds in association with lingual salivary glands named Von-Ebner glands (VEG). Sonic hedgehog (Shh) is a key regulator of anterior taste buds, where it represses taste specification, however, its role in the development of CVP/VEG complex has not been explored. Here we tested the temporal and spatial requirement of Shh in CVP/VEG complex development by genetic fate mapping and loss of function analyses. Shh is expressed in the early CV placode at E12.5, well before the VEG primordia have emerged. At birth, Shh-descendent placode cells identified by genetic fate tracking populate the apical epithelium of the circumvallate papilla, as well as the trenches, as immature taste cells. In adults, by contrast, Shh-descendent cells are lost from the trenches, and instead persist only as a scanty population in the apical epithelium. Conditional deletion of Shh in Shh-expressing cells commencing at the CVP placode stage (E12.5) results in impaired trench formation and to a complete absence of VEG at birth. These findings indicate, in contrast to its repressive function in anterior taste precursor specification, that Shh is required for CVP development. Thus, our data support the growing consensus that molecular regulation of the anterior and posterior taste fields differ significantly. Moreover, our findings indicate that Shh expressed by developing taste epithelium is a key regulator of salivary gland morphogenesis.

Program/Abstract # 107

Shroom3-dependent apical constriction requires an association with the adherens junctions through p120 catenin

Plageman, Timothy F.; Lang, Richard, Cincinnati Children's Hospital, Cincinnati, United States

During eye morphogenesis, the lens placodal cells elongate and adopt a wedge or conical shape in a process termed apical constriction (AC). This cell shape change drives lens pit invagination and requires the cytoskeletal protein Shroom3. Shroom3 activity is dependent on its interaction with Rock1, which stimulates the activation and contraction of the apically positioned actomyosin network. It has been shown in other models of AC that the contraction of actomyosin filaments generates force on the apical junctions pulling them toward the middle of the cell and effectively reducing the apical circumference. In lens placodal cells, we have similarly observed apically positioned myosin-containing filaments associated with adherens junctions at the point of deformation. It is currently unknown how the contractile actomyosin network in the lens placode is associated with the apical junctions and Shroom3-dependent AC machinery. To determine if Shroom3 genetically interacts with essential components of adherens junctions, Shroom3 targeted mice were bred with those containing conditional alleles of E-cadherin, N-cadherin, b-catenin, and p120 catenin (p120). Surprisingly, we found that Shroom3/p120 double heterozygotes displayed severe neural tube and eye morphogenetic defects at high penetrance suggesting that Shroom3 and p120 may be functioning together during epithelial morphogenesis. When conditionally removed from the lens pit we observed that p120 and Shroom3 deficient lens pits are similarly misshapen and that like Shroom3, p120 is required for lens pit AC. In addition, we found that p120 is required for Shroom3 induced AC in cultured cells. Together, these data suggest a potential interaction between the Shroom3-dependent AC complex and the apical junctions through p120 catenin.

Program/Abstract # 108

An essential role for claudins in neural tube closure in chick

Baumholtz, Amanda; Collins, Michelle; Simard, Annie; Ryan, Aimee (McGill University, Montreal, Canada)

Neurulation is a developmental process that results in the rolling up of a flat sheet of epithelial cells into an elongated tube. While the process of neurulation has been extensively studied, the genes that regulate the morphogenesis of the neural tube remain poorly understood. We have completed expression analyses of 17 members of the claudin family of tight junction proteins during neurulation in chick embryos. At neurulation, claudin family members exhibited three expression patterns: uniform expression across the ectoderm, reduced expression in the neural ectoderm and enriched expression in the neural ectoderm. To determine if claudins play a role in neural tube closure, we used the C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE) to knock down claudins in the ectoderm of chick embryos at the neural plate stage. Embryos were cultured with bacterially purified C-CPE using the *ex ovo* cornish pasty method. After 20 hours, GST-treated embryos developed normally while C-CPE-treated embryos had an open neural tube, a shortened anteroposterior (AP) axis and abnormally shaped somites. Neural tube defects (NTDs) were classified according to the level of the opening along the AP axis which corresponds to the human phenotype: 50% completely open (craniorachischisis), 25% open at anterior end (anencephaly), and 25% open at posterior end (spina bifida). Preliminary in situ hybridization analysis of the GST-C-CPE-treated embryos revealed that genes expressed in the neural and non-neural ectoderm have a normal expression pattern. These data suggest that claudins are required for neural tube closure and not for the initial differentiation of cells in the neural ectoderm.

Program/Abstract # 109

Cofilin1 and PTEN are involved in two cell autonomous processes required for cephalic neural tube closure.

Grego-Bessa, Joaquim; Anderson, Kathryn, Memorial Sloan Kettering Cancer Center, New York, United States

Closure of the mouse neural tube (NTC) is regulated by different genes along the anterior- posterior body axis. For example, Planar Cell Polarity mutants show NTC defects in the trunk, excluding the head; in contrast, Shroom mutants show NTC defects exclusively in the head. In this work we define new players that regulate closure of the cephalic neural tube. Cofilin 1 (Cfl1) is an actin binding protein that regulates actin dynamics by severing actin filaments. Strong Cfl1 mutants die at midgestation with prominent exencephaly. We find that Cfl1 mutants have dramatic defects in apical-basal polarity where a single cell can have two apical domains at opposite poles of the cell, as shown by ectopic localization of apical markers. As vesicular trafficking is required for cell polarity, we analyzed different vesicular markers in the neural plate. In WT they are localized along the apical-basal axis of neuroblasts, but in Cfl1 mutants they appear to accumulate to the apical surface, suggesting that Cfl1might regulate apical-basal polarity and NTC by regulating vesicular trafficking. The tumor suppressor gene PTEN can regulate proliferation, cell size, apoptosis and cell polarity. We found that conditional deletion of PTEN in the epiblast is lethal at E9.5 and mutants fail to close the cephalic neural tube. In this tissue, loss of PTEN does not affect proliferation, cell size or cell death, but instead prevents elongation of neuroblasts and

the mutant neural plate fails to become pseudostratified. The data suggest that Pten acts through stabilization of microtubules to control morphogenesis of the cranial neural plate.

Program/Abstract # 110
Cdon mutation and fetal ethanol exposure synergize to produce midline signaling defects and holoprosencephaly spectrum disorders in mice
Hong, Mingi; Krauss, Robert, Mount Sinai School of Medicine, New York, United States
Holoprosencephaly (HPE) is a remarkably common congenital anomaly characterized by failure to define the midline of the forebrain and midface. HPE is associated with heterozygous mutations in Sonic hedgehog (SHH) pathway components, but clinical presentation is extremely variable, and many mutation carriers are unaffected. It has been proposed that these observations are best explained by a multiple-hit model, in which the penetrance and expressivity of an HPE mutation is enhanced by a second mutation or the presence of cooperating, but otherwise silent, modifier genes. Non-genetic risk factors are also implicated in HPE, and gene-environment interactions may provide an alternative multiple-hit model to purely genetic multiple-hit models; however, there is little evidence for this contention.We report here a mouse model in which there is dramatic synergy between mutation of a bona fide HPE gene (Cdon, which encodes a SHH co-receptor) and a suspected HPE teratogen, ethanol. Loss of Cdon and in utero ethanol exposure in 129S6 mice give little or no phenotype individually, but together produce defects in early midline patterning, inhibition of SHH signaling in the developing forebrain and a broad spectrum of HPE phenotypes. Our findings argue that ethanol is indeed a risk factor for HPE, but genetically predisposed individuals, such as those with SHH pathway mutations, may be particularly susceptible. Furthermore, gene-environment interactions are likely to be important in the multifactorial etiology of HPE.

Program/Abstract # 111
FGF8 regulates multiple levels of neurogenesis in the zebrafish, from neural progenitor maintenance to differentiation
Dean, Benjamin, Vanderbilt University, Nashville, United States
The habenular nuclei are part of an evolutionarily conserved conduction system in the dorsal diencephalon of the vertebrate brain. The habenular nuclei are sites of pathogenesis and therapeutic intervention in addiction and depression. There is no clear understanding of how these crucial brain structures develop. During CNS development, fibroblast growth factors (FGFs) direct myriad developmental programs including the proliferation, migration and differentiation of neurons. FGF8 has been implicated in early habenular development. Murine hypomorphic mutants of fgf8 fail to specify dorsal diencephalic neurons including the habenular nuclei, due to altered anteroposterior (A/P) and dorsoventral (D/V) patterning (Martinez-Ferre et al., 2009). By contrast, we have found that an fgf8 null mutation in zebrafish does not alter A/P or D/V patterning in the dorsal diencephalon. Rather, zebrafish fgf8 mutants generate a reduced population of cells in the vicinity of the habenular nuclei, and most of these cells fail to differentiate. We have shown that this phenotype is in part due to reduced proliferation and increased cell death. However, it remains unclear what aspect of proliferation is perturbed, how fgf8 impacts differentiation and which FGF receptors and pathways mediate these programs. We will use the zebrafish fgf8 mutant to study how this brain region generates the appropriate number of neurons and how habenular precursor cells undergo the transition from undifferentiated precursor cells into mature neurons. Towards this goal, we will take advantage of the amenability of zebrafish embryos to transgenic overexpression, in vivo time lapse imaging and small molecule inhibition of signaling pathways.

Program/Abstract # 112
miR-153 regulates SNAP-25, synaptic transmission and neuronal development
Wei, Chunyao, Vanderbilt University, United States; Thatcher, Elizabeth (Worcester, United States); Olena, Abigail; Carter, Bruce; Broadie, Kendal; Patton, James (Nashville, United States)
SNAP-25 is a core component of the trimeric SNARE complex mediating vesicle exocytosis during membrane addition for neuronal growth, neuropeptide/growth factor secretion, and neurotransmitter release during synaptic transmission. Here, we report a novel microRNA mechanism of SNAP-25 regulation controlling neuronal development, neurosecretion, synaptic activity, and movement in zebrafish. Loss of miR-153 causes dramatic overexpression of SNAP-25 in neurons and consequent hyperactive movement in zebrafish embryos. Conversely, overexpression of miR-153 causes severe SNAP-25 down regulation resulting in near complete paralysis, mimicking the effects of treatment with Botulinum neurotoxin. Underlying the movement defects, perturbation of miR-153 function causes dramatic developmental changes in motorneuron patterning and branching and miR-153-dependent changes in synaptic activity at the neuromuscular junction are consistent with the observed movement defects. These results indicate that the precise control of SNAP-25 expression by miR-153 is critically important for proper neuronal growth patterning as well as neurotransmission.

Program/Abstract # 113
Requirement of microtubule based processes in dendrite maintenance
Lee, Jiae, University of Washington, Seattle, United States

Development of a cell into its proper shape is critical for its function. This is especially true in neurons, which display a great diversity in morphologies. I am using the *Drosophila melanogaster* peripheral nervous system (PNS) neurons to identify the cellular machinery involved in ensuring that dendrites maintain their proper shape. I carried out an EMS-induced mutagenesis screen to unravel the intrinsic factors involved in dendrite establishment and maintenance. From this screen, I have identified mutants that the dendrite branches distal from the cell body were lost, whereas the proximal area towards the cell body showed exuberant branching. This demonstrates the presence of distinct domains within the dendrites is required for proper maintenance of dendrite arborization. For further characterization of these genes, I monitored the progressivity of the phenotype, and the velocity and intracellular distribution of trafficking organelles. Since defects in dendrite maintenance have obvious implications for neuronal function and pathologies that are hallmarks of many neurodevelopmental diseases, understanding of the intrinsic signals will likely facilitate therapeutic intervention in MR and neurodevelopmental diseases with progressive dendrite pathologies.

Program/Abstract # 114
Talin: A master regulator of Cell-ECM adhesion-dependent morphogenesis
Ellis, Stephanie; Fairchild, Michael; Czerniecki, Stefan; Pines, Mary; Tanentzapf, Guy, University of British Columbia, Vancouver, Canada

Morphogenesis of a complex body plan requires coordinated regulation of cell adhesion molecules and the cytoskeleton to form distinct, organized tissues. Integrin adhesion receptors mediate ECM attachment and connect to the cytoskeleton through the adapter protein, talin. Talin interacts with many binding partners including integrin and F-actin. A delicate balance of these multiple interactions offers a means of fine-tuning integrin function and linkage to the cytoskeleton. Using targeted point mutations, we systematically investigate the role of different domains of talin during *Drosophila* embryogenesis. Our results suggest that morphogenetic events requiring short term, transient adhesions, such as germband retraction and dorsal closure, are highly sensitive to mutations in talin that compromise the ability to quickly disassemble adhesive contacts and linkage to the cytoskeleton. Conversely, in the embryonic and larval musculature, where myotendinous junctions form adhesive contacts that grow and persist over several days, talin interactions that strengthen attachment between integrins and the surrounding ECM are of greatest importance. Finally, using FRAP in the living embryo, we find that disruption of key domains in talin alters the dynamics of talin at adhesions, suggesting talin may be a master regulator of adhesion stability and cytoskeletal dynamics. Altogether, we demonstrate how the ability of talin to switch between multiple binding partners comprises an essential mechanism for modulating integrin function to elicit distinct developmental outcomes.

Program/Abstract # 115
Regulation of nonmuscle myosin II during *Drosophila* cellularization
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The *Drosophila* cellular blastoderm is formed by the enclosure of approximately 6,000 peripheral syncytial nuclei by plasma membrane. The bases of the newly formed cells are partially closed by the constriction of a network of actomyosin rings at the leading edge of membrane addition. We find that nonmuscle myosin II activity is required for both contraction and formation of these actomyosin rings. Mutation of the regulatory subunit of nonmuscle myosin II, myosin regulatory light chain (MRLC, Sqh), causes severe cytoskeletal defects during cellularization: actomyosin rings do not properly form, are disorganized, and do not contract. A number of different serine/threonine kinases, including Rho kinase (Rok), myosin light chain kinase (MLCK), and citron kinase, can phosphorylate and activate MRLC. We are investigating the roles of these potential MRLC regulators in controlling actomyosin dynamics at the cellularization front. Rok has been shown to be a key regulator of actomyosin during many morphogenetic events in *Drosophila*. Most of the defects in the cellularization actomyosin cytoskeleton in rok mutant embryos are caused by earlier embryonic defects; however, cellularization-specific defects to appear to be present. Mutation of one of the *Drosophila* MLCK genes, Strn-Mlck, produces no actomyosin cytoskeletal defects during cellularization. Mutation of another *Drosophila* MLCK gene, drak, cause striking defects in the organization of actomyosin rings and abrogates most MRLC phosphorylation during cellularization. Our results suggest that specific changesin the actomyosin cytoskeleton during cellularization are controlled by the regulation of myosin II activity by specific MRLC regulatory proteins.

Program/Abstract # 116

Structural changes of the nuclear envelope impact murine embryonic stem cell differentiation

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Murine embryonic stem cells can be differentiated as embryoid bodies or by treatment with retinoic acid and differentiation is associated with changes in chromatin conformation. It is thought that the nuclear lamina plays a role in organizing chromatin, with a high affinity for heterochromatin. We have investigated the roles of the nuclear envelope (NE), including the outer and inner nuclear membranes (ONM and INM), in ES cell differentiation. Using transmission electron microscopy we found that the lumen between the ONM and INM is 60% larger in ES cells when compared to differentiated cells, although actual nuclear pore density remained unaltered. Gene expression profiling revealed that the synthesis of several nuclear lamina and envelope proteins, including Syne1, lamin A/C and emerin are upregulated during differentiation. Differentiation was also associated with enrichment of heterochromatin attached to the nuclear lamina. Surprisingly, mice deficient for Syne1, emerin and/or laminA/C are capable of completing embryonic development. We have also found that ES cells deficient of NE proteins show only subtle changes in differentiation in vitro. However, overexpression of exogenous laminA and other NE structural proteins in undifferentiated ES cells changed the propensity of the cells to differentiate. We conclude that the NE and lamina undergo drastic structural changes during the differentiation of embryonic stem cells and that these changes exert an impact upon gene expression and differentiation.

Program/Abstract # 117

Eya1 mice as models for understanding middle ear developmental defects

Joshi, Leena, King's College London, United Kingdom

The mammalian middle ear is composed of three bony ossicles, the malleus, incus and stapes which function to conduct sound from the external ear via the tympanic membrane, to the inner ear through the oval window. Normal development of these three ossicles and the formation of a tissue and liquid free middle ear space are integral for this transduction of sound, defects resulting in conductive deafness. Branchio- oto- renal syndrome is a disorder of craniofacial development which causes developmental defects in both the middle and inner ear structures and accounts for 2% of profoundly deaf children worldwide. 40% of patients with this syndrome possess mutations in the Eya1 gene, one of several genetic factors influencing the development of this syndrome. Eya1 heterozygous mice exhibit variable hearing loss and possess extra ossicle like structures between the malleus and incus. We show that the extra ossicles originate from the incus as a result of ectopic joint formation. This provides us with a tool to investigate the mechanisms of ossicle morphogenesis and cartilage joint formation in the middle ear. Eya1 mice are further predisposed to developing otitis media (middle ear infections) in adult life, implicating Eya1 as a regulator of both embryonic and postnatal middle ear development.

Program/Abstract # 118

***In vivo* knock down of Wnt signalling components via shRNA in the inner ear anlage**

Funke, Constanze; Sienknecht, Ulrike J., Carl von Ossietzky University Oldenburg, Germany

Development of the vertebrate inner ear relies on elaborate morphogenesis. Finally, the resulting 3-dimensional apparatus consists of the dorsal semicircular canals and underneath the vestibular macula organs. Amniotes (land vertebrates), in addition, extend ventrally the cochlear duct housing a dedicated auditory organ. Involvement of Wnt signalling in both cell proliferation and programmed cell death necessary for proper morphogenesis has been shown for other organ systems. However, studies on the function of endogenous Wnts or Wnt inhibitors in the inner ear are still sparse. Our hypothesis is that Wnt signalling is capable of maintaining cells in an undifferentiated proliferative state and it also regulates apoptosis during chicken inner ear morphogenesis. Here we introduce a method for regional loss-of function of otherwise vital genes, adapted for studies of inner ear development. Gene silencing is carried out via shRNA (short hairpin RNA). Briefly: Genes such as Wnt9a or SFPR2 are targeted by custom-designed hairpins with a non-collapsing spacer and an additional restriction site to facilitate sequencing. We utilize a primer extension method with two oligos of different length (one hairpin containing template oligo and a short generic primer for PCR extension). Injection and electroporation of the hairpin expression vector into the otocyst is performed at embryonic day (E)3 prior to inner ear morphogenesis. Embryos are then harvested at desired stages of development. Gene knock down efficiency is evaluated on histological serial sections via RNA in-situ hybridization and the ear morphology is analyzed with the aid of immunohistology. Supported by CvO University Oldenburg

Program/Abstract # 119

Cardiac contractility and blood flow regulate cardiac form

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(Queens College, Flushing, United States); Leung, Alanna (Townsend Harris High School, Flushing, United States); Karp, Ariel (Queens College, Flushing, United States); Singleman, Corinna (Queens College, CUNY Biology, United States)

Cardiac morphogenesis is a tightly orchestrated dance between cardiac form and function. Cardiomyocytes begin to contract well before a complete heart tube forms and these contractions are fundamental to generating flow through the heart. This flow is detected as shear stress within the heart and directs further morphogenesis of the heart thus changing the flow patterns and continuing the cycle. We have identified a dominant zebrafish mutant in the atrial specific myosin myh6. Interestingly, this mutation results in modified blood flow leading to two distinct mutant phenotypes; an overly muscular heart that becomes constricted or a dilated myocardium. Close examination of these cardiomyopathies demonstrated two divergent contractility phenotypes; a non-contracting atrium and partially-contracting atrium. In the non-contracting mutants, the heart constricts, reducing the amount and efficiency of blood flow, resulting in death at around 5 days post fertilization. Partial-contractility results in embryos with a dilated myocardium, both in the atrium and ventricle, yet the majority of these fish are viable and live to adulthood with ongoing defects in cardiac form and presumably function. We are currently examining the blood flow and sheer stresses in myh6 mutants during early development and are conducting micro-array analysis to identify genes involved in initiating these two distinct phenotypes. We expect these studies to further develop the use of the myh6 mutants as a model for cardiomyopathy and generate an understanding of the molecular regulation of these divergent cardiomyopathies.

Program/Abstract # 120

Myocardial progenitors in the pharyngeal regions migrate to distinct conotruncal regions

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The cardiac progenitor cells for the heart outflow tract (OFT) reside in the visceral mesoderm of the pericardial coelom caudal to the developing OFT and mesodermal core of the anterior pharyngeal arches, which are defined as the SHF (secondary heart field) and AHF (anterior heart field), respectively. Although SHF/AHF is known to contribute to form conotruncal region, the destination of progenitor cells reside in each second lineage of heart forming region is not clarified. Using chick embryos, we injected fluorescent-dye into the SHF or AHF at stage 14 (ED 2), and the destinations of the labeled cells were examined at stage 31 (ED 7) by fluorescent stereoscopic microscope. To further examine, hearts were fixed, serial sections were cut, stained with anti-sarcomeric α-actinin and defined the distribution of the labeled cells. Dye-labeled cells from the right SHF were found in the α-actinin-positive myocardium on the left dorsal side of the OFT, and cells from the left SHF were detected on the right ventral myocardium of the OFT. Dye-labeled cells from the right and left AHF in the anterior two pairs of pharyngeal arches migrated to regions of the ventral wall of the OFT close to the aortic and pulmonary valves, respectively. These observations indicate that myocardial progenitors from the SHF and AHF contribute to distinct conotruncal regions, and that cells from the SHF migrate rotationally into the OFT while cells from the AHF in a non-rotational manner. Results suggest that spatiotemporal abnormal development in each of second lineage of heart forming regions may cause the specific spectrum of conotruncal heart defects.

Program/Abstract # 121

Ectodysplasin regulates hormone-independent mammary ductal morphogenesis via NF-kappaB

Voutilainen, Maria; Lindfors, Päivi; Lefebvre, Sylvie; Ahtiainen, Laura; Fliniaux, Ingrid; Rysti, Elisa; Murtoniemi, Marja (University of Helsinki, Helsinki, Finland); Schneider, Pascal (University of Lausanne, Epalinges, Switzerland); Schmidt-Ullrich, Ruth (Center for Molecular Medicine, Berlin,, Germany); Mikkola, Marja (University of Helsinki, Helsinki, Finland)

The mammary gland development begins during embryogenesis but is only completed during adulthood. Whereas the hormone dependent ductal growth during adulthood has been extensively studied relatively little is known about the molecular pathways controlling the early stages of ductal growth. Ectodysplasin (Eda), a member of the tumor necrosis factor family, is one of the regulators of skin appendage development in vertebrates. In activating mutations in Eda lead to hypohidrotic ectodermal dysplasia, which is characterized by hair abnormalities, missing teeth, and inability to sweat. The function of Eda in mammary gland development has not been studied in detail. We have previously shown that transgenic overexpression of Eda in developing ectoderm (K14-Eda mice) leads to formation of ectopic mammary placodes. Here we report that NF-kB, downstream of Eda, is a novel regulator of embryonic and pre-pubertal mammary ductal morphogenesis. Excess of Eda caused precocious and accelerated branching morphogenesis that was NF-kB-dependent.The opposite was seen with loss of Eda or inhibition of NF-kB which led to ductal trees with fewer branches. We have identified PTHrP, Wnt10a and Wnt10b, two Egf-family ligands (amphiregulin and epigen), as putative transcriptional targets of the Eda/NF-kB pathway. Using an ex vivo embryonic mammary bud culture system that we have

developed, we show that application of recombinant PTHrP, Egf-family ligands, or Wnt3A proteins stimulated branching of embryonic mammary glands suggesting that these pathways may cooperatively mediate the effects of Eda. Moreover, we show that in contrast to wild-type male mice, ductal growth proceeded in K14-Eda males similarly to transgenic females.

Program/Abstract # 122
Twisted gastrulation, an extracellular BMP binding protein, is required for postnatal mammary gland morphogenesis
Forsman, Cynthia, University of Minnesota Genetics, Minneapolis, United States

The role bone morphogenetic proteins (BMPs) play and how they are regulated in the postnatal mammary gland (MG) remains virtually unknown. It has been established that BMPs are involved in embryonic MG development and can be dysregulated in breast cancer. This study examines the role of Twisted gastrulation (TWSG1), an extracellular BMP binding protein that can modulate BMP signaling, during postnatal MG development. LacZ staining demonstrates that TWSG1 is expressed in the myoepithelium of the postnantal MG but immunolocalization studies show TWSG1 in both the myoepithelium and the epithelium suggesting a role for TWSG1 in epithelial/stromal communication. Global deletion of Twsg1 leads to a delayin ductal elongation, hyperplastic terminal end buds, occluded lumens and cell shedding. pSMAD1/5/8 level and the expression of BMP target genes (Msx2, Gata-3) are reduced, consistent with a decrease in BMP signaling. Apoptosis of excess body cells is required for lumen formation and in the Twsg1-/- MG apoptosis is reduced which may contribute to occluded lumens seen in these MG. Furthermore, shed cells and some cells within the luminal compartment are K14-positive, while normally K14-positive cells are restricted to the myoepithelial layer suggesting that regulation of BMP signaling by TWSG1 is required for myoepithelial compartmentalization. Altogether, these data show that TWSG1 facilitates BMP signaling and plays a role in ductal morphogenesis in the postnatal MG.

Program/Abstract # 123
Planar cell polarity proteins differentially regulate ECM organization during zebrafish gastrulation
Jessen, Jason; Williams, Blairanne; Mundell, Nathan, Vanderbilt University Medical Center Medicine, Nashville, United States

During zebrafish gastrulation, planar cell polarity (PCP) is defined as the elongation and mediolateral alignment of cells engaged in polarized behaviors including collective migration. A decade ago it was shown that homologs of proteins regulating PCP in fly cuticular structures also control PCP in gastrula cells. Loss of PCP gene function in mutants such as trilobite/vang-like 2 (vangl2) produces a phenotype characterized by shortened and broadened embryonic body axes. While it is thought that disrupted membrane protrusive activity underlies the PCP defect in vangl2 mutants, the establishment of PCP must be coordinated with, and perhaps also regulates, dynamic changes in extracellular matrix (ECM) organization. Previously our lab demonstrated that membrane type-1 matrix metalloproteinase (Mmp14) is required for PCP exhibiting a strong genetic interaction with knypek/glypican4, a Wnt co-receptor necessary for gastrulation cell movements. Subsequently we showed that a fibronectin- and laminin-containing ECM network develops coincidently with the onset of PCP. We have now demonstrated that Vangl2 regulates the endocytosis and cell surface proteolytic activity of Mmp14. Furthermore, our data show that Mmp14 acts as a downstream effector of Vangl2 signaling during gastrulation. Similar to vangl2 mutants, wild-type embryos injected with prickle antisense morpholinos have reduced fibronectin. By contrast, glypican4 and frizzled7a/7b mutant embryos exhibit increased fibronectin fibrillogenesis without an increase in fibronectin protein levels. These data suggest that while Vangl2/Prickle affect proteolysis of ECM substrates, Frizzled/Dishevelled signaling likely influences ECM organization by impacting intercellular adhesion.

Program/Abstract # 124
Dynamin is required for the maintenance of EVL integrity and the progression of epiboly
Lepage, Stephanie; Bruce, Ashley, Univ of Toronto, Canada

Epiboly, the first morphogenetic cell movement that occurs in the zebrafish embryo, is the process by which the blastoderm thins and spreads to engulf the yolk cell. This process requires the concerted actions of the deep cells, which make up the embryo proper, and the two extra-embryonic tissues, the enveloping layer (EVL) and yolk syncytial layer (YSL). One mechanism predicted to contribute to the progression of epiboly is the endocytic removal of yolk cell membrane, just ahead of the advancing blastoderm, in the region of the YSL. Using a drug-based approach, we demonstrate that marginal endocytosis occurs in a Dynamin-dependent and Clathrin-mediated manner and that inhibition of either Dynamin or Clathrin results in a severe epiboly delay. In contrast, localized expression of dominant-negative (DN)-Dynamin 2 in the yolk cell reduced marginal endocytosis, but epiboly still progressed normally. This suggests that endocytic removal of yolk cell membrane is dispensable for the successful progression and completion of epiboly. Instead,

these data imply that Dynamin is primarily required in the blastoderm during epiboly. Consistent with this finding, inhibition of Dynamin using the inhibitor dynasore caused profound defects in EVL cell morphology, polarity and tissue integrity. Scattered EVL cells adopted a rounded morphology, lost contact with their neighbours and were occasionally extruded basally from the epithelium. Expression of DN-Rho was able to rescue both the epiboly delay and constricted EVL cell phenotype; however, this occurred independently of Myosin II since the effects of dynasore treatment could not be rescued by the Myosin II inhibitor blebbistatin. Members of the Ezrin/Radixin/Moesin (ERM) protein family are key regulators of cortical tension and epithelial integrity in other systems. EVL cells of dynasore treated embryos had a striking reduction in cortically localized phosphorylated ERM. In addition, morpholino knockdown of a single ERM family member, ezrin, phenocopied the dynasore-induced epiboly delay and EVL defects. Taken together, these data suggest that Dynamin maintains EVL integrity and promotes epiboly progression by maintaining ERM activity at the EVL cell cortex and antagonizing Rho signaling.

Program/Abstract # 125
Functional studies of Fam132a/C1qdc2, a secreted molecule downstream of Stat3 signaling, during zebrafish gastrulation
Liu, Yinzi; Sepich, Diane; Shin, Jimann; Solnica-Krezel, Lilianna, WUSTL, Saint Louis, United States
Convergence and extension (C&E) are evolutionarily conserved vertebrate gastrulation movements, which narrow the germ layers mediolaterally and elongate them anteroposteriorly. Many pathways have been implicated in regulating these processes, including Stat3 signaling in zebrafish. Conserved from *Drosophila* to mammals, Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway mediates diverse biological processes through transcriptional regulation of downstream targets in response to cytokines and growth factors. During zebrafish gastrulation, activated Stat3 is required cell-autonomously for the anterior migration of the axialmesodermal cells originating in the gastrula organizer, and also regulates convergence of lateral mesendodermal cells non cell-autonomously. However, the underlying mechanisms remain poorly understood. Here, we identified Fam132a, or family with sequence similarity 132a, C1q domain containing 2 (C1qdc2), a conserved protein of unknown function, as a downstream target of Stat3 signaling by comparing gene expression profiles between stat3-deficient and wild-type gastrulae. We found that zebrafish fam132a is both maternally and zygotically expressed. Fam132a-GFP fusion protein appears to be secreted into the extracellular space and forms puncta during blastula and gastrula stages. Interestingly, over expression of fam132a resulted in not only independent C&E defects, but dorsoventral patterning defects as well. We will report ongoing loss-of-function analysis of fam132a through transcription activator-like effector nuclease(TALEN).

Program/Abstract # 126
Gastrulation in high-resolution: New insights into an important process of development.
Martik, Megan; McClay, David, Duke University, Durham, United States
Gastrulation is a complex orchestration of movements by cells that are specified early in development. Until now, it was thought that lateral rearrangement of endoderm cells by convergent extension was the main contributor to sea urchin archenteron elongation. Our project characterizes, at high-resolution, the repertoire of cellular movements contributing to the length of gut. We have performed cell transplantation to live image and analyze a subset of labeled endoderm cells in the optically clear sea urchin embryo. We have found that the endoderm cells that initially invaginate remain contiguous throughout extension, so that, if convergent extension is present, it is not a major contributor to elongation. We have also found, unexpectedly, that endoderm cells proliferate as they move to elongate the archenteron. Our descriptive studies of the cellular processes during gastrulation have allowed us to begin investigating their molecular control. The sea urchin endomesoderm gene regulatory network (GRN) describes the cell fate specification of the future larval gut; however, the GRN does not describe specific cell biological events driving morphogenesis. We plan to dissect the transcriptional circuitry of the GRN responsible for the cell biological events of gastrulation. Our ability to connect the endomesoderm GRN to the morphogenetic events of gastrulation will provide a framework for characterizing this remarkable sequence of cell movements in the simplest of deuterostome models at an unprecedented scale.

Program/Abstract # 127
Functions of p120-catenin in the developing mouse embryo.
Hernández-Martínez, Rocío, Sloan-Kettering Institute, United States; Anderson, Kathryn, V., Sloan-Kettering Institute, New York, U.S.A.
p120-catenin is an member armadillo family proteins that regulate intercellular junctions. The best-defined role of p120-ctn is to promote the stabilization of cadherins on the cell surface through inhibition of endocytosis. In addition, it is has been observed that p120-ctn also functionally interacts with specific Rho-GTPases family that are important regulators of

cytoskeletal dynamics and cell migration. In the mouse, tissue-specific gene targeting experiments have shown that p120-ctn may act through different mechanisms to promote cell-cell adhesion or cell migration indifferent cell types. In the mouse deletion of p120-ctn is embryonic lethal. We are characterizing the phenotypes of p120-ctn mutant embryos to determine a possible mechanism linking signaling and migration during early morphogenesis of the embryo. Embryos lacking p120-ctn show morphogenetic defects including axial duplication and defects in mesoderm migration. The production of mesoderm cells at the correct position in the embryo depends on the earlier movement of Anterior Visceral Endoderm (AVE) cells, and we observe that AVE migration is abnormal in p120-ctn null embryos. Currently we are analyzing changes in cell behavior of the Hex-GFP expressing AVE cells using time-lapse imaging in early embryos. In addition we are analyzing whether these defects are associated with changes in E-cadherin. To determine the specific roles of p120-ctn in the later embryo we ablated the gene specifically in the epiblast and we are characterizing that phenotype. Our findings will define the functions of p120-ctn in normal development and will contribute to understand its roles in diverse types of cancer cells.

Program/Abstract # 128

The polarity complex Par6b/Par3 is required for the normal pattern and function of cadherins in ectoderm cells

Wang, Sha; Cha, Sang-wook; Wylie, Christopher Cincinnati Children's Hospital Med Center, Cincinnati, United States)

The Par (partitioning defective) proteins Par6b and Par3 are expressed in *Xenopus* ectoderm. Here we show that the Par6b/ Par3 complex is required for the normal pattern and function of cadherins expressed by the ectoderm cells. Depletion of Par6b in the non-neural ectoderm (the presumptive epidermis) causes a loss of E-cadherin. Cell-cell adhesion is retained at first due to the continued expression of C-cadherin. Embryos start to shed epidermis around stage 32 when epidermal C-cadherin is switched off. The neural ectoderm is also affected by Par6b depletion, which causes disruption of N-cadherin expression at the cell surface and failure of neural fold closure. Depletion of Par3 shows similar effects as Par6b knockdown, suggesting that Par6b and Par3 regulate cadherins as one functional complex or in the same pathway. Expression of the apical marker Crumb3 and the baso-lateral marker Lgl2 suggests that Par6b depletion causes a change of cell polarity leading to Crumb3 being stabilized all around the membrane of the non-neural ectoderm cells, whilst Lgl2 level is reduced. This may explain the loss of baso-laterally expressed E-cadherin. In addition, the tight junctions that structurally define the apical-basolateral border are also reduced by depletion of Par6b. These data together show that cadherins are under the control of the Par6b/Par3 complex, and that this complex controls the boundary between apical and baso-lateral membrane in the early *Xenopus* ectoderm.

Program/Abstract # 129

Claudins are required for ureteric bud branching during kidney morphogenesis

Khairallah, Halim; El Andalousi, Jasmine; Ryan, Aimee; Gupta, Indra (McGill, Monntreal, Canada)

The claudin family of integral tight junction proteins has documented roles in tissue and organ morphogenesis, including tubule and lumen formation of epithelial cell layers. In the adult mouse kidney, claudin family members are expressed along the nephron and determine the specific paracellulartransport properties of each nephron segment. The function of claudin proteins during kidney morphogenesis remains unclear. During kidney development, the ureteric bud emerges from the nephric duct and undergoes branching morphogenesis: the ureteric bud elongates and divides in a series of repeated bifurcations at the ureteric bud tips which ultimately give rise to the collecting ducts of the adult kidney. We have shown that claudin-3 is expressed in the nephric duct and ureteric bud and promotes tubulogenesis in vitro. Based on our in vitro studies, we hypothesize that claudins are required for ureteric bud branching. Using RT-PCR and in situ hybridization analysis we found that 15 claudins are expressed at critical time points during mouse kidney development. Removal of multiple claudins was performed by growing mouse embryonic kidney explants in the presence of the C-terminus of Clostridium perfringens enterotoxin (C-CPE), which is known to bind to specific claudins and remove them from tight junctions. Explants treated with C-CPE demonstrated a decrease in ureteric bud tip number and an increase in ureteric bud stalk length compared to controls. We have targeted individual claudins using morpholinos and shown that knockdown of claudin-3 decreases ureteric bud branching. In conclusion, our data suggest that claudins have a role in ureteric bud branching during kidney morphogenesis.

Program/Abstract # 130

MAPK pathway is required for branch point determination

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Congenital renal malformations are common birth defects affecting approximately 1% of the infants and often arising from the defects in ureteric bud (UB) development. Glial cell line-derived neurotrophic factor (GDNF) signaling through the RET receptor tyrosine kinase is essential for ureteric bud outgrowth but the events that occur downstream of RET to promote ureter morphogenesis remain largely obscure. In addition, the cellular mechanisms by which GDNF/RET signaling promotes kidney growth through branching morphogenesis remain to be discovered. We have focused our studies on the function of mitogen activated protein kinase (MAPK) pathway in the ureteric bud (UB) lineages. Mice lacking MAPK activity specifically in the UB initiate renal morphogenesis normally and are capable of undergoing nephrogenesis, but fail to form complex UB patterns. The analysis of Hoxb7CreGFP; Mek1F/F; Mek 2kidney phenotype revealed that only a fraction of previously identified GDNF target genes are regulated through this pathway. The results also suggest specific role for MAPK pathway in branch point determination potentially through the maintenance of epithelial integrity.

Program/Abstract # 131

Calcium/NFAT signaling is essential for mesenchymal-epithelial transition during nephron formation

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During kidney development, a subpopulation of stem cells in the metanephric mesenchyme (MM) undergoes mesenchymal-epithelial transition (MET) to form the tubular segments of the nephron. To elucidate the mechanism of MET, we developed a culture system for the maintenance and propagation of MM progenitor cells and have demonstrated that WNT4 induces MET by a non-canonical Wnt/calcium pathway and not by a canonical Wnt/β-catenin pathway as previously believed. WNT4 stimulated calcium influx and phospholylation of CaMKII in MM cells, and Ionomycin, a calcium ionophore, induced MET in MM, indicating that activation of the calcium signaling pathway is sufficient for MET induction. Leukemia inhibitory factor (LIF) also induced MET in MM and similarly activated calcium signaling and induced phosphorylation of CaMKII. Reporter activity for the transcription factor, Nuclear Factor Activated T-cells (NFAT), which has been implicated in calcium signaling, was greatly elevated in MM cells treated with WNT4 or LIF, and expression of a constitutively active form of NFAT in MM cells up regulated MET markers, consistent with a possible role for NFAT in this morphogenesis. GSK3β normally blocks the nuclear accumulation of NFAT by phosphorylation, and GSK3β inhibitor CHIR99021 causes NFAT dephosphorylation, allowing its translocation to the nucleus for transcription. In our system, CHIR99021 activated an NFAT reporter and induced MET in MM cells. Finally, induction of MET by WNT4, LIF or CHIR99021 was blocked by the NFAT inhibitor cyclosporin A, but not a β-catenin peptide inhibitor, suggesting that NFAT is required for MET and that these very different signaling molecules share a common calcium-dependent process for MET. These results demonstrate that the calcium/NFAT pathway is essential for morphogenesis of nephronic stem cells and provide insight into key signaling mechanisms involved in kidney development.

Program/Abstract # 132

Incidence of vesicoureteric reflux and other urinary tract abnormalities in OSR1 deficient mice

Watt, Christine; El Andalousi, Jasmine; Fillion, Marie-Lyne; Gupta, Indra, McGill, Montreal, Canada

Congenital abnormalities of the kidney and urinary tract (CAKUT) are a cause of chronic kidney disease and encompass a spectrum of phenotypes including vesicoureteric reflux (VUR), the retrograde movement of urine from the bladder to the kidney, duplex systems and/or urinary tract (UT) obstruction. This broad range of phenotypes is attributed to aberrations at different key developmental time points. The kidney and UT arise from the ureteric bud (UB), an epithelial structure derived from reciprocal signaling between the nephric duct and surrounding metanephric mesenchyme. The distal portion of the UB will branch and form the kidney, while the proximal portion will elongate to form the ureter. Initially, the ureter is connected to the nephric duct via the common nephric duct and subsequently separates, moving to its final insertion point in the bladder. We have identified the C3H mouse as a model of fully penetrant VUR and identified a 22Mb susceptibility locus on proximal chromosome12, the Vurm1 locus that contains many candidate genes including, Odd Skipped Related 1 (Osr1), a gene encoding a zinc finger protein, that has been implicated in kidney and UT development. Osr1 is expressed in intermediate and undifferentiated metanephric mesenchyme and homozygous null mice are anephric. Analysis of B6.129S1-Osr1tm1Jian/J+/-(Osr1+/-) mice has shown an increased incidence of VUR:35% in Osr1+/- (5/14) compared to 3% in wild-type littermates (1/26). Other UT abnormalities in addition to VUR are also observed, including duplex kidneys, bifidureters, and urinary tract obstruction. This data suggests that haploinsufficiency of OSR1 has a critical impact on urinary tract development.

Program/Abstract # 133

Three-dimensional modeling of the zebrafish liver network reveals regulators of biliary morphogenesis.

Justin M.Nussbaum; Hasan, Ayesha; Sakaguchi, Takuya (Lerner Research Institute).

A primary function of the liver is to produce bile and transfer it from hepatocytes to the biliary network, a complex series of ducts comprising specialized epithelial cells (cholangiocytes). While the structure of the mature biliary network is well characterized, the cellular and molecular mechanisms by which it differentiates and branches out to form a complex network are poorly understood. Identifying regulators of morphogenesis is currently hindered by lack of an efficient and accurate means of quantitating changes in network development. To overcome this barrier, we have combined three-dimensional confocal imaging with a novel computer-based algorithm to identify individual structures within cellular networks and render them as skeletonized structures. After rendering the network as a series of discrete points, quantitative data describing the dimensions of each structure within the network (intersections, connecting branches, terminal branches etc...) and their interactions with each other are then computed. We also demonstrate the utility of this tool for quantitatively tracking biliary network development in individual samples through time by coupling it to live multiphoton confocal imaging. Furthermore, we demonstrate the practical utility of these tools through the identification of multiple regulators of biliary morphogenesis through small molecule and forward genetic screens.

Program/Abstract # 134

Apical contraction of the actomyosin network initiates branching morphogenesis of the embryonic chicken lung

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During development of the lung, branching morphogenesis sculpts the airway epithelium to maximize the surface area available for gas exchange. The epithelial tube undergoes iterative rounds of morphogenetic routines including bud formation, extension, and bifurcation to build the complex tree-like structure of the lung. Despite many biochemical signals implicated in regulation of the branching process, little is known about the physical mechanisms that build the airways. Here, we used the embryonic chicken lung to investigate remodeling of the actomyosin network and extracellular matrix (ECM) during bud initiation. Lungs from 4-6-day-old embryos were immunostained and visualized using confocal microscopy. 3D reconstruction of confocal stacks revealed that filamentous actin was enriched at the apical surface of the emerging bud, and colocalized with phosphorylated myosin light chain. Moreover, inhibition of myosin contractility blocked bud initiation, indicating that apical contraction of the actomyosin network is required to fold the epithelium into a nascent bud. Furthermore, among several ECM proteins we found a distinct localization of tenascin-C (TNC). In addition to the basement membrane, TNC was deposited preferentially in mesenchyme adjacent to the expanded and elongated buds, but not in mesenchyme surrounding a newly forming bud. Together, our observations suggest that apical contraction is the cellular machinery that induces nascent bud formation in the developing chicken lung, and TNC might indicate the location of mechanical signals that act on the mesenchyme through the growing epithelial buds.

Program/Abstract # 135

Computational mechanobiology of peristalsis in embryonic lung

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The lung is optimized for efficient transport of air in its lumen, yet it develops with a liquid-filled lumen. It is well established that both prenatal occlusion and airway peristalsis (AP) increase branching morphogenesis, but the mechanisms of their actions remain undetermined. Given that both occlusion and AP affect both transport and mechanics in the lung, it is important to understand the mechanics and transport in the control and treatments. We present a study of the fluid-tissue interactions of the pseudoglandular embryonic lung and its lumen contents. Our analysis suggests that some hypothesized mechanosensing mechanisms may be irrelevant in the context of airway branching.

Program/Abstract # 136

Over-expression of receptors for advanced glycation end-products (RAGE) causes anomalous epithelial cell survival and differentiation in the embryonic murine lung

Reynolds, Paul, Brigham Young University, United States

RAGE is a multi-ligand membrane receptor highly expressed in the developing lung and in inflammatory lung disease. However, the contributions of RAGE to pulmonary organogenesis remain poorly characterized. In order to test the hypothesis that RAGE misexpression affects lung morphogenesis, conditional transgenic mice were generated that over-express RAGE. Over-expression of RAGE throughout embryogenesis resulted in severe lung hypoplasia and perinatal lethality. Flow cytometry and immunohistochemistry employing cell-specific markers demonstrated anomalies in key epithelial cell types following RAGE up-regulation. Electron microscopy identified significant morphological disturbances including blebbing of epithelium and loss of basement membrane integrity. Possible RAGE-mediated mechanisms leading to the disappearance of pulmonary tissue were then evaluated. A time course of lung organogenesis demonstrated that increased RAGE expression primarily alters lung morphogenesis beginning at E16.5. TUNEL immunostaining and blotting for active caspase-3 confirm a shift toward apoptosis in lungs from RAGE over-expressing mice compared to controls.

Elevated nuclear translocation of NF-κB was also discovered in lungs from transgenic mice. An evaluation of genes regulated by NF-κB demonstrated elevated expression of Fas ligand, suggesting increased activity of the Fas-mediated signaling pathway in which ligand-receptor interaction triggers cell death. These data provide evidence that RAGE expression must be tightly regulated during organogenesis and that RAGE signaling during branching morphogenesis may provide insight into the progression of developmental lung anomalies including bronchopulmonary dysplasia.

Program/Abstract # 137

Glycosaminoglycan biosynthesis is required non-cell-autonomously for correct patterning of the dorso-anterior *Drosophila* eggshell by the Epidermal Growth Factor Receptor ligand Gurken

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Graded EGF-R signaling within the follicle cell epithelium occurs in response to a germline-derived ligand, Gurken (Grk), secreted dorso-anteriorly near the position of the oocyte nucleus. High Grk levels specify dorsal midline, intermediate levels specify position of the dorsal appendages, and lowest levels allow expression of Pipe ventrally to later define the dorsoventral axis of the embryo. Prior studies by Li-Mei Pai's lab showed that over-expression of Dally-like, a membrane-linked heparan sulfate proteoglycan (HSPG), results in a shallower extracellular gradient ofGrk and a reduced gap between the dorsal appendages, indicating loss of high-Grk domain, while the Pai lab and David Stein's lab report that Fringe Connection (frc) and Sulfateless (sfl), enzymes involved in HS synthesis and sulfation, respectively, are not required for eggshell patterning. We examined strong mutant alleles of protein O-xylosyl transferase (oxt), the first and rate-limiting enzyme acting in glycosaminoglycan (GAG) synthesis. Egg chambers containing anterior or complete oxt-mutant follicle cell clones show increases in size of the dorsal gap between dorsal appendage primordia from 3-4 cells wide to up to 6-7 cells wide, while small clones do not exhibit cell-autonomous changes in fate. These findings suggest loss of follicle cell-derived GAGs is associated with expansion of the high-Grk domain. The most notable change in Grk distribution is its ectopic presence within germline vesicles in affected egg chambers. One possibility suggested by this data is that there is reduced uptake and degradation of Grk by follicle cells when it is not bound to GAGs, leading to increased EGF-R signaling and perhaps alternative uptake into the oocyte.

Program/Abstract # 138

Mirror and Paxillin act downstream of Tramtrack69 to regulate tube morphogenesis in the *Drosophila* ovary

Peters, Nathaniel C., University of Washington Genome Sciences, United States

Epithelial tubes are essential for organ and tissue function, and faithful tubulogenesis requires precise orchestration of cell signaling, shape change, migration, and adhesion. Epithelial follicle cells in the *Drosophila* ovary undergo morphogenesis to form a pair of tubes, the apical lumens of which act as molds for the egg shell respiratory filaments, or dorsal appendages (DAs); this system is a robust and accessible model for studying epithelial tube patterning, formation, and expansion. The Tramtrack69 (TTK69) transcription factor controls DA lumen volume by regulating apical surface area and tube expansion; the twin peaks mutant reduces TTK69 levels specifically late in oogenesis, inhibiting tube expansion and stunting the DAs. Microarray analysis of wild type and twin peaks ovaries, followed by in situ validation of candidates, identified mirror and paxillin as TTK69 targets. mirror encodes a homeodomain protein that patterns the epithelium prior to DA tubulogenesis, but post-patterning RNAi-knockdown disrupted DA tube expansion, indicating that mirror may have a novel role in DA tubulogenesis. paxillin expression is highest in the DA tube cells just prior to tube expansion, and RNAi-knockdown of paxillin resulted in DA tube defects. We are now determining whether Paxillin, a scaffolding protein, interacts with Integrin-based cell adhesions, JNK signaling, and/or GPCR signaling, all of which are required for DA tubulogenesis, and whether TTK69 regulates paxillin via a mirror-dependent pathway. Further characterization of TTK69 targets, such as paxillin and mirror, will help illuminate the network of transcription factors, signaling pathways, and cytoskeletal regulators required for epithelial tube morphogenesis.

Program/Abstract # 139

ErbB signaling within Schwann cells controls quiescence of zebrafish lateral line progenitor cells through regulation of Wnt and FGF signaling.

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The Zebrafish lateral line is a mechanosensory system consisting of neuromasts containing support cells and sensory hair cells. The formation of the lateral line has proven an excellent model for the study of developmental events, such as collective cell migration. Here we show that it is also a unique model to study stem cell biology. Previous work from our laboratory revealed a role for glia in negatively regulating proliferation and differentiation of lateral line stem cells. Mutations in erbb2, erbb3b and nrg1-3 have increased neuromast numbers. We show by transplantation experiments and

glial specific overexpression of a dominant negative ErbB (DN-ErbB) that erbb is required in glia while nrg1-3 is required in neurons. Treating embryos with the ErbB inhibitor AG1478 or overexpression of DN-ErbB after glial migration also leads to increased neuromast number suggesting that ErbBs are continuously required within glia to inhibit precocious differentiation of neuromasts. Wnt and Fgf signaling pathways, required for early neurogenesis in the lateral line, are also upregulated in ErbB mutants. We find that activating Wnt or inhibiting FGF signaling induces proliferation of interneuromast cells. Conversely extra neuromasts are reduced by inhibition of Wnt or activation of FGF signaling. Thus, ErbBs are inhibiting progenitor proliferation non-cell autonomously by regulating Wnt and Fgf pathways. Elucidating the interactions between ErbB and Wnt and FGF is not only important for understanding the control of cell proliferation during normal development but will also contribute to our understanding of how misregulation of the ErbB signaling pathway contributes to cancer.

Program/Abstract # 140

***In vitro* embryonic axial elongation morphogenesis using mammalian stem cells**

Marikawa, Yusuke; Tamashiro, Dana Ann A., University of Hawaii, Honolulu, United States

During mammalian development, the primitive streak forms at the posterior end of the embryo, and continuously generates germ layers to build a body that is elongated along the anterior-posterior axis. To investigate the mechanisms of axial morphogenesis in mammalian embryos, we previously created an in vitro model of axial elongation using embryoid body (EB) of mouse P19 embryonal carcinoma cells. In this system, aggregated P19 cells that are cultured in hanging drops of medium, up-regulate the expression of various posterior genes, and also exhibit axial elongation morphogenesis. Here, we used P19 EB as a tool to search for genes that are essential for axial elongation morphogenesis. Genes that are strongly expressed at the posterior end of the embryo were selected based on microarray screening of dissected mouse embryos and whole mount in situ hybridization analysis. Candidate genes were knocked down by stable transfection of specific shRNA plasmids in P19 cells, and the ability of knockdown P19 cells to display axial elongation morphogenesis was examined. We found that the knockdown of Chst7 (carbohydrate [N-acetylglucosamino] sulfotransferase 7) or Dgkk (diacylglycerolkinase kappa) interfered with elongation of P19 EB, which implicates that these two genes play essential roles in axis morphogenesis. We are now investigating how these genes are involved in the regulation of axial morphogenesis. In addition, we have developed a culture condition that enabled EB of human embryonic stem cells (hESCs) to exhibit elongation morphogenesis in vitro. We are currently determining whether elongation of hESC EB is equivalent to that of P19 EB with respect to molecular regulations.

Program/Abstract # 141

A single-cell resolution Notch signaling reporter strain mice

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Live cell imaging is an essential tool for understanding the highly dynamic and coordinated events that drive cell lineage specification and morphogenesis during mammalian development. To elucidate the critical role of signaling pathways and begin to assay signal responsiveness, signaling reporter strains can be engineered by placing signaling responsive elements to direct the expression of reporter genes. To date, several transgenic Notch reporter strains have been generated marking the sites of active Notch signaling during development. Here we present the construction of a fluorescent protein-based single-cell resolution Notch signaling reporter designed for live visualization and tracking of individual cells in vivo in mouse embryos and adults and ex vivo in stem cells such as ES cells. We have placed a CBF (also called RBP-Jk and CSL) responsive element (CBFRE) containing 4 copies of the mouse CBF1 binding sites and an SV40 minimal promoter in front of a fluorescent protein fusion comprising human histone H2B linked to the Venus fluorescent protein. We have used the CBFRE::H2B-Venus construct to generate transgenic ES cells and a strain of transgenic mice designed to report a transcriptional readout of Notch activity. Characterization of developmental and adult stages of the resulting CBFRE::H2B-Venus strain reveal discrete and specific expression of the transgene at previously characterized and uncharacterized sites of Notch signaling. Our current validation of the CBFRE::H2B-Venus strain will be presented and should allow us to determine whether it faithfully reports Notch activity at single cell resolution in vivo.

Program/Abstract # 142

Crosstalk between cell cycle and cytoskeletal rearrangements during hair follicle morphogenesis

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Organogenesis depends on reciprocal interactions between tissues which result in transcriptional changes that regulate cell cycle progression and cytoskeletal reorganization and drive organization of cells into organs. Epidermal development exemplifies this idea, where in response to inductive signals from the underlying dermis, a single layer of epithelial

progenitor cells that cover the surface of the embryo either stratify to form epidermis or invaginate to form hair follicles (HFs). Dramatic morphological changes take place during the first steps of HF formation at the placode stage. Dermal cells are recruited to form the dermal condensate that will later give rise to the dermal papilla, while overlaying epidermal cells assume more elongated columnar morphology. Additionally, placode downgrowth is asymmetric, such that HFs grow at an acute angle towards the anterior of the mouse. Several signaling pathways, e.g. Wnt, sonic hedgehog (Shh), and transforming growth factor beta 2 (TGF-β2) regulate HF development, but how various pathways interact and regulate cell cycle and cytoskeleton resulting in HF downgrowth and differentiation remains largely unknown. We use a novel lentivirus-based in vivo gene knockdown approach to explore these fascinating questions.

Program/Abstract # 143
Mechanisms of Integrin-linked kinase modulation of hair follicle morphogenesis.
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Integrin-linked kinase (ILK) is essential for hair follicle (HF) morphogenesis. Hair follicle development arrests at stage 4-5 in ILK-deficient epidermis. To identify the processes modulated by ILK in developing HF, we examined alterations in factors important for HF morphogenesis in embryonic ILK-deficient epidermis. LEF1 expression in embryonic day (E) 15.5 epidermal condensates is not altered in ILK-deficient epidermis indicating that ILK is not required for initial activation of the Wnt pathway during HF specification. In contrast, LEF1 expression decreased in ILK-deficient E17.5 hair pegs (stage 4-5). At E17.5, sonic hedgehog (Shh) signaling participates in furthering HF development. Significantly, Gli1, a marker and mediator of Shh signaling, is expressed in E15.5-E17.5 mutant epidermis. In contrast, in the absence of ILK P-cadherin expression is decreased, and this is accompanied by abnormal spatial expression of E-cadherin. This suggests that ILK is required to modulate the pathways involved in upregulation of P-cadherin in coordination with downregulation of E-cadherin during normal HF development. ILK-deficient HFs also show aberrant laminin 511 deposition in regions between the hair matrix and the dermal papilla. In the latter, expression of key markers, such as CD133, is also impaired. Thus, in the absence of ILK the second wave of Wnt signaling is altered during HF formation at stage 4-5, which is associated with alterations in E- and P-cadherin expresion and, potentially, cell-cell communication. ILK is also likely necessary for normal interactions between the developing HF epithelium and the underlying mesenchymal cells in dermal papilla. Supported with funds from the Canadian Institutes of Health Research.

Program/Abstract # 144
Olfactory microvillous neurons arise from the neural crest in a Sox10-dependent manner
Saxena, Ankur; Peng, Brian; Bronner, Marianne, California Institute of Technology, Pasadena, United States

Development of the vertebrate olfactory system involves a complex interplay between elements of the central and peripheral nervous systems. Classically, the olfactory ectodermal placode has been presumed to form all olfactory sensory neurons. In contrast, here we show that cranial neural crest is the primary source of microvillous sensory neurons within the olfactory epithelium of zebrafish. Using photo conversion-based fate mapping and live cell tracking coupled with laser ablation, we followed neural crest precursors in transgenic zebrafish embryos as they migrated from the neural tube to the nasal cavity. A subset of these cells, coexpressing the transcription factors Sox10 and neurogenin1, ingressed into the olfactory epithelium and differentiated into microvillous neurons. Loss-of-functionanalysis revealed a critical role for Sox10 in microvillous neurogenesis. In sum, these results not only challenge the dogma regarding the origin of olfactory sensory neurons but also provide important insights into the early events that build the nascent olfactory system.

Program/Abstract # 145
Development of gustatory papillae in the absence of Six1 and Six4.
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Six-Homeo family genes code transcription factors, and a deficiency in them leads to defects in the sensory organs. Six1 was expressed in the taste bud-bearing lingual papillae of mice, and loss of Six1 affected the development of these gustatory papillae as previously reported. We found that embryos lacking both Six1 and Six4 showed severer abnormalities than those lacking Six1 alone during morphogenesis of their gustatory papillae. Six4 was broadly distributed in the epithelium of the lateral lingual swellings at embryonic day (E) 11.5, and in the tongue epithelium, mesenchyme, and muscles at E12.5. From E14, Six4 expression pattern was similar to Six1. In the fungi form papillae, Six4 expression was observed in the epithelium at E14-E16.5. In the circumvallate and foliate papillae, Six4 was expressed in the trench wall of these papillae at E15.5-P0. Although Six4-deficient mice had no abnormalities, Six1/Six4-deficient mice showed distinct morphological changes: fusion of the lateral lingual swellings was delayed, and the tongue was developed poorly. The primordia of fungi form papillae appeared earlier than those in the wild-type or Six1-deficient mice, and the papillae

rapidly increased in size; thus fusion of each papilla was evident. The circumvallate papillae showed severe defects; for example, invagination of the trenches started asymmetrically, forming longer and shorter trenches. The foliate papillae elevated initially, and resulted in stunted trenches. We conclude that Six1 and Six4 function synergistically to form gustatory papillae during development of the tongue.

Program/Abstract # 146

WNT signaling controls parasympathetic ganglion formation during submandibular gland development

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Submandibular gland (SMG) organogenesis involves the coordinated development of the epithelium and neural crest-derived parasympathetic ganglia (PSG), which condense and associate with the primary epithelial duct. FGFR signaling is required for both epithelial and PSG development as these structures are absent in Fgf10-/- embryos. Signals from the PSG maintainthe keratin 5+ (Krt5+) progenitor cells in the epithelium but it is not known whether the epithelium plays an inductive role in PSG development. We hypothesized that secreted factor(s) from the epithelial duct promote PSG condensation and association with the epithelium. Wnt-reporter mice show that WNT signaling occurs in PSG neurons early in development. We performed microarray analysis of early SMG end buds and ducts, identifying increased Wnt gene expression in the duct. Exogenous WNT proteins promote PSG proliferation and WNT inhibitors disrupt the epithelial-PSG association. To determine if FGFR signaling affects WNT signaling in the PSG we treated SMGs with FGF7 and FGF10, which reduced WNT signaling, PSG condensation and association with the duct, and the number of Krt5+ progenitor cells. We also used a genetic model to increase endogenous FGFR signaling, deleting the signaling antagonists Sprouty1 and Sprouty2 (Spry1/2DKO), which results in a similar but more severe phenotype. In conclusion, we have identified a novel role for WNT signaling in promoting PSG proliferation and association with the epithelial duct during SMG development, which establishes neuronal-epithelial communication required for progenitor cell maintenance and SMG development.

Program/Abstract # 148

Reprogramming of thymic epithelial cells in response to hyperactivation of Wnt/Beta-Catenin signaling during embryonic development

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Wnt/beta-catenin signaling is involved in many developmental processes, and in particular plays a critical role in cell fate determination. Hyperactivation of the pathway has been shown to cause cellular transdifferentiation from one mature epithelial cell type to another. In the current work we describe a mouse mutant in which the Wnt/beta-catenin signaling pathway is ectopically activated in thymic epithelial cells (TECs) from E11.5 of embryonic development. This resulted in a dramatic alteration in thymus structure: the thymus was devoid of lymphocytes, failed to become vascularized and was encased in a dense mesenchymal capsule. Furthermore, the epithelial cells no longer formed the characteristic 3D network, ceased proliferation and showed an altered epithelial marker profile, with a loss of ‘TEC’ markers and gain of ‘epidermis’ markers. Experiments are in progress to determine the ability of this transformed mutant thymus to integrate into normal adult skin following transplantation. The Foxn1 transcription factor is required for epithelial cell differentiation and proliferation in the thymus and skin, and it is intriguing to propose that tissue-specific roles and control mechanisms exist for Foxn1 in the two organs. Interestingly, we observed a transient downregulation of Foxn1 in the mutant thymus. We propose that suppression of Wnt signaling in TECs is required for Foxn1 to assume its thymus-specific role, and that ectopic activation of the pathway causes a “switch” to its skin role. We are interested to define the molecular mechanisms that underlie the altered epithelial cell fate observed in these mutants, and specifically those that control the tissue-specific roles of Foxn1 in the thymus versus skin.

Program/Abstract # 149

YY1 control of Vegf expression in the visceral endoderm is essential for yolk sac angiogenesis

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Yin Yang-1 (YY1), a multifunctional protein essential for embryonic development, is aptly named based on its function as both a repressor and as an activator of gene transcription. Even though YY1 has been shown to control cell proliferation and chromatin remodeling in cells, its function in particular cell types during embryogenesis is largely unexplored. A conditional knock-out (cKO) strategy using the FoxA3-Cre line was used to assess the role of YY1 in the embryonic endoderm and visceral endoderm (VE). Although cKO embryos appear normal until E8.5, by E9.5 Yy1 cKO embryos are delayed and display both a loss of VE cell-polarity as well as an apparent disruption of yolk-sac angiogenesis. The

abnormally dilated capillaries in the cKO yolk sac fail to be remodeled into large blood vessels, displaying a delay in vessel maturation including a reduction in smooth muscle actin. Prior to the onset of these phenotypes at E9.5 we found a significant increase in the percent of cleaved Caspase-3 positive cells in the yolk sac mesoderm of E9.0 cKO embryos suggesting that loss of Yy1 in the VE promotes apoptosis and delays differentiation in the adjacent YY1-positive yolk-sac mesoderm. Vascular endothelial growth factor (VEGF), a secreted growth factor known to be required for blood vessel development and for protection from apoptosis, is downregulated in cKO embryos by E9.0. Because YY1 was recently shown to directly bind to the Vegf promoter and activate Vegf expression, it seemed likely that the primary target of YY1 activity in the VE was Vegf. To test this hypothesis, cKO embryos were cultured from E8.5 through E9.5 with exogenous VEGF and we found that VEGF rescued many of the defects noted in vivo. Taken together, these results suggest that the role of YY1 in the VE is to promote angiogenesis and to moderate apoptosis in the adjacent mesoderm by activating Vegf expression.

Program/Abstract # 150

Uncovering the function of TMED2 during trophoblast differentiation

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Transmembrane emp24 domain trafficking protein 2, (TMED2) is a member of the p24 family of proteins involved in vesicle transport between the ER and Golgi. During vesicular transport between the ER and Golgi p24 proteins function as receptors for both cargos and coat proteins. Our group showed that Tmed2 is required for normal embryo and placental development in mouse and that syncytiotrophoblast cells of the mouse labyrinth placenta failed to differentiate in homozygous mutant embryos. In human placenta, we showed expression of TMED2 between 5.5 and 40 weeks of gestation in all trophoblast cell types. We noted that early in gestation TMED2 was more highly expressed in cytotrophoblast cells versus syncytiotrophoblast. Surprisingly, we found that TMED2 was more highly expressed in a choriocarcinoma cell line, BeWo, which can be induced to differentiate and form syncytiotrophoblast when compared to the JEG-3 cell line, which does not fuse to form syncytiotrophoblast. We hypothesized that TMED2 is required for fusion of trophoblast cells during syncytiotrophoblast differentiation. To test this hypothesis we are examining the function of TMED2 during trophoblast differentiation of BeWo and Jeg-3 cell lines. We will show our plans to ectopically express TMED2 in Jeg-3 cells and to knockdown TMED2 expression in BeWo choriocarcinoma cells with shRNA. Our work suggests that TMED2 is required for trafficking cargoes that are essential for placental development.

Program/Abstract # 151

Interference with glutamate signaling induces neural tube defects: Implications for antiepileptic drug action during neural tube formation

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Failure of neural tube closure leads to malformations known as neural tube defects (NTDs). Offspring of epileptic women exhibit higher incidence of NTDs due to the use of antiepileptic drugs (AEDs) during pregnancy. The mechanisms through which these drugs induce NTDs remain unclear. We hypothesize that neurotransmitter signaling is present at neural plate stages and is important for neural tube formation. AEDs induce NTDs by impairing embryonic neurotransmitter signaling. Immunostaining and calcium imaging experiments reveal that glutamate is expressed throughout the neural plate and its cells are responsive to glutamate and NMDA. The AED valproic acid (VPA) inhibits these responses. We then investigated whether NMDA receptor-mediated signaling influences neural tube formation by incubating early neural plate stage embryos with the NMDA receptor antagonist, D-AP5 until neural tube closure was completed in control siblings. D-AP5 increases the incidence of NTDs in a dose-dependent manner. In parallel, knocking down the NMDA receptor subunit NR1 inhibits the responsiveness of neural plate cells to NMDA and induces open neural tube phenotype. Inhibiting NMDA receptors increases the number of phospho-histoneH3-expressing neural plate cells, suggesting that glutamate signaling regulates cell proliferation during neural tube formation. Interestingly, the induction of NTDs by VPA is accompanied by increases in BrdU incorporation, in PCNA-labeled and total number of neural plate cells, indicating that VPA is interfering with the normal cell cycle progression. These findings suggest that glutamate signaling regulates cell cycle exit during neural tube formation and AEDs may interfere with this action.

Program/Abstract # 152

Developmental Retardation of Male Rat Brain, Testis Gonocytes According to Bisphenol A in vivo Exposure Time

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Bisphenol A (BPA) is a endocrine disruptor chemicals (EDCs) and acts like estrogenic chemical. EDCs mainly occur reproductive, nervous systemic disorders. Especially BPA, used in plastic like nursing bottle and resins which is utilized in

food packaging and dentistry, is often exposed in life. Animal studies indicate that exposure to BPA may affect brain and testis gonocytes development in embryos. However the detailed phenotypes and pathogenetic degree according to exposure time, especially with regard to the sex relative hormone of brain, testis gonocytes and sperm analysis have not been clarified. We therefore studied developmental retardation of brain, testis gonocytes according to BPA exposure time. And we confirmed them with tissue, sex relative hormone level and sperm analysis. We orally injected with 20 μg/kg of BPA dissolved in sesame oil to pregnant female rat and 200 μg/kg to newborn male rat by period which is embryonic day 0, embryonic day 12 and neonatal to postnatal day (PND) 20. Histological characteristics of male rat brain and testis gonocytes were processed immunohistochemistry and hormone was measured serum hormone level by radioimmunoassay and AR, GnRH-R, FSH, LHβ, ERα,β mRNA expression by quantitative RT-PCR at PND 20. And we measured sperm motility and number by computer assisted sperm analysis (CASA) at 8 weeks. In this study, we confirmed differences of development of brain and testis gonocytes according to BPA exposure time. And we also confirmed differences of sex relative hormone level and reduction of reproductive capacity according to development of brain and testis gonocytes. As BPA has been exposed human populations, further studies are warranted to assess the effects of BPA on human fertility.

Program/Abstract # 153

Requirement of Co-Smad independent BMP canonical Smad signaling for the specification process of the anterior rhombic lip during cerebellum development

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Cerebellum is an important organ in the central nervous system for coordinating body movement and balancing. Its development involves complicated cellular and molecular events controlled by various signaling pathways. Bone morphogenetic protein (BMP) has showed to be involved in these processes.However, the detail molecular mechanism of the Smad proteins usage, the downstream mediator of BMP signaling pathway, is not clear. In this study, we utilized the En1-driven Cre to conditionally inactivate Smad1, Smad5 and Smad4 in the mouse embryonic cerebellum. Our results demonstrated that Smad1and Smad5 are required in a functional redundant manner for the specification process of the anterior rhombic lip (ARL) during cerebellum development. Inactivation of both Smad1 and Smad5 resulted in the reduction of granule cell precursor numberand the loss of nuclear transitory zone leading to the loss of parts of deep cerebellar nuclei. In addition, the migration of Purkinje cells was also affected. Surprisingly, inactivation of Smad4 only resulted in mild cerebellar defects. The Msx2 expression in the ARL was not abolished suggesting that R-Smads were still transcriptional active in the absence of Smad4. Thus, our results support a co-Smad independency in the BMP signaling during the cerebellum development and challenge our current understanding of the BMP canonical Smad signaling.

Program/Abstract # 154

Embryonic DNA repair and gender are risk factors in ethanol embryopathies in oxoguanine glycosylase 1 (OGG1) knockout mice: A role for oxidatively damaged DNA and protection by a free radical spon trapping agent

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Reactive oxygen species (ROS) have been implicated in the mechanism of Fetal Alcohol Spectrum Disorders (FASD). To determine the involvement of ROS-mediated embryonic oxidative DNA damage, DNA repair-deficient oxoguanine glycosylase 1 (OGG1) knockout (KO), heterozygous (HET) or wild-type (WT) embryos were exposed in culture to ethanol (EtOH) (2 or 4 mg/ml) on gestational day (GD) 9 (plug = GD 1), with or without pretreatment with the free radical spin trap phenylbutylnitrone (PBN) (0.125 mM). Visceral yolk-sacs were genotyped for DNA repair status and gender. EtOH caused a concentration-dependent decrease in anterior neuropore closure (ANC), somite development, turning, crown-rump length, yolk sac diameter and head length ($p < 0.001$) in all 3 ogg1 genotypes, with a further ogg1 gene-dependent decrease in KO embryos for ANC, somite development, turning, crown-rump length and head length ($p < 0.05$), and a genotype-dependent correlation between head length and ANC($p < 0.01$). PBN pretreatment blocked most EtOH embryopathies ($p < 0.001$), although slightly so in KO embryos. Oxidatively damaged DNA determined as8-oxo-2'-deoxyguanosine (8-oxodG), which is repaired by OGG1, was measured in single GD 11 embryos 6 hours after maternal EtOH treatment (4 g/kg ip). Preliminary data suggest that EtOH-initiated 8-oxodG was greater in KO embryos. Head length and ANC were reduced in female embryos independent of treatment or ogg1 genotype, whereas the ratio of female to male KOs was increased compared to HET and WT embryos ($p < 0.001$). These results suggest that ROS-initiated embryonic DNA oxidation is involved in EtOH embryopathies, and embryonic DNA repair status and gender may be determinants of embryopathic risk. (Support: CIHR)

Program/Abstract # 155

Valproic acid induces p53 activation via hyperacetylation and increases cellular apoptosis leading to limb

malformations in murine limb buds

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Valproic acid (VPA), a common anticonvulsant and antidepressant, is an established human teratogen, causing spina bifida and limb malformations. It is also a known inhibitor of histone deacetylases (HDACs) which are involved in chromatin remodeling and cellular signaling. Other HDAC inhibitors have been shown to cause apoptosis in cancer cell lines. We hypothesize that VPA-induced HDAC inhibition triggers cellular apoptosis, leading to limb teratogenesis. To test this hypothesis, we compared the effects of VPA and its inactive analog, valpromide (VPD), using an in vitro limb bud culture system. GD12 murine embryonic forelimbs were cultured in the absence or presence of VPA or VPD(0.6, 1.8 or 3.6 mM) for 6 days and stained with toluidine blue. Limbs were cultured for 1, 3, 6 or 12h and used for Western blot quantification of histone4 acetylation, p53 acetylation and cleaved-caspases 9 and 3 or for the mRNA quantification of p53 target genes, Bcl2 and Survivin, by qRT-PCR. VPA significantly increased limb malformations that included oligodactyly and missing digits. At 3h, VPA induced the hyper acetylation of both histone 4 and p53. At 6h, both Bcl2 and Survivin were downregulated and at 12h caspases 9 and3 were both activated. In contrast, VPD caused a small significant effect on limbs only in the highest concentration group; no changes were observed in the acetylation of histone 4 or the cleavage of caspase 3 at any concentration.Together, these data show a sequential activation of the intrinsic apoptotic pathway and suggest that VPA induces apoptosis via p53 acetylation and activation of the downstream pathway. We propose this plays a role in VPA-induced teratogenesis. These studies were supported by CIHR and FRSQ.

Program/Abstract # 156

Renal lineage and self-renewing potential of GDNF-expressing cells

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Nephrogenesis initiates when the ureteric bud (UB), invades the metanephric mesenchyme (MM), inducing it to epithelialize and differentiate into nephrons. GDNF, expressed in the MM, is a critical factor for renal development and signals to the UB tips via Ret receptors. We generated a mouse line for inducible Cre-mediated recombination in GDNF-expressing cells, and used it to show that these cells are self-renewing progenitors that also give rise to the condensing mesenchyme, nephron tubules, Bowman’s capsule and podocytes. Interestingly, between E7 and E9, GDNF-expressing cells contribute to the UB lineage, while after E9 they contribute exclusively to the MM lineage. To test whether progenitor number is important for nephron number and kidney size, GDNF-expressing cells were depleted by mating GDNF-Cre-ERT2 and R26DTA mice followed by Tamoxifen injection. When the vast majority of progenitors are ablated (Tam injection at E9.5), the kidneys fail to form. If recombination is induced later, at E12.5, only a subset of progenitors is ablated. At birth, these animals present a significant reduction in kidney size and nephron number. By adulthood, kidney size recovers to ~80%of normal, but the nephron number fails to recover, remaining <50% of normal.While heterozygosity for Sprouty1, a negative regulator of GDNF signaling, can rescue renal hypoplasia in GDNF+/- mice, it failed to rescue kidney size when progenitors had been ablated, arguing that the renal hypoplasia is a direct consequence ofprogenitor depletion, and not simply due to reduced GDNF expression. In summary, GDNF-expressing cells are renal progenitors but their self-renewal potential is limited and their depletion critically impairs nephron number in mice.

Program/Abstract # 157

Pdx-1 as a potential regulator of epithelial organization in the developing pancreas

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Current efforts for the treatment of diabetes are focused on the generation of functional beta cells in vitro for cell replacement therapies; however, attempts have been unsuccessful and factors that could be required for the generation of functional islets remain unknown. Our lab has shown that the developing pancreatic epithelium undergoes a transient stratification when multipotent progenitor cells (MPCs) are set aside. These MPCs emerge in specific locations within the pancreatic epithelium and give rise to the ductal, exocrine and endocrine lineages. We hypothesize that this transient stratification is important for commitment of the MPCs to their different lineages, and that defects in this process may skew cell fate or prevent differentiation altogether. Pdx-1 is a transcription factor required for pancreatic development, and for the specification of endocrine fate, particularly that of beta-cells. However, much is unknown about its downstream targets and whether it plays a role in the epithelial organization of the pancreas. We know that pancreatic developmental failure in Pdx-1 homozygous null mice coincides with the time point at which the stratified pancreatic epithelium resolves back to a monolayered epithelium. We have observed that “de-stratification” of the pancreas fails in these null embryos. We have also observed a significant decrease in the levels of E-Cadherin and b-catenin, suggesting that factors controlling epithelial 3D organization are targets of Pdx-1. Understanding the developing pancreatic epithelium will allow for the elucidation of the programming of beta-cell fate.

Program/Abstract # 158
Transdifferentiation of liver to pancreas
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Cell-based therapies in diabetes research are mainly focused on the use of stem cells to produce pancreatic beta cells in vitro for transplantation. An alternative strategy is to generate pancreatic tissue from other tissues already present in the body by transdifferentiation. Using transgenic *Xenopus* tadpoles we showed that Pdx1-VP16 converts liver cells into all pancreatic cell types (both endocrine and exocrine), while Ptf1a-VP16 converts liver cells to only exocrine cells. However which genes are activated by Pdx1-VP16 or Ptf1a-VP16 in this process is unknown. In order to identify the genetic networks controlled by Pdx1-VP16 and Ptf1a-VP16 during the transdifferentiation of liver to pancreas we identified what gene expression changes occurred immediately after these genes were expressed in the liver. This was achieved by driving the expressionof Pdx1-VP16 and Ptf1a-VP16 in *Xenopus* tadpoles using the liver-specific TTR (transthyretin) promoter, which is activated in transgenic tadpoles at stage 44. We dissected out livers at stage 44/45 (just after activation of TTR promoter), extracted RNA from these livers and performed microarray to compare the genes expression profile of transgenic livers with that of control liver samples. The microarray data obtained were analyzed. Functional analysis of the selected candidate genes will be presented in the poster.

Program/Abstract # 159
Discovering the molecular pathways controlling progenitor differentiation in pancreatic development and regeneration
Parsons, Michael J.; Huang, Wei; Delaspre, Fabien, Johns Hopkins University, Baltimore, United States
We previously characterized a population of pancreatic Notch-responsive cells (PNCs) in developing zebrafish larvae that are closely associated with the pancreatic ducts. Using long-range lineage tracing we showed that these PNCs are the cellular origins of both adult endocrine cells and centroacinar cells (CACs). CACs resemble their larval predecessors in their appearance, molecular markers and progenitor characteristics. We used several different models of pancreas damage in the adult zebrafish and have shown that CACs are directly involved in tissue regeneration. Following a chemical screen to find drugs that induce PNCs to differentiate into endocrine cells, we have identified several pathways that maintain the larval progenitors in an undifferentiated state. Furthermore, we have developed a transgenic cre/lox system to test pathway components in the PNCs in a cell-autonomous manner. Using this method we have investigated components of the retinoic acid (RA) and Notch signaling pathways. We have demonstrated that the RA and Notch pathways converge to control the formation of the emerging pancreatic endocrine system during embryogenesis. In the future, we will test the same pathway components controlling larval pancreas formation in damaged adult pancreas to ascertain their role in regeneration.

Program/Abstract # 160
Loss of Brachyury in the mouse notochord results in axial skeletal defects and urorectal malformations
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The T-box transcription factor Brachyury (T) is a key regulator of mesoderm formation during early development, with loss of T leading to embryonic lethality around E10.0. Here we characterize an miRNA-based in vivo knockdown mouse model of T, termed KD3-T, which exhibits a hypomorphic phenotype. This phenotype results from a failure to maintain the notochord domain of T expression, without loss of expression in the pre-somitic mesoderm. This results in the notochord taking on a neural character and losing its ability to act as a signalling center. KD3-T animals survive until birth, which allowed us to examine later roles for T in the development of notochord-derived structures. KD3-T embryos display axial skeletal defects caused by apoptosis of paraxial mesoderm posterior to the hindlimb, which is accompanied by urorectal malformations resembling the murineuro-recto-caudal syndrome. Overall, our data demonstrate that a failure to maintain T in the notochord is sufficient to elicit a caudal regression phenotype, although the initial body axis is normally patterned and formed. By utilizing a novel in vivo miRNA-based gene knockdown system, we have uncovered a notochord-specific, tailbud-independent role for T in embryonic development.

Program/Abstract # 161
The chromatin remodeling complex subunit Baf60c regulates essential gene expression programs in heart development
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Yuqing (Mouse Imaging Centre, Toronto, Canada); Christodoulou, Danos; Seidman, Christine; Seidman, Jonathan (Harvard Medical School, Boston, United States)

Mammalian BAF (Brm/BRG1 Associating Factor) complexes are ATP-dependent chromatin remodeling complexes. Identification and characterization of unique BAF complex assemblies functioning in specific cell types suggest that tissue-specific chromatin remodeling mechanisms offer another layer of control on the orchestration of gene expression during mammalian development. Baf60c is one of the three Baf60 subunits in the mouse. Baf60c is highly expressed in the mouse heart from the cardiac crescent stage. Baf60c can associate with cardiac transcription factors including Tbx5, Nkx2-5, and Myocardin. To understand the role of Baf60c in mouse heart development, a Baf60c conditional knockout mouse line was established. Constitutive loss of Baf60c in embryos resulted in hypoplastic myocardium and embryonic death by E14.5. RNA-seq of E10.5 and E12.5 Baf60c knockout embryo hearts detected a broad down-regulation of gene groups essential for cardiomyocyte functions, including sarcomere structure and contraction apparatus genes, as well as energy metabolism genes. High frequency echocardiography showed that Baf60c knockout embryo ventricle walls were hypocontractile. Deletion of Baf60c with Myh6::Cre in myocardium at later developmental stage also caused abnormal heart growth and animal mortality. The hearts of Myh6::Cre, Baf60cflx/- mice were severely dilated and hypoplastic. All the Myh6::Cre, Baf60cflx/- mice died before 4 months age. Echocardiography and electron cardiography detected contractionand conduction defects. At both embryonic and adult stages, transmission electron microscopy showed disarrayed sarcomere and irregular Z-disks. In summary, Baf60c is a cardiac-specific chromatin-remodeling factor that has critical roles in both embryonic and postnatal heart growth. It takes part in regulation of gene groups essential for cardmiomyocyte identity and function. The composition of the potential cardiac BAF complexes and the mechanismof Baf60c function in regulating cardiac growth in conjunction with transcription factors and other chromatin remodeling complexes are to be elucidated.

Program/Abstract # 162

Role of endothelin-A receptor in cardiac neural crest cell development

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Congenital cardiovascular malformations are the most common birth defects affecting children. Several of these defects occur in structures developing from neural crest cells (NCC). These NCC originate from the neural fold and migrate ventrally to populate the pharyngeal arches. During cardiovascular development, cardiac NCC (CNCC) participate in the asymmetric remodeling of the pharyngeal arch arteries (PAA) into the great vessels and the septation of the cardiac outflow tract (COFT) into the pulmonary and aortic outflows. One of the key signaling pathways regulating CNCC development involves the Endothelin-A receptor (Ednra). The absence of Ednra signaling in the mouse causes severe cardiovascular defects, including persistent ductus arteriosus and coarctation of the aorta. However, the exact function of Ednra signaling in CNCC is unknown. CNCC fate mapping in the Ednra mouse indicated that the migration of these cells is aberrant in the COFT of the mutant embryos, but not in the pharyngeal arches. This defect appeared to be independent of CNCC proliferation and apoptosis changes. From these results, we hypothesized that CNCC migration is arrested prematurely because they fail to activate specific cell migratory mechanisms and to distinguish themselves from the CNCC in the PAA. We analyzed CNCC migration in the COFT by looking at migratory markers like Cdc42 and RacGTP. We identified genes differentially expressed in the two subpopulations of CNCC regulated by Ednra signaling. Our results revealed specific changes that could explain the CNCC migratory behavior and the Ednra mouse phenotype. It appears that Ednra signaling has a dual function: controlling CNCC patterning in the PAA and CNCC migration in the COFT.

Program/Abstract # 163

Ccm3 functions in a manner distinct from Ccm1 and Ccm2 in a zebrafish model of CCM vascular disease

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Cerebral cavernous malformations (CCMs) are vascular anomalies of the central nervous system that arise due to mutations in genes coding three unrelated proteins: CCM1 (KRIT1); CCM2 (Malcavernin/OSM) and CCM3 (PDCD10). Biochemical and mutant studies suggest that CCM1 and CCM2 act as part of a physical complex to regulate vascular morphogenesis and integrity. In contrast, mouse Ccm3 mutant and in vitro cell culture data suggests an independent role for Ccm3. We report that inhibition of zebrafish ccm3a/b causes heart and circulation defects distinct from those seen in ccm1 (santa) and ccm2 (valentine) mutants, and leads to a striking dilation and mispatterning of cranial vessels reminiscent of the human disease pathology. We show that knockdown of ccm3 causes endothelial cells of the cranial vasculature to form an increased number of protrusions not seen in ccm1/2 mutants. ccm3, but not ccm2, defects can be rescued upon overexpression of stk25b, a GCKIII kinase previously shown to interact with CCM3. This suggests that Stk25b activity is downstream of Ccm3 in the cranial vasculature. In addition, morpholino knockdown of the GCKIII gene stk25b results in heart and vasculature defects similar to those seen in ccm3 morphants. Finally, additional loss of ccm3 in ccm2 mutants

leads to a synergistic increase in cranial vessel dilation. These results support a model in which CCM3 plays a role distinct from CCM1/2 in CCM pathogenesis, and acts via GCKIII activity to regulate cranial vasculature integrity and development. Our current work is focused on determining the specific cellular defects caused by disruption of Ccm3/Stk25 signaling and elucidating Ccm3 and Stk25b residues important for proper cranial vasculature development.

Program/Abstract # 164

Fibroblast-growth factor 8a (fgf8a) synergistically interacts with ethanol to perturb proper skull development.

McCarthy, Neil; Swartz, Mary; Eberhart, Johann, Austin, United States

The processes that shape the skull are intricate and highly susceptible to teratogens, including ethanol. Fetal exposure to ethanol can result in fetal alcohol spectrum disorder (FASD). FASD is highly prevalent and variable, and can include neurological and craniofacial defects. Susceptibility to FASD is in part due to genetic predisposition, but we know little of these genetic factors. To identify these genes, we screened zebrafish mutants for dominant enhancers of ethanol teratogenicity. From this screen, we initially characterized a synergistic interaction between ethanol and platelet-derived growth factor receptor alpha (pdgfra) caused by the inhibition of mTOR function, downstream of PI3K signaling. This analysis suggests a model in which other PI3K-dependent pathways would interact with ethanol. Indeed, fibroblast growth factor 8a (fgf8a) also interacts with ethanol and causes a loss of the parachordal cartilages of the posterior neurocranium. Eitherm TOR inhibition, via rapamycin, or fgf3;fgf8a double loss-of-function recapitulates the ethanol-induced defects. Using the broad Fgf inhibitor SU5402, we find that Fgf signaling between 36 and 48 hours post fertilization is necessary for the formation of the parachordals. Collectively, these data suggest that Fgf signaling is required in either the differentiation or survival of parachordal precursor cells and that ethanol perturbs this process by inhibiting Fgf-dependent mTOR signaling. Further analyses will include characterizing the precursors for the parachordal cartilages and how ethanol disrupts the development of these precursors.

Program/Abstract # 165

Zebrafish craniofacial cartilage morphogenesis is controlled by elements of the Wnt/PCP signaling pathway

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The Wnt/Planar Cell Polarity (PCP) pathway is composed of multiple molecules that control a wide variety of normal and disease processes during development. One such process is the lengthening of the anterior-posterior axis of the embryo via convergence and extension. The large-scale zebrafish mutagenesis screens identified multiple convergence and extension mutations found in Wnt/PCP genes. A sub-class of the identified genes also seemed to have defects in proper craniofacial formation. To better understand the role that the Wnt/PCP pathway plays in craniofacial development we set out to identify which of the known Wnt/PCP genes are critical for proper head cartilage formation and how they affect chondrocyte morphogenesis. In this study we evaluated multiple known Wnt/PCP mutants and confirmed that two mutants with convergence and extension defects, glypican 4 (gpc4; knypek), and wnt5b (pipe tail), also exhibited craniofacial defects. We did not find major defects in specification or migration of the neural crest, which gives rise to the cranial cartilages. Furthermore, the number of chondrocytes within the gpc4 and wnt5b mutant craniofacial cartilage elements is similar as compared to wild type siblings. Cell transplantation experiments indicate that Wnt5b acts in a paracrine fashion while Gpc4 is needed cell-autonomously in the developing chondrocytes. These results suggest that the intercalation driven cartilage elongation requires Wnt5b signaling received by chondrocytes with the help of Glypican 4. At the same time a core Wnt/PCP member, Vangl2, seems not to be involved in this process, suggesting that stacking chondrocytes utilize only a specific branch of this pathway.

Program/Abstract # 166

A comprehensive timeline of quail small intestine development

Thomason, Rebecca T.; Winters, Niki; Bader, David, Vanderbilt University, Nashville, United States

The early embryonic gut is a tube composed of splanchnic mesoderm and endoderm. Many studies have investigated specific tissue layers or time points in gut development. However,an inclusive timeline of the morphological events that occur throughout small intestine development is lacking. We utilized immunofluorescence, morphometric analysis, histology, and transgenic quail embryos to examine mesothelial formation, mesenchymal growth, vascular development, and smooth muscle morphogenesis. Two basement membranes are evident at E1.9, underlying the endoderm and subjacent to the outer epithelium. At E6, the outer epithelial basement membrane stabilizes and a mesothelium is first identified. Following this transition, we observe mesenchyme expansion, until villi form at E10. After the formation of villi, the average area of the mesenchyme decreases as the small intestine lengthens. Using Tg(tie1:H2B-eYFP) quail embryos, the nuclei of endothelial cells are first visualized in the mesenchymal space at E2.1, then organized into two layers at E6, and

in the villi at E14. At E11, the major blood vessels, circumferentially covering the midgut, are first detected. The visceral smooth muscle layer was first apparent at E6, both circular and longitudinal layers at E12, and subepithelial myofibroblasts were identified in the villi at E14. Taken together, these dataprovide a comprehensive timeline of intestinal development. Developmental processes not normally correlated may have both a temporal and morphological relationship. This timeline will benefit both researchers examining intestinal development and clinicians studying congenital syndromes that may originate from a combination of disrupted developmental processes.

Program/Abstract # 167
Identification of a novel developmental mechanism in the generation of mesothelia
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Mesothelial cells form the surface layer of all coelomic structures and are essential to organ function. During development, these cells undergo an epithelial to mesenchymal transition (EMT) to provide the precursors for the vasculature and stromal cells to all coelomic organs investigated to date. Furthermore, in the adult, mesothelial cells stimulated by disease or injury retain the ability to undergo EMT to generate fibroblasts and vascular smooth muscle cells mimicking their developmental behavior. Despite the broad contribution of this cell type to developing organs and adult disease, our current understanding of the genesis of this cell type is confined to a single organ, the heart, in which an exogenous population of cells, the proepicardium, migrates to and over the myocardium to give rise to the cardiac mesothelium and coronary vasculature. It is unknown whether this pattern of development is specific to the heart or applies broadly to other coelomic organs. Using two independent long term lineage tracing studies, we demonstrate that mesothelial progenitors of the intestine are intrinsic to the gut tube anlage. Furthermore, a novel chick-quail chimera model of gut morphogenesis reveals these mesothelial progenitors are broadly distributed throughout the gut primordium and are not derived from a localized and exogenous proepicardium-like source of cells. These data demonstrate an intrinsic origin of mesothelial cells to a coelomic organ and provide a novel mechanism for the generation of mesothelial cells.

Program/Abstract # 168
Lasp regulates actin filament dynamics in *Drosophila* myofibril assembly.
Fernandes, Isabelle; Schoeck, Frieder, McGill University, Montreal, Canada
The actin cytoskeleton plays a key role in a number of motile and morphogenetic processes. The coordinated assembly and disassembly of actin filaments promotes cell shape changes, mediates motility, contractility, and many other processes. In each case, actin dynamics is finely regulated by a large number of actin-binding proteins (ABPs) that control actin filament polymerization, nucleation and crosslinking. One group of ABPs is the nebulin family. To date, this family comprises 5 members, each containing from 2 to 185 actin-binding nebulin repeats. In our previous work, we showed that Lasp is the single member of this family in *Drosophila*. Lasp has an N-terminal LIM domain, two nebulin repeats, and a C-terminal SH3 domain. Lasp null mutants are homozygous viable, but male sterile. The stem cell niche is not properly anchor and actin cone migration is impaired, resulting in failure of spermatid individualization. Recently, we discovered that Lasp also functions in myofibril assembly. In Lasp mutants, sarcomere length is reduced and muscle contractility is weaker than in wild type flies. These results are consistent with a function of nebulin family proteins as scaffolding and actin filament organizing proteins. Using a double-tagged Lasp, I performed a pull down assay to identify interacting partners. As expected Lasp binds muscle-specific actin isoforms. More surprisingly, most of the isolated proteins are components of thick filaments, suggesting new functions for Lasp. We will report which domain of Lasp regulates actin dynamics, protein interactions and proper localization in sarcomere assembly. By deciphering Lasp function in both Z-disc and A-band, we will obtain further insights into the mechanism of myogenesis.

Program/Abstract # 169
Muscle type-specific expression and function of Zasp52 isoforms in *Drosophila*
Schoeck, Frieder; Katzemich, Anja; Fernandes, Isabelle, McGill University, Montreal, Canada
Zasp52 is a member of the PDZ-LIM domain protein family in *Drosophila*, which comprises Enigma, ENH, ZASP, Alp, CLP36, RIL, and Mystique in vertebrates. *Drosophila* Zasp52 colocalizes with integrins at myotendinous junctions and with α-actinin at Z-discs,and is required for muscle attachment as well as Z-disc assembly and maintenance. Here we document thirteen Zasp52 splice variants giving rise to six different LIM domains. We demonstrate stage-and tissue-specific expression in different muscle types for Zasp52 isoforms encoding different LIM domains. In particular, LIM1b is expressed only in heart muscle and certain somatic muscles, implying muscle-specific functions in Z-disc assembly or maintenance. We will present the phenotype of certain isoform-specific RNAi knockdowns.

Program/Abstract # 170

***Drosophila* Zasp52 has a dual role in Z-disc maintenance and myofibril assembly**

Katzemich, Anja; Schoeck, Frieder, McGill University, Montreal, Canada

Zasp52 is a multi-domain protein, composed of an N-terminal PDZ domain, ZM motif, and four LIM domains, and is found predominantly in *Drosophila* muscle. It is part of the PDZ-LIM domain protein family, which comprises Enigma, ENH, ZASP, Alp, CLP36, RIL, and Mystique invertebrates. In *Drosophila* embryos, Zasp52 was shown to co-localize with alpha-actinin at Z-discs and with integrins at myotendinous junctions. Mutations in Zasp52 cause first in star larval lethality with defects in Z-disc assembly and maintenance as well as muscle attachment. During *Drosophila* indirect flight muscle (IFM) development in the pupa, Zasp52 is present at very early stages of myofibril assembly. It localizes with alpha-actinin in rudimentary Z-bodies along the assembling myofibril, before other muscle proteins show any periodicity. Live imaging of endogenous GFP-Zasp52 also demonstrates that Zasp52 is present in the developing Z-disc throughout embryonic myofibrillogenesis. RNA interference against the last exon encoding the most C-terminal LIM domain results in the depletion of all high molecular weight isoforms. This gives rise to viable but flightless adult animals. IFM sarcomeres show thin and interrupted Z-discs as well as distorted H-zones. In some regions of the IFM, sarcomeres were torn apart severely. These findings suggest that Zasp52 is required for the establishment of normal Z-discs in the IFM and subsequent sarcomere stability after onset of contractility. We will propose a model of IFM myofibril assembly based on electron microscopy, confocal microscopy and live imaging of IFM development in wild type and Zasp52 mutants.

Program/Abstract # 171

Sulf1 modulates FGF and BMP signaling to pattern trunk muscle, pigmentation, and lateral line

Meyers, Jason; Planamento, Jessica; Krulewitz, Neil, Colgate University, Hamilton, United States; Pownall, Mary (University of York, York, United Kingdom)

Heparan sulfate proteoglycans (HSPGs) are glycosylated extracellular or membrane-associated proteins. The sulfated domains of heparan sulfate polysaccharide chains can interact with many growth factors and receptors, modifying their activity or diffusion. The pattern of sulfation can be modified by secreted extracellular sulfatases, which remove specific sulfates from the heparan sulfate chains. Changes in sulfation patterns can change growth factor gradients and activities, thus precise expression of sulfatases is believed to be necessary for normal development. We have examined the role of the sulf1 gene, which encodes a 6-O-endosulfatase, in trunk development of zebrafish embryos. Sulf1is expressed in the developing trunk musculature and notochord. Knockdown of sulf1 with antisense morpholinos results in a lack of a myoseptum, improper pigmentation along the mediolateral stripe, and improper migration of the lateral line primordium. All of these phenotypes are consistent with alteration of signaling along the myoseptum. Sulf1 knockdown results in a decrease in muscle pioneer cells and loss of sdf1 expression along the mediolateral trunk musculature, but these can be rescued by pharmacological inhibition of BMP signaling, which also restores pigmentation patterning. Lateral line migration and deposition depend on proper sdf1 expression and FGF signaling respectively, both of which are disrupted in sulf1 morphants. Pharmacological activation of FGF signaling can rescue proper spacing of neuromast deposition in these fish. Together this data suggests that sulf1 serves a crucial role in modulating both BMP and FGF signaling to allow proper morphogenesis of trunk musculature, pigment cells, and lateral line neuromasts.

Program/Abstract # 172

Prox1 modulates the neuromast deposition frequency in the migrating posterior lateral line primordium

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Mechanosensory organs called neuromasts in zebrafish are generated by periodical deposition from the trailing end of migrating posteriorlateral line primordium (pLLP) as it migrates from the otic vesicle to the tip of the tail. The longevity of the migrating primordium is maintained by the activity of Wnt effector, Lef1, in the leading zone of primordium. In contrast, formation, maturation of proto-neuromasts and its deposition is regulated by the activity of FGF signaling in the trailing zone. In this study we have examined the role of the homeo domain transcription factor Prox1 in the migrating pLLP. Prox1 is broadly expressed in the pLLP, which contains 2-3 proneuromasts at progressive stages of maturation. Its expression is driven by dual signaling system of Wnt signaling in the leading zone and FGF signaling in the trailing zone. Knockdown of prox1 with morpholinos showed the less frequent deposition of neuromasts and delayed formation and maturation of proto-neuromasts in the pLLP. These changes were associated with expanded expression of Wnt effector lef1 and reduction of pea3, target gene of FGF signaling, in the pLLP. Our results suggest a previously unaddressed role for Prox1 in switching between Wnt and FGF signaling determining the frequency of neuromast deposition in the pLLP.

Program/Abstract # 173
The role of non-muscle myosins in *C. elegans* gonad architecture
Pisio, Amanda; Kachur, Torah; Pilgrim, Dave, University of Alberta, Edmonton, Canada

Non-muscle myosins (NMM) are hexamers of heavy, essential light and regulatory light chains that use ATP to move actin filaments. NMMs play a role in many processes, such as cytokinesis, where they form actomyosin complexes that lead to cell shape changes. *C. elegans* have two non-muscle myosins, NMY-1 and NMY-2. NMY-2 has been studied in numerous processes in the worm; however NMY-1 has only been studied during elongation of the embryo where it is redundant with NMY-2. NMMs are mostly regulated through phosphorylation of their regulatory light chain by upstream kinases and phosphatases. While *C. elegans* has two genes encoding NMM heavychains, the only known regulatory light chain is MLC-4. We would expect, therefore, that in tissues where both NMM heavy chains are expressed, NMY-1 and NMY-2 hexamers will be co-ordinately regulated through MLC-4. We can examine this prediction in the *C.elegans* gonad. At the distal tips of the gonads, mitotic germ cells are syncytial and as they migrate towards the proximal end of the gonad they mature into oocytes, grow in size and, close the ring channel that attached them to the syncytium. The gonads of nmy-2 mutants entirely lack this cellularization process, and therefore no oocytes are formed. In contrast, in nmy-1 mutants the gonads show a premature cellularization of the oocytes. This difference in phenotype may indicate that the two myosins work in opposition which is interesting considering they share the same regulatory light chain. However, in order to answer questions involving the regulation of these two opposing myosins, it is necessary to first localize the proteins and characterize the effect of their loss. I shall be presenting the results of this characterization.

Program/Abstract # 174
Profilin controls soma-germline interaction and differentiation upon exit from the stem cell niche in the *Drosophila* testes
Fairchild, Michael J.; Tanentzapf, Guy, University Of British Columbia, Vancouver, Canada

The *Drosophila* testes house a stem cell niche containing both somatic and germline stem cells that regulate each other's proliferative capacity. Upon exit from the stem cell niche the soma encapsulates the germline. This association and communication between the somatic and germline daughter cells ensures proper differentiation of the germline into mature spermatids. In order to better understand the morphogenic events underlying regulation of stem cell maintenance and differentiation we undertook a forward genetic screen of all cytoskeletal genes in the somatic cells of the testes using tissue specific RNAi knockdowns. Using sterility as a phenotypic assay we identified upwards of 25 cytoskeletal genes required in the soma, among them chickadee, the *Drosophila* homologue of the actin binding protein profilin which we analyzed in detail. We found that profilin is enriched in the somatic cells of the testes, both in the somatic stem cells and their early daughter cells during germline encapsulation. As profilin is an essential gene we investigated somatic cell survival and found that profilin deficient somatic cells not only survive but also overproliferate. While profilin deficient somatic cells were often found to be in contact with the germline we found that some germline cells were not encapsulated, forming germline tumors. We found that this may be due to profilin deficient somatic cells having defects in EGFR-MapK signal transduction which controls germline encapsulation. We further analyzed the tumors formed by unencapsulated germline cells and found them to express a range of spermatogonial markers as well as germline stem cell markers that are usually spatially restricted to the stem cell niche.

Program/Abstract # 175
Gap Junction-Mediated Regulation of Germline Differentiation and Soma Proliferation
Smendziuk, Christopher M.; Messenberg, Anat; Islam, Fayeza; Tanentzapf, Guy, University of British Columbia, Vancouver, Canada

In animals, two tissue types populate the gonads: the germline, which gives rise to gametes, and the soma, which gives rise to all other tissues that support gamete formation. Gametogenesis is a complex process requiring intricate cooperation between the soma and germline. A key feature of gametogenesis is the involvement of two specialized stem cell populations that produce the soma and the germline. Stemcells in the testes of *Drosophila melanogaster* serve as a superb model system to dissect the cellular and molecular mechanisms that regulate stem cells. Studies in the fly testis have illustrated that soma-germline interactions control stem cell behaviour and we wish to explore the mechanisms that underlie soma-germline interactions. Flies containing mutations in the gene zero population growth/innexin4 (zpg) are sterile and possess tiny gonads. Zpg encodes an innexin, a gap junction protein. Previous studies indicate that Zpg functions in the germline to regulate germline stem cells but the precise role of Zpg has not yet been elucidated. Our preliminary data supports the idea that Zpg may mediate signalling from the germline to the soma. We have uncovered previously uncharacterized defects in the soma in zpg mutants, including overproliferation. In addition, we have analyzed the function of the eight *Drosophila* innexins in the testes. Our observations support the assertion that Zpg helps form gap

junctions between the germline and soma. In order to further analyze the function of Zpg, we have begun a detailed structure/function analysis of the Zpg protein. Altogether, our studies are beginning to provide mechanistic insight into the role of gap junctions in germline-soma communication and stem cell regulation.

Program/Abstract # 176
The role of DAZ family proteins in heat stress response of male germ cells
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Mammalian male germ cells should be maintained below body temperature for proper development. Here, we investigated how male germ cells respond to heat stress. A short exposure of mouse testes to core body temperature induced phosphorylation of eIF2a and the formation of stress granules (SGs) in male germ cells. We observed that DAZL, a mouse homologue of human DAZ, was translocated to the SGs upon heat stress. Furthermore, the SG assembly activity was significantly diminished in the early male germ cells of Dazl-knockout mice. The DAZL-containing SGs played a protective role against heat stress-induced apoptosis by the sequestration of specific signaling molecules, such as RACK1, and the subsequent blockage of the apoptotic MAPK pathway. Based on these results, we discuss the functional roles of DAZ family proteins in heat stress response of male germ cells.

Program/Abstract # 177
Effects of Ginsenoside-Rg1 on activity of mitochondria of cryopreserved boar sperm after thawing
Kim, Joo Won; Kim, Sung Won; Park, Cheol Ho; Park, Soo Jung; Hwang, You Jin; Kim, Dae Young, Gachon University, Incheon, Republic of Korea

Ginsenoside is one of the glycosides of plant-kind that has different structure comparing to common theirs and it was well known as anti-tumor, anti-oxidant and anti-aging. Ginsenoside-Rg_1, a known neuroprotective drug, had been also studied that is promoting DNA synthesizing. When sperm is cryopreserved for long-time storage, reactive oxygen species (ROS) appear on boar sperm within mitochondria which are damaged to DNA. Therefore ROS rate should be cared because it is not meaningful if cells' mitochondria gets damaged and works not properly thawed. Ginsenoside-Rg_1 had added into boar sperm for cryopreservation so that it could help protect DNA of thawed sperm which increase viability rate. In this study, we not only used flow cytometric assay of which is for evaluating mitochondrial activity rate, but also Western blot had been used as detecting the mitofusin-2 (Mfn-2) of mitochondria in boar sperm. Here we report that Ginsenoside-Rg_1 had been affected to motility and viability of boar spermatozoa. Moreover, Ginsenoside-Rg_1 can be used as protective additives for the suppression of intracellular mitochondrial oxidative stress by cryopreservation. *This research was supported byBasic Science Research Program through the National Research Foundation ofKorea (NRF) funded by the Ministry of Education, Science and Technology (No.2012-0001770).

Program/Abstract # 178
Effects of erythritol on boar sperm during washing through percoll gradients
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Percoll solution consists of silica beads coated with polyvinylpyrrolidone (PVP) used for the purification of sperm. Centrifugation through discontinuous Percoll gradients is useful for flow cytometric assay, because removal of a number of debris. However, the reactive oxygen species (ROS) generated by use of percoll during washing of boar sperm affect both motility and fertility. The aim of this research was to optimize protocol for washing of boar sperm. It is a matter to which a study of cyopreservation of sperm is the most important thing sperm can do to maintain optimal condition. When freezing and thawing cryopreserved spermatozoa, boar sperm was prepared by Percoll gradient centrifugation and added Erythritol which decrease ROS levels in boar sperm. Fresh and thawed sperm were measured the motility with computer-assisted analysis of sperm motility (CASA) and concentration of ROS with flow cytometric assay. The results of sperm motility washing different Percoll gradients in extender added Erythritol had higher than low concentration of Erythritol in extender. The Pecoll treatments which added Erythritol increased purity of sperm and reduced that damage is associated with ROS during washing. These results suggest that the Percoll treatments using Erythritol help to optimize condition of boar sperm after washing. *This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No.2012-0001770).

Program/Abstract # 179
Effects of the Wnt/ß-catenin signaling pathway on zebrafish primordial germ cell migration

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In multicellular organisms, primordial germ cells (PGCs) are specified distant from the site of gonad development. In mammals, PGCs migrate along the midline to the genital ridge, where they interact with somatic cells and initiate gonadogenesis. In zebrafish, migration is guided by the chemokine SDF-1a, which is recognizedby the CXCR4b receptor in PGCs. Aberrant PGC migration can lead to gonadal malformations, infertility and possibly germ cell tumors (GCTs). Recently, the Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of GCTs, indicating a potential role for this pathway in PGC biology. Our goal is to use zebrafish to dissect the role of Wnt signaling in PGC migration. Using small-molecule antagonists of Wnt/β-catenin signaling, we found that inhibiting Wnt during periods of active PGC movement impairs PGC migration. Importantly, drug treatment did not affect the patterning of SDF-1a, CXCR4b or CXCR7b, which provide essential guidance cues to migrating PGCs. To address a possible cell autonomous role of Wnt signaling, we targeted expression of mutant RNA constructs, designed to manipulate the Wnt pathway, specifically to PGCs. Wnt knockdown specifically in PGCs results in aberrant migration. Furthermore, our results revealed a novel mechanism linking SDF-1a/CXCR4b signaling and the Wnt pathway in PGCs. We also sought to determine the fate of ectopic PGCs in zebrafish and their possible role in causing extra gonadal GCTs. Daily monitoring of ectopic PGCs revealed that they are not cleared, but persist in their ectopic positions. Future analyses will clarify the mechanism by which Wnt signaling regulates PGC migration as well as the potential role of this pathway in the pathogenesis of GCTs.

Program/Abstract # 180

Spermatogenesis in *Peltophryne gundlachi* and *P. cataulaciceps* (Anura: Bufonidae), two Cuban endemic toads

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Peltophryne gundlachi (Ruibal, 1959), Cuban high-crested toad, and *Peltophryne cataulaciceps* (Schwartz, 1959), Cuban pine toad are habitat restricted, their populations are declining by pollution, chytridiomycosis and habitat loss. Both hold some endangered category by IUCN Red List. Little is known about the biology of these species and is crucial to know the begining of its development through the characterization of their sex cells. The main goal of this paper is to present the elements of spermatogenesis and thus getting a better understanding of this development process that would allow both recommend actions on reproductive biology and also contribute to its conservation and taxonomy. Three males of each species were collected in the Isle of Youth in July 2011 and ethically euthanized. Gonad fragments were fixed in Bouin's fluid, Paraformaldehyde and Glutaraldehyde to be processed for light microscopy, fluorescence and TEM respectively. The results indicate that sexual cells are found in diverse degrees of development inside the seminiferous tubules. During the cellular differentiation and proliferation, different cellular types (spermatogonia, spermatocytes I II, early, mid and late spermatids, and spermatozoa) have a cystic organization, associated with Sertoli´s cells. The spermatozoa are characterized by an extraordinary nuclear compactation and undulantig membrane was observed in them. There is a Bidder's organ in the cranialis part of each testicle and for the first time numerous pigment-containing cells were found in it. These cells are also randomly distributed in the tunica albuginea and testicular interstitium. This could be a strategy to protect the gonads from UV rays or a sign of pollution.

Program/Abstract # 181

Atypical Wnt receptor involvement in hematopoietic stem cell specification and leukemia

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Signaling by Wnt family ligands regulates embryonic development, cellular homeostasis, and is dysregulated in disease, especially cancer. We have recently shown that wnt16 is required for hematopoietic stem cell specification (HSC) through regulation of somitic gene expression. Knockdown of Wnt16 during development leads to defects in somitic Notch ligand expression that is required for somite patterning and HSC specification from hemogenic endothelium of the dorsal aorta. We have now identified the Wnt16 receptors involved in this process. Wnt16 genetically interacts with the atypical Wnt receptors Ryk andRor1. Ryk and ror1 are expressed in the developing somite at the right time and place to transduce Wnt16 signaling. Knockdown of either receptor leads to decreases in expression of the Notch ligand deltaC (dlc) and a decrease in expression of the HSC marker runx1, thus receptor knockdown recapitulates the Wnt16 phenotype. The severity of hypomorphic phenotypes of ligand or receptor, achieved through low-dose morpholino injection, enhance one another, demonstrating that these genes function in a linear genetic pathway. Thus Wnt16 and Ror1 and/or Ryk are likely to be physiological ligand/receptor pairs. Because Wnt ligands use different domains to bind Ror and Ryk receptors, it is possible that Wnt16/Ror1/Ryk form a tripartite complex. Misexpression of WNT16 and ROR1 is a feature of specific B cell leukemias, including pre-B ALL resulting from the t(1;19) translocation and CLL. Our results point to the possibility

that developmental patterning downstream of Wnt16/Ror1, required for HSC specification, is aberrantly reawakened in leukemic transformation.

Program/Abstract # 182
Interactions between transplanted mouse embryonic stem cell-derived neural progenitors and endogenous brain vasculature
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Embryonic Stem Cell-derived Neural Progenitors (ESNPs) have potential clinical uses as replacement therapies for a variety of brain disorders. However, how transplanted cells recruit a blood supply, or how they migrate in the host brain, remains unclear. Angiogenesis can occur in the adult mouse brain in response to several insults, for example a tumor, ischemia, or an epileptic seizure. Conversely, the adult brain vasculature has been shown to act as a scaffold and signal source for migrating neural progenitors in the SVZ and SGZ. We have used an ESNP transplant paradigm to investigate both the ability of transplanted cells to recruit endogenous blood vessels and their migration in the host brain. We observe that ESNPs transplanted into the hippocampi of adult mice initially display a relatively avascular core, which after 4 weeks becomes abundantly vascularized. We also observe proliferating (Ki67+) endothelial (CD31+) cells in the transplant and surrounding hippocampus, but not on the contralateral side that did not receive a transplant. We find putative fractones (slender laminin-positive stems attached to blood vessels) in and near the transplant, but never on the contralateral side. These data suggest that the transplanted ESNPs stimulate an angiogenic response in the host brain. We also observe transplanted cells migrating on endogenous blood vessels. In addition, ESNPs cultured with hippocampal slices associate with the blood vessels in the slices. These data suggest that transplanted ESNPs use endogenous blood vessels as a scaffold for migration.

Program/Abstract # 183
Presynaptic input from corticotropin-releasing hormone-expressing neurons promotes adult-born neuron circuit integration
Garcia, Isabella; Huang, Longwen; Arenkiel, Benjamin, Baylor College of Medicine, Houston, United States
The brain undergoes neurogenesis throughout life, but the programs guiding newborn neuron circuit integration are unknown. Thousands of neurons are born daily in the olfactory system, yet only half form long-lasting circuits. Many activities, including learning, stress, and neuropathology affect this process.To reveal how activity influences neurogenesis, we aimed to identify the cell types providing inputs onto newborn neurons in the mouse olfactory bulb (OB). By targeting newborn neurons for transsynaptic tracing with Rabies Virus, we mapped local Corticotropin-Releasing Hormone (CRH)-expressing neurons with extensive inputs onto newborn neurons. CRH is implicated in diverse medical and psychological conditions, many of which influence neurogenesis. To elucidate the role for CRH input onto newborn neurons, we tested the following hypothesis: Presynaptic input from CRH-expressing neurons promotes newborn neuron circuit integration and synapse formation. Using novel viral tracing, mouse genetics, electrophysiological recordings, and optical imaging, we have found that 1) local CRH-expressing neurons are presynaptic to newborn neurons, 2) newborn neurons dynamically express the CRH receptor during periods of circuit integration, and 3) newborn neuron integration and survival is negatively impacted in mouse models lacking CRH or its receptor. We are currently investigating the programs that underlie CRH signaling to promote newborn neuron synapse formation, survival, and circuit integration in the OB. Our discovery that CRH-expressing neuron sprovide presynaptic inputs onto newborn neurons represents a novel and important mechanism to promote synapse formation and circuit integration in the adult mammalian brain.

Program/Abstract # 184
Genome-wide analysis of the basic Helix-Loop-Helix gene family in planarians identifies factors involved in neurogenesis
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Nervous system development requires the spatial and temporal specification of diverse neuronal populations, a process that is partly regulated by transcription factors from the basic Helix-Loop-Helix (bHLH) proneural gene family. Due to the limited regenerative capacity of most well-studied model organisms, little is known about the potential role these factors might play during replacement of damaged or missing neural tissues. We are characterizing the bHLH gene family in the freshwater planarian *Schmidtea mediterranea*. Following amputation, these animals can completely regenerate their central nervous system (CNS) from a population of pluripotent adult stem cells they maintain throughout their lives. Furthermore, planarians possess an easily-accessible nervous system with diverse neural subtypes, making them excellent models to

examine stem cell-based CNS regeneration invivo. To investigate the role of planarian bHLH genes, we identified 44 genes encoding bHLH domains in the *S.mediterranea* genome. Expression analysis using whole mount in situ hybridization revealed that a majority of these genes are enriched in the stem cells and/or CNS. We are currently using RNA interference to test the roles of these genes during CNS regeneration. Thus far, we have identified candidate bHLH genes that regulate neurogenesis such as achaete-scute and atonal homologs, which are known to play major roles in neural specification in other organisms. Understanding the factors that control neurogenesis in planarians will help us elucidate potential molecular mechanisms that direct stem cells in vivo to regenerate new neurons in adult animals. [Supported by NSERC grant to B.J.P., and CIRM Grant RN2-00940-1 to R.M.Z.]

Program/Abstract # 185

Epigenetic regulation of planarian stem cells by the SET1/MLL family of histone methyltransferases

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Chromatin regulation is a fundamental mechanism underlying stem cell pluripotency and differentiation and the establishment of the gene expression profiles of different cell types. A complete understanding of chromatin state changes during stem cell regulation will enhance our ability to identify pathways leading to developmental disorders and disease. To examine chromatin regulation in stem cells in vivo, we study regeneration in the freshwater planarian *Schmidtea mediterranea*. These animals possess a high concentration of pluripotent stem cells known as neoblasts, which are capable of restoring any damaged or lost tissues after injury or amputation. The SET1/MLL family regulates gene expression by methylating lysine4 of histone H3. This mark leads to an active chromatin state and recruitment of RNA polymerase II, thus promoting transcription. In order to identify set1/mll genes involved in neoblast regulation, we searched the *S.mediterranea* genome and found six homologues (set1, mll1/4, mll2/3, trr, mll5.1 and mll5.2). Using whole-mount in situ hybridization, we determined that four of these genes (set1, mll2/3, trr, and mll5.1) are expressed in the neoblasts. RNA interference (RNAi) knockdown of set1, mll1/4, trr, and mll5.2 results in animals that fail to regenerate properly after amputation. Most notably, set1 RNAi leads to a partial loss of the stem cell population. Future experiments will focus on identifying genes targeted by the SET1/MLL family in planarians and expanding the work to include other histone methyl transferases to help uncover epigenetic mechanisms that underlie stem cell regulation. [Supportedby CIRM Grant RN2-00940-1 to R.M.Z. and by NIH-IRACDAPostdoctoral Fellowship GM68524-08 to A.H.]

Program/Abstract # 186

Follistatin is required for head regeneration in the planarian, *Schmidtea mediterranea*

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Planarians possess an extraordinary capacity for regeneration. Upon amputation, these freshwater flatworms replace missing tissues and organ systems, partly through the function of pluripotent stem cells called neoblasts. Importantly, axial polarity respecification during regeneration enables the proper repatterning of new tissues and the formation of organs in the correct place. Anterior/posterior polarity in planarians requires Wnt signals from the tail and Wnt inhibition by Notum and secreted Frizzled-related proteins (sFRPs) in the head. However, the mechanisms underlying reestablishment of these signaling centers after amputation remain unknown. Here, we show that the *Schmidtea mediterranea* homolog of follistatin plays an essential role during head regeneration. follistatin expression is limited to a small number of notum+ cells in the anterior of the animal and to non-neural cells distributed near the planarian nervous system. After amputation, Smed-follistatin (RNAi) animals fail to regenerate cephalic ganglia and fail to reinitiate sFRP1 and notum expression in new anterior tissue. Concomitant RNAi of Smed-activin or Smed-ActRI rescues these phenotypes, suggesting that Follistatin antagonizes Activin signaling during regeneration. We propose a model in which a Follistatin/Activin axis controls respecification of axial patterning during planarian regeneration. We also hypothesize that Follistatin and Activin signaling might influence specification of cell fates during the regenerative process.

Program/Abstract # 187

Novel antibodies to track cell differentiation in planarians

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Planarians are well known for their ability to replace any of their tissues from a population of adult pluripotent stem cells (neoblasts). However, our knowledge of the spatial distribution and temporal sequence of neoblast differentiation during normal tissue homeostasis or regeneration is limited. To gain insights into the cellular events underlying regeneration, we have developed new monoclonal antibodies (mAbs) for the planarian *Schmidtea mediterranea*. Thus far, we have

generated nine mAbs that are specific to various cell types including the epidermis, musclefibers, protonephridia, and multiple neural populations. We have also characterized the staining patterns of these mAbs in regenerating planarians and found that a subset distinctly label cells within the regeneration blastema. We are currently co-staining planarians with the mAbs and other well-characterized tissue markers to further characterize the specific cell populations they label. To track cell differentiation, we are using these mAbs in conjunction with a new bromodeoxyuridine (BrdU) soaking protocol to track differentiation of mAb positive cells from neoblasts. We have optimized BrdU penetrance and yield a higher BrdU labeling success rate compared to currently published feeding protocols when intact worms were evaluated shortly after pulsing. Our work has produced novel markers to visualize planariant issues and a new method for introducing BrdU to quantify their differentiation. These tools will improve our understanding of how the neoblasts reconstitute different tissues during regeneration and aid in phenotypic screenings of gene knockdown experiments in planarians. This work was supported by CIRM GrantRN2-00940-1 to RMZ.

Program/Abstract # 188

Blastemal growth in regenerating *Giraradia tigrina* is inhibited by xenoestrogens

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Xenoestrogens are chemicals that mimic estrogen by direct binding to estrogen receptors. Many of these chemicals are industrial enviromental pollutants such as bisphenol A (BPA) and 4-octylphenol (4-OP). Prior work in our lab has shown that regeneration is disrupted by exposure to BPA and 4-OP in several species of planarians. In this work we use a quantitative method to measure blastemal growth in planarian regeneration and analyze the results with a logistic growth model. Using the model we show that xenoestrogens affect not only the rate of regeneration but also the size of the resulting blastema in a dose dependent manner.

Program/Abstract # 189

Elucidating the mechanism of proximal tubule regeneration in the pronephros *Xenopus laevis* tadpoles

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While the renal system is critical for maintaining homeostatic equilibrium within the body, it is also susceptible to various kinds of damage. Tubule dysfunction in particular contributes to acute renal failure and chronic kidney disease in millions of patients worldwide. Since current treatments are highly invasive and often unavailable, we are exploring the regenerative capacity of renal structures, specifically focusing on tubule restoration. Previously we have shown that *Xenopus laevis* tadpoles have this capacity to regenerate proximal tubules following partial nephrectomy. We have also validated the renal identity of the regenerate and demonstrated its ability to functional normally, providing the first evidence of renal regeneration in an amphibian system. We are now investigating the mechanism by which this regenerative event occurs, focusing our studies on the three week period following damage. An increase in the active form of caspase 3 was observed shortly after injury suggesting a role for apoptosis during the initial wound healing phase. This phenomenon has also been observed in the mammalian metanephric repair model, and thus appears to be a conserved aspect of the renal regeneration process. We are currently conducting inhibitor studies to assess whether this apoptotic event is critical for successful regeneration. Additionally, we are interested in the role that matrix metalloproteinase 9 (Xmmp-9) plays in this regenerative process. While Xmmp-9 hasbeen found to be essential for proper renal development, its role in renal repair has never been examined. Previously we have observed a biphasic pattern of Xmmp-9 up-regulation during renal regeneration, first during the initial wound healing process, and again later during tubule restoration. We are now investigating the roles that this protease may be playing during these two independent phases. Preliminary evidence suggests that Xmmp-9 may have an inhibitory effect on renewal of proximal tubule tissue.

Program/Abstract # 190

Calcium-mediated electrical activity manifests in regenerating tissues and is required for appropriate muscle regeneration

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Xenopus laevis tadpole can regenerate its tail after amputation with complete recovery of muscle, notochord and spinal cord. The cellular and molecular mechanisms underlying this phenomenon are still unclear. The goal of this study is to elucidate the mechanisms underlying muscle regeneration. We hypothesize that Ca2+- mediated electrical activity manifests in regenerating tissues and that this activity is necessary for proper muscle regeneration. Ca2+ imaging experiments show that cells in the regenerating tail exhibit spontaneous Ca2+ transients as early as 2 h post amputation (hpa). The level of activity appears to be temporally regulated and ceases by 30 hpa. Ca2+ transients last for 5-70 s with amplitudes in the range of 200-270% above baseline, have a rise time of 5-23 s and a frequency of 3-24/h per active cell.

Incubation of regenerating cells with either the anesthetic MS222 or intracellular Ca2+- release inhibitors Ryanodine and Xestospongin C, inhibits spontaneous activity by 60%. To investigate the impact of inhibiting Ca2+-mediated activity on the success of muscle regeneration we incubated amputated tadpoles with MS222, Ryanodine, Xestospongin C, or the cell-permeant Ca2+ chelator BAPTA-AM. Whole-mount immunostaining with muscle markers reveals that inhibiting Ca2+ transients decreases the extent of regeneration by 60-80% after 72 hpa. These findings suggest that regenerating tissues exhibit Ca2+ transients, mediated by Ca2+ influx and release from stores, which are necessary for muscle repair. Understanding how Ca2+-mediated electrical activity contributes to the repair of injured tissues may lead to improved therapies for tissue regeneration.

Program/Abstract # 191
An investigation of the role of transforming growth factor beta (TGFß) during multi-tissue regeneration.
Gilbert, Richard WD; Vickaryous, Matt; Viloria-Petit, Alicia, University of Guelph, Guelph, Canada
The transforming growth factor beta (TGFb) signaling pathway has a number of well documented roles in wound healing and is becoming increasingly appreciated as a vital component of multi-tissue regenerative processes in amphibians (e.g., *Xenopus* and axolotls). For amniotes (mammals and reptiles), less is known in part because of the lack of an appropriate model organism capable of multi-tissue regeneration. With this in mind, we examined the localization of several key components of the TGFb signaling pathway during tail regeneration of the leopard gecko (*Eublepharis macularius*). As for many lizards, the gecko is able to spontaneously and repeatedly regenerate its tail. We focused on characterizing TGFb 1+2, TGFb 3, phosphorylated Smad2 (pSmad2), as well as target genes such as Snail, Slug and Zeb2, at various stages of regeneration. We demonstrate that there is a sharp increase in TGFb ligand availability during both early and late regeneration combined with a localized increase in pSmad2 in both the regenerative epidermis and blastema like structure located basally to the epidermis. This activity has diverse effects on known Smad target genes including Snail, Slug and Zeb2. These genes encode transcription factors that have been implicated in driving cell motility and stem cell like features. Our characterization of the spatial and temporal expression of TGFb ligands suggests the possible role of epithelial to mesenchymal transitions during multi-tissue regeneration.

Program/Abstract # 192
Retinal regeneration following targeted rod photoreceptor destruction
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Zebrafish have the remarkable ability to fully regenerate their retinas following damage. The genes and regulatory mechanisms involved in retinal regeneration are largely unknown. MicroRNAs (miRNAs) are an intriguing possibility as one possible mechanism that controls the regeneration process. Previously, deep sequencing was used to create miRNA libraries throughout retinal regeneration following treatment with constant intense light to identify miRNAs involved in this process. This treatment, however, non-selectively destroys all photoreceptors. A new method has been developed to selectively destroy rod photoreceptors. Here we characterize regeneration progress in the retina following rod destruction. Once regeneration is characterized we can perform deep sequencing analysis to identify miRNAs and mRNAs specifically involved in rod regeneration.

Program/Abstract # 193
Analysis of gene expression in mantle and interneuromast cells reveals genes that are differentially regulated during hair-cell regeneration
Steiner, Aaron; Kim, Taeryn; Hudspeth, A. James, The Rockefeller University and HHMI, New York, United States
Hearing loss is an increasingly common problem in modern societies. Because the majority of hearing deficits are attributable to the death of sensory hair cells in the cochlea, therapeutic regeneration of these cells represents an attractive avenue to recovery. Although hair cells of the adult mammalian cochlea do not regenerate, those of nonmammalian sensory systems regenerate throughout life. In the zebrafish lateral line, for example, hair cells extirpated by chemical treatment regenerate fully within 48 hours. Each neuromast, the sensory unit of the lateral line, comprises a cluster of hair cells encircled by mantle cells that are connected to other neuromasts by interneuromast cells. Both of the latter cell types have been proposed to harbor hair-cell progenitors. We have developed a transgenic line of zebrafish,Tg (tnap:mCherry), that expressesred-fluorescent mCherry in both mantle and interneuromast cells. Fluorescence-activated cell sorting from transgenic larvae followed by RNA-seq and microarray-based expression analyses enables us to identify genes that are expressed more highly in these cells than in other cell types. In situ hybridization confirms that many of these genes are specific markers of mantle and interneuromast cells. Similar analyses following hair-cell ablation reveal genes that are differentially regulated during regeneration. Proteins encoded by these genes include cell-cycle regulators and components of known signaling pathways as well as several novel proteins. Knockdown and overexpression of candidate genes are

currently underway to determine how they affect regeneration. This approach will help elucidate the molecular mechanisms of hair-cell regeneration.

Program/Abstract # 194
Expression of stem pluripotency-inducing factors during RPE reprogramming
Luz-Madrigal, Agustin; Grajales-Esquivel, Erika; Di-Lorenzo, Ashley; Dannenfelser, Janessa; Del Rio-Tsonis, Katia, Miami University, Oxford, United States

The embryonic chicken can regenerate its retina by the reprogramming of the retinal pigmented epithelium (RPE) and by the activation of stem/progenitor cells present in the ciliary margin (CM) in the presence of fibroblast growth factor 2(FGF2). Recently, it has been demonstrated that somatic mammalian cells can be reprogrammed in vitro to generate induced pluripotent stem cells (iPSC) by the ectopic expression of Oct4, Nanog, Sox2, Klf4, c-Myc or Lin28 (ONSKCL). However, there is limited information concerning the reprogramming during the process of retina regeneration in vivo. Here, we test the hypothesis that reprogramming of the RPE can share similarities to the reprogramming of somatic cells that generate iPSC. Therefore, we analyzed the expression of stem cell pluripotency factors during chick development and RPE reprogramming. The analysis of ONSKCL expression showed that only Sox2, c-Myc and Klf4 mRNAs were detected by RT-PCR in the CM. Furthermore, Sox2 was detected by immunofluorescence in the CM and central retina but not in the RPE ofdeveloping embryos at day 4-7. Upon retina removal, while Sox2, c-Myc and Klf4 remained expressed in the CM, these genes were induced in the RPE. Their expression was maintained up to day 3, and at that time, FGF2 was required to keep the expression. These results suggest that the injury itself is sufficient to induce the expression of stem cell pluripotency-inducing factors. However, FGF2 is required to maintain the expression during RPE reprogramming. We also observed up-regulation of Lin28 and the pro-neural transcriptional factor Ascl1a in the RPE post-retinectomy suggesting that these two factors might also contribute to the process of RPE reprogramming. Finally our results demonstrate that Oct4 and Nanog are dispensable during the process of chick retina regeneration and indicate that reprogrammed RPE cells do not generate pluripotent cells. However, our results do suggest that retina regeneration through RPE reprogramming share similar mechanisms to the generation of iPSC cells.

Program/Abstract # 195
The role of microRNAs as downstream effectors of RARß-mediated retinoid signalling during spinal cord regeneration in the adult newt.
Lepp, Amanda C.; Carlone, Robert, Brock University, St. Catharines, Canada

Urodele amphibians possess the unique ability to regenerate lost structures, including spinal cord, following tail amputation. Little is known regarding the coordination of molecular pathways that control the formation of the tail blastema, as well as the outgrowth and patterning of the regenerating spinal cord. Our lab has been involved in studies examining the role of retinoic acid signalling in this epimorphic regenerative phenomenon. We have previously demonstrated that inhibition of RARβ-mediated retinoic acid signalling using a specific antagonist, LE135, significantly inhibits tail and spinal cord regeneration by delaying the formation and outgrowth of an ependymal tube caudal to the amputation plane. Our current focus is to identify the downstream effectors of RARβ-mediated retinoid signalling and elucidate the mechanism by which they contribute to regeneration. We have utilized a microarray approach with microRNA-based profiling to identify 18 highly conserved microRNAs that display significant changes in expression in tail regenerates treated with LE135 compared to DMSO control regenerates during the first 48 hours post amputation. Initially we have chosen seven of these microRNAs (miR-133a, miR-1, miR-26a,miR-145, miR-223, miR-1306 and let7c) for further investigation. miR-133a is expressed in the ependymal cells surrounding the central canal of the spinal cord in adult newt tail tissues. This miRNA is significantly down regulated in these cells during the first three weeks following tail amputation. Moreover, the spatial and temporal pattern of expression of miR-133a is consistent with a role for this micro RNA as a mediator of RARβ signalling in this process. Analysis of the patterns of expression and putative functions of the other microRNAs as downstream regulators of retinoid signalling during caudal spinal cord regeneration are currently underway.

Program/Abstract # 196
Two-Photon microscopy to capture live cell behavior in the hair follicle stem cell niche
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Stem cells and niche components are responsible for the timely orchestration of the regeneration process that leads to highly organized tissues. Despite recent progress in our understanding of stem cell biology, the dynamic interaction between stem cells and the niche is not well understood. A current challenge in the field is having access to a well-defined stem cell niche in which the orderly development of stem cells can be observed, characterized and manipulated *in vivo*. To

learn which dynamic behaviors occur during mammalian regeneration, we took advantage of the unique accessibility of the skin hair follicle and set up a novel imaging approach to study a physiological regeneration process non-invasively by two-photonmicroscopy. By these means, we have studied the behavior of the epithelial stem cells and their progeny during physiological hair regeneration and how the mesenchymal niche influences their behavior. Consistent with earlier studies, stem cells are quiescent during initial stages of hair regeneration, whereas the progeny are more prone to divide. Moreover, stem cell progeny cell divisions are spatially and temporally coordinated within follicles. In addition to cell divisions, coordinated cell movements within the progeny allow hair follicle rapid expansion. Finally, we demonstrate the requirement for the mesenchymal niche for hair regeneration through targeted cell ablation and long-term tracking of live hair follicles. Thus, we have established an in vivo approach that has led to the discovery of unpredicted mechanisms of growth regulation, and enabled us to precisely investigate functional requirements of stem cell niche components during the process of physiological regeneration.

Program/Abstract # 197

Uncovering the conserved stem cell functions of the Piwi/piRNA pathway in *Hydra*

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Piwi proteins have conserved functions in the somatic and germline stem cells of metazoans. They associate with Piwi-interacting RNAs (piRNAs, ~26-31 nucleotides) to form Piwi/piRNA complexes that regulate gene expression at both the epigenetic and post-transcriptional levels. However, the gene targets of the piRNA pathway have remained enigmatic due to the complex nature of both piRNA populations and bilatarian organ systems. *Hydra* provides a relatively simple and tractable metazoan system for studying adult stem cells in vivo. The adult *Hydra* polyp is composed of three distinct cell lineages, each supported by a separate population of stem cells. Our results show that there are two Piwi proteins (Piwi1 and 2) in *Hydra*, both of which are expressed in the cytoplasm of all *Hydra* stem cells, specifically enriched in perinuclear granules. We have sequenced ~15 million *Hydra* piRNAs associated with Piwi1 and Piwi2 and found that they have the same characteristics as bilatarian piRNAs, with primary and secondary piRNAs bound specifically to Piwi1 and Piwi2 respectively. Stable transgenic *Hydra* lines are being generated to test the function of *Hydra* Piwi proteins. Epithelial stem cells appear to require Piwi for proliferation and/or survival. Conversely, overexpression of Piwi in the epithelial cells leads to rapid proliferation and abnormal cellular morphology. These observations may suggest a conserved role for Piwi, as it is ectopically expressed in several human cancers. To identify the post-transcriptional target genes of the piRNA pathway, we are mapping the piRNAs to our de novo assembly of the *Hydra* transcriptome. This analysis will be extended by comparing the expression levels of putative targets in Piwi knockdown and overexpression cells as compared to the wild type. Cnidarian gene sets, such as that of *Hydra*, exhibit the same complexity found in vertebrate genomes, thus the identification of piRNA pathway targets in our work will likely provide important insights into the function of this pathway in more complex animals.

Program/Abstract # 198

Derivation of a phylogenetically conserved pluiripotent stem cell signature using transcriptomic analyses

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For many multicellular organisms, adult somatic stem cells (ASCs) are responsible for a stunning array of biological processes including tissue regeneration, homeostasis, and longevity. A major impetus in the field of regenerative medicine is to identify the factors responsible for maintaining ASC identity. It is hypothesized that a “stemness” program exists in ASCs, though the genetic mechanisms which define it remain elusive. The adult freshwater planarian (flatworm) possesses a large population of pluripotent ASCs (~10-20% of the cells in its body), which confers their ability to regenerate. Here, we exploit this abundant source of ASCs to study the molecular basis of the stem cell program. Planarian stem cells were sorted by flow cytometry to achieve three distinct cell populations on the basis of mitotic state and include: ASCs, their progeny, and terminally differentiated cells. Each lineage was RNA-deep sequenced and their transcriptomes compared to those of mouse and human embryonic stem cells (ESCs) so as to distil a stem cell gene signature. 385 highly enriched stem cell specific genes were cloned and screened by large-scale RNAi in the planarian. Of these, 100 genes to date were found to be indispensable for stem cell functions during regeneration. Among these ASC/ESC genes are candiates with no previous association to stem cell function, including dystrophin, DMD1, linked to muscular dystrophy in humans, and the

thyroid adenoma associated protein, THADA, putatively involved in TRAIL-induced apoptosis thus in mediating the steady state between cell death and proliferation. Through this and future work, we are beginning to identify the key "stemmness" factors that truly represent regulators of regeneration.

Program/Abstract # 199
MicroRNA mediated regulation of naïve and primed pluripotent states
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Two distinct phases of pluripotency have been proposed in the early mammalian embryo, a naïve state found in the epiblast of the 3.5 days post coitum (dpc) mouse embryo and in embryonic stem cells (ESCs) and a primed state, found in the epiblast of the 5.5-6.5 dpc embryo and in epiblast stem cells (EpiSCs). These two states of pluripotency are thought to be regulated by different mechanisms as different combinations of growth factors are required for their maintenance both in vivo and in vitro. MicroRNAs (miRNAs) are small non-coding RNAs that repress gene expression post-transcriptionally. In ESC miRNAs are required for proper proliferation and for exit from the naïve pluripotent state, however little is known about their roles in the primed phase of pluripotency. We have found that in contrast to the naïve pluripotent state in the primed pluripotent state miRNAs are required for cell survival but not for the initiation of differentiation, either in vitro or in vivo. Profiling in embryos from 5.5 dpc to 8.5 dpc has identified four miRNA families that account for over 75% of the total miRNA content at these stages. These miRNAs show dynamic expression during the initial phases of epiblast differentiation and are likely to be responsible for the defects observed in embryos and EpiSCs lacking miRNAs. This work provides insight into how miRNAs regulate the different pluripotent states and contributes to the understanding of the regulatory networks involved in stem cell homeostasis, pluripotency and differentiation during early embryo development.

Program/Abstract # 200
Examining the evolutionarily conserved functions of Piwi proteins in *Hydra*
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Piwi proteins and their associated Piwi-interacting RNAs (piRNAs) are evolutionarily conserved, having been observed throughout the animal kingdom to participate in stem cell regulation and preserving genetic integrity. In the vertebrates and ecdysozoans investigated thus far, Piwi proteins and piRNAs are highly enriched in the germline. Interestingly, Piwi proteins are expressed in the multipotent or totipotent stem cells of other bilaterians and in non-bilaterian animals, implying a wider role of such genes throughout the animal kingdom than previously thought. More importantly, this suggests an ancestral significance of Piwi protein functions in stem cells. We have generated antibodies against two Piwi orthologs, Piwi1 and Piwi2, in the basal metazoan, *Hydra*. Both Piwi proteins are specifically expressed in the interstitial stem cells—multipotent stem cells that give rise to somatic and germline lineages—and localize to perinuclear foci which are largely reminiscent of the nuage. This organelle has been implicated as a site for piRNA-mediated transposon silencing and the ping-pong amplification loop of piRNA biogenesis. Piwi1 and Piwi2 foci are frequently observed to colocalize or collocate with each other, suggesting that piRNA biogenesis via ping-pong amplification might be taking place between the two *Hydra* Piwi proteins. Both Piwi1 and Piwi2 foci progressively diminish in size and intensity in differentiating intermediates of the interstitial cell lineage and are not observed in fully differentiated cells. Our preliminary data suggests that piwi2 is involved in the maintenance of interstitial stem cells. We have also isolated piRNAs from *Hydra* and the piRNA population which is bound to Piwi2.

Program/Abstract # 201
Forward genetics identifies Edf1 as a novel regulator of epidermal development and stem cell quiescence
Lee-Wölfel, Sunjin; Kong, Yong; Weatherbee, Scott, Yale University, New Haven, United States.

The outermost layer of the skin, the epidermis, plays a key role in animal survival by acting as a barrier to prevent infection and desiccation. Stem cells in the interfollicular epidermis (IFE) undergo a series of cell fate choices during the differentiation program to form a stratified epidermis. The appropriate balance between proliferation and differentiation is crucial for epidermis function, and alterations in this process can cause human diseases, such as psoriasis and skin cancer. However, the factors that regulate cell fate choices of stem cells in the IFE are not well understood. To identify new mediators involved in these processes, we performed a forward genetics screen in mice and identified a novel regulator of skin development, the Epidermal differentiation factor 1 (Edf1) gene. Mice carrying a homozygous mutation in Edf1 develop a hyperproliferative, poorly differentiated epidermis. We have shown that Edf1 function is essential to curb stem

cell proliferation and for normal differentiation of their progeny. We further demonstrate that Edf1 and the cell cycle regulator Stratifin (Sfn; 14-3-3sigma) act together to regulate keratinocyte differentiation and epidermal barrier formation. The transcription factor p63 is a master regulator of epidermal development and strongly expressed in the stem cell compartment. Edf1 mutants, however, exhibit increased levels of p63 throughout the IFE and reduction of p63 dosage in Edf1 mutants rescues many aspects of the phenotype, indicating that Edf1 modulates p63 levels. Together, our findings identify Edf1 as a novel regulator of epidermal stem cell proliferation and differentiation that regulates p63 expression and acts with Sfn to balance these processes.

Program/Abstract # 202
Hh signalling is a key regulator for somatic stem cells in the *Drosophila* testis
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Proper control of stem cell maintenance versus differentiation is essential for tissue homeostasis. In the *Drosophila* testis a niche located at the tip regulates two types of stem cells, germline stem cells (GSCs) and cyst stem cells (CySCs), in a concerted manner. Much light has been shed on how GSCs are regulated, but there is now growing interest in the regulation of CySCs. Here we show that Hedgehog (Hh) signaling is a key regulator of CySCs in the testis, while only indirectly affecting GSCs. Loss of Hh signaling in CySCs results in premature differentiation and consequent loss of the cells. Overactivation of the pathway leads to an increased proliferation and an expansion of the stem cell compartment. As Hh signaling is also a regulator of the somatic cells in the mammalian testis and the fly ovary this may reflect a higher degree of homology between these systems than previously expected.

Program/Abstract # 203
Regulating the transition from proliferation towards differentiation in the zebrafish retinal stem cell niche
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The eyes of fish grow continuously due to the presence of actively cycling stem cells. These retinal stem cells are maintained in a small region near the periphery of the eye termed the ciliarymarginal zone (CMZ). Cells within the CMZ are organized such that the leas tdetermined stem cells are nearest the periphery, the proliferative neuroblasts more medial, and the post-mitotic differentiating cells adjacent to the central retina. To investigate how CMZ cells transition from quiescence to proliferation to terminal cell cycle exit and differentiation, we are analyzing zebrafish mutants with small eyes that fail to grow. In the flotte lotte (flo) mutant, CMZ cells remain proliferative, lose the ability to enter their final, neurogenic divisions, and subsequently die. Interestingly, when flo mutant cells are transplanted into the CMZ of wild-type retinae, they progress from proliferation towards differentiation, revealing that the differentiated retinal environment limits proliferation of precursors emerging from the CMZ. Clues to the identity of one of the signals required for environmentally enforced differentiation come from our analysis of a new mutant, egghead (egh). In egh embryos, retinoic acid (RA) synthesis is up-regulated throughout the entire peripheral domain of the CMZ. Increasing RA levels in wild-type eyes during the period of CMZ neurogenesis blocks retinal growth and promotes precocious cell cycle exit and differentiation. Together, our data support a model in which extrinsic signals impinge on distinct phases of the cell cycle to maintain the composition of the CMZ and ensure that an appropriate number of new neurons emerge from the retinal stem cell niche.

Program/Abstract # 204
Fern leaf evolution and development
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The evolution and development of leaves in land plants has been debated for more than a century. Multiple lines of evidence indicate that megaphylls originated independently up to nine times, six of which have occurred within ferns. Most research on the developmental network necessary to specify leaves has been done on angiosperms, and comparable studies are largely lacking for ferns. Therefore, questions remain about the homology of megaphylls. To address this gap in our understanding of fern leaf evolution, we are studying some of the gene families that play a role in angiosperm leaf development. We are cloning and analyzing two of these genes across ferns: Class-1 KNOX and Class III HD-Zips. Our analyses include 10 out of 11 orders of extant ferns. Results suggest that both gene families duplicated early in the diversification of ferns, and at least two copies of each gene family are present in each fern taxa sampled. We have analyzed the expression patterns of several paralogs of these two families and found that they resemble that of angiosperms homologs. There are no functional model systems in ferns. Instead, we are using the natural variation of leaf morphology present in a group of closely related species in the fern genus *Elaphoglossum*, in an attempt to correlate their leaf

morphology variation with the expression patterns of the genes orthologous to the angiosperm leaf genes. Our molecular phylogenetic analyses indicate that divided leaves in the group evolved at least four times independently. So far, our expression studies across ferns indicate that the same leaf developmental network was coopted for megaphyll development in ferns and angiosperms suggesting a deep homology of megaphylls in these two groups.

Program/Abstract # 206
Conservation of Myogenic Regulatory Factor function
Meedel, Thomas H., Rhode Island Col Dept of Biol, Providence, United States; Izzi, Stephanie; Colantuono, Bonnie; Sullivan, Kelly (Rhode Island College, Providence, RI, United States)
Recent studies on the developmental basis of animal evolution indicate that changes in regulatory gene activities often occur without extensive modification of the proteins they encode, instead occurring via changes in cis-regulatory elements. Myogenic Regulatory Factors (MRFs) of the MyoD family are a possible exception to this concept since vertebrate and invertebrate myogenesis typically differs in its reliance on these genes. Our studies with Ci-MRF, the *Ciona intestinalis* MRF, lead us to an alternative explanation; that is MRFs are indeed functionally conserved proteins, and differences in their roles invertebrate and invertebrate myogenesis result from the dissimilar regulatory settings in which they function. We tested this idea using a simple in vivo assay in which MRFs were expressed in the notochord of *Ciona* embryos, and their ability to direct myogenesis in that tissue was determined. We first used naturally occurring variants and synthetic constructs of Ci-MRF to evaluate the roles of three conserved features of MRFs: a cysteine/histidine rich domain just N-terminal to the b-hlh domain; Helix III near the C-terminus, and an alanine-threonine dipeptide of the basic domain known as the “myogenic code”. As in vertebrate MRFs, all three were required for normal Ci-MRF activity. We also created chimeric proteins by replacing the C/H domain and b-hlh domain of Ci-MRF with the corresponding domains of selected vertebrate and invertebrate MRFs and found that these too were able to direct myogenesis. Our studies support the hypothesis that MRFs are functionally conserved over extensive evolutionary distances.

Program/Abstract # 207
Evolution of gene regulatory networks for novelty
McCauley, Brenna; Hinman, Veronica (Carnegie Mellon U., Pittsburgh, United States)
Changes to developmental gene regulatory networks (GRNs) underlie the advent of novel morphologies. The means by which novelty arises during evolution are not well understood, but the co-option of networks or network subcircuits from distinct developmental contexts is thought to be an important mechanism. While there are many examples of possible co-option in the literature, how the ancestral developmental programs change to accommodate these novel subcircuits has not been explored in depth. Echinoderm embryos are an excellent model system to examine this question, as sea urchins possess at least two types of mesoderm, the skeletogenic mesenchyme (SM) and pigment cells, that are not present in basal sea stars. Furthermore, the GRN underlying sea urchin mesoderm development is extremely well-characterized, providing a fundamental starting point for examining network changes associated with novelty. Comparisons of transcription factor expression in the mesodermal territories of sea urchins, sea stars, and the new model system, the sea cucumber, *Parastichopus parvimensis*, revealed a pattern of broad conservation coupled with subtle changes in gene regulatory state associated with the presence of novel mesodermal cell lineages. Our results suggest that the creation of a novel regulatory territory precedes the acquisition of a new cell fate and provide evidence for the step-wise evolution of traits.

Program/Abstract # 208
Hedgehog signaling is dependent on ciliary trafficking proteins in the sea urchin embryo.
Warner, Jacob, Duke University, Durham, United States; McClay, David (Duke University, Durham, United States)
A recent frontier in Hedgehog (Hh) signaling research is the requirement of the primary cilium and intra-flageller transport (IFT) proteins for signal transduction. Studies in vertebrates have shown that proper trafficking of Hh pathway components within the primary cilium is essential for Hh signaling. This machinery is not necessary, however, for Hh signal transduction in protostomes such as *Drosophila*. As a basal deuterostome, the sea urchin occupies a unique phylogenetic position and can provide crucial insight into the evolution of hedgehog signaling. Here we provide evidence that, as in vertebrates, Hh signaling in the sea urchin relies on the presence of cilia. It has been previously shown by Robert Morris and John Scholey that Kinesin II is essential for cilia assembly. Indeed, knockdown of Kinesin II using an antibody phenocopies Hh morphants. Likewise, knocking down the microtubule associated protein, Costal2, also produces a phenotype consistent with inhibition of Hh signaling. These findings lead to a model of sea urchin Hh signaling that closely resembles the pathway as it functions in vertebrates. This indicates the necessity of cilia for Hh signal transduction is not vertebrate specific as previously speculated but in fact deuterostome specific.

Program/Abstract # 209

***Drosophila* Zasp52 and Zasp66 act partially redundantly in Z-disc assembly and maintenance**

Liao, Kuo-An, McGill University, Montreal, Canada; Katzemich, Anja; Schöck, Frieder (Montreal, Canada)

Zasp52 is the only member of the PDZ-LIM domain protein family in *Drosophila*, which comprises Enigma, ENH, ZASP, Alp, CLP36, RIL, and Mystique in vertebrates. *Drosophila* Zasp52 colocalizes with integrins at myotendinous junctions and with a-actinin at Z-discs, and is required for muscle attachment and Z-disc assembly and maintenance. There is, however, a closely related PDZ-only domain protein in *Drosophila*, called Zasp66, which also localizes to Z-discs. Here we show that Zasp52 and Zasp66 precisely colocalize at the Z-disc and that depletion of Zasp66 by RNAi causes mild defects in Z-disc structure. Additionally, knockdown of Zasp66 with one copy of Zasp52 removed causes higher pupal lethality than knockdown of Zasp66 alone, demonstrating a strong genetic interaction. Finally, the Zasp52 Zasp66 double mutant shows higher embryonic lethality than the Zasp52 mutant alone, suggesting that these two proteins act partially redundantly in Z-disc assembly. To assess the mechanism of this redundancy, we have started to analyze proteins interacting with Zasp52 and Zasp66 by affinity purification and mass spectrometry and will discuss our results. In particular, we demonstrate that both Zasp52 and Zasp66 biochemically interact with a-actinin, which tethers thin filaments to the Z-disc. This interaction appears to be mediated by the PDZ domain and the Zasp motif, which are present in both Zasp52 and Zasp66.

Program/Abstract # 210

Identifying cis-regulatory element changes that underlie gene expression and phenotypic evolution between species.

Salomone, Joseph R., University of Dayton Biology, Dayton, United States; Rogers, William; Williams, Thomas (University of Dayton, Dayton, United States)

Contributing substantially to the diversity of Earth's animal life are differences in regulatory DNA, particularly in cis-regulatory elements (CREs) that contain information about where, when, and to what level genes are expressed. The variation in abdominal pigmentation in fruit fly species provides a model to study both the evolution of CREs and the gene expression patterns they control. The bric-a-brac (bab) locus codes for the Bab1 and Bab2 proteins that are repressors of abdominal pigmentation development. In the species *Drosophila melanogaster*, pigmentation and Bab expression are sexually dimorphic due to the activity of a CRE called the dimorphic element. As abdominal pigmentation patterns vary between related species, our overarching hypothesis is that these phenotypic differences stem from changes in Bab expression via modifications to the dimorphic element. To test this hypothesis we are studying the Bab1 and Bab2 expression patterns and the regulatory capabilities of dimorphic elements for 4 related species with different abdominal pigmentation patterns. To trace how modern dimorphic element regulatory activities evolved, we derived and resurrected ancestral dimorphic element sequences at key nodes in the phylogeny of the studied species. Ongoing functional studies aim to identify the evolutionarily relevant mutations responsible for the evolved Bab expression patterns.

Program/Abstract # 211

The role of toolkit genes in the evolution of complex wing, thorax, and abdominal color patterns in *Drosophila* guttifera

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Animal color patterns such as zebra stripes, leopard spots, and the myriad variants of butterfly wing color patterns are known to play important ecological and physiological roles in the life of animals and are crucial for the survival of species. Scientists first tried to solve the secret of animal patterns with mathematical approaches to find models that could explain how these patterns developed. In 1952, Turing proposed the famous reaction-diffusion model in which a short-range acting activator molecule diffuses from a source to stimulate color production, while a long-range acting inhibitor molecule prevents pigmentation. Using the spectacularly ornamented fruit fly *Drosophila guttifera*, we developed a transgenic protocol to study the development and evolution of color patterns. We identified that the Wingless morphogen had evolved a new function in the *D. guttifera* lineage by activating the yellow gene on pre-existing structural landmarks on the wing, causing black melanin spots around sensory organs, tips of the veins, and crossveins. We are currently expanding this work by investigating if the melanin patterns on different body parts of *D. guttifera* evolved by the same mechanisms involving Wingless, or if they are a product ofconvergent evolution. We optimized an in situ hybridization technique for the developing thorax and abdomen and showed that the yellow gene is expressed in a pattern precisely foreshadowing the four longitudinal melanin stripes on the thorax, and the six rows of abdominal spots that decorate the body of the adult *D.*

guttifera fly. We will use the in situ hybridization technique to identify candidate regulators that govern the complex yellow expression pattern.

Program/Abstract # 212
A potential patterning difference underlying the oviparous and viviparous development in the pea aphid
Bickel, Ryan, University of Nebraska, Lincoln, Lincoln, United States; Cleveland, Hillary; Barkas, Joanna; Belletier, Nicollette; Davis, Gregory K., Bryn Mawr College Dept of Biology, Bryn Mawr, United States

The pea aphid, *Acyrthosiphon pisum*, exhibits several environmentally cued, discrete, alternate phenotypes (polyphenisms) during its life cycle. In the case of the reproductive polyphenism, differences in day length determine whether mothers will produce daughters that reproduce either sexually by laying fertilized eggs (oviparous sexual reproduction), or asexually by allowing oocytes to complete embryogenesis within the mother without fertilization (viviparous parthenogenesis). Oocytes and embryos that are produced asexually develop more rapidly, are yolk-free, and much smaller than oocytes and embryos that are produced sexually. Perhaps most striking, the process of oocyte differentiation is truncated in the case of asexual/viviparous development, potentially precluding interactions between the oocyte and surrounding follicle cells that might take place during sexual/oviparous development. Given the important patterning roles that oocyte-follicle cell interactions play in *Drosophila*, these overt differences suggest that there may be underlying differences in the molecular mechanisms of pattern formation. Our preliminary work comparing the expression of homologs of torso-like and tailless, as well as activated MAP kinase, suggests that there are important differences in the hemipteran version of the terminal patterning system between viviparous and oviparous development. Establishing such differences in the expression of patterning genes between these developmental modes is a first step toward understanding how a single genome manages to direct patterning events in such different embryological contexts.

Program/Abstract # 214
Key regulator for developmental and evolutionary switch from Rohon-Beard cells to dorsal root ganglia
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In the body of adult vertebrates, dorsal root ganglia (DRG) convey a variety of sensory modalities to the central nervous system. However, during fish and amphibian development, intramedullary sensory neurons, Rohon-Beard (RB) cells, are responsible for juvenile mechanosensation. As DRG start to process mechanosensory inputs, RB cells undergo cell death by apoptosis. In *Xenopus* RB cells, the first expression of Six1 appears right before the onset of cell death, eliciting the idea that Six1 mediates the developmental switch from RB cells to DRG. Indeed, forced expression of Six1 in early *Xenopus* embryo resulted in precocious switching from RB cells to DRG, on the contrary, knockdown of Six1 prevented the switching. Furthermore, genetic ablation of both Six1 and Six4 caused the emergence of RB-like cells in mice. These results allow us to hypothesize that the precocious expression of Six1 in mouse sensory neuron development leads to the disappearance of RB cells and investigate the molecular mechanism defining the onset of Six1 expression. Expression of Six1 in mouse DRG was mediated by one of conserved Six1 enhancers. *Xenopus* orthologous enhancer activated transcription when the endogenous Six1 expression is turned on in *Xenopus* RB cells. However, the mouse enhancer activated transcription in RB cells earlier than the *Xenopus* element, indicating that the heterochronic shift of Six1 expression is caused by alteration of a single enhancer. Taken together, our findings suggest that the difference in the architecture of primary sensory neurons is caused by the change of cis-regulatory element and that the precocious expression of Six1 is a key process that facilitates the phylogenetic disappearance of RB cells in amniotes.

Program/Abstract # 215
Characterization of the bone-forming cells of the turtle plastron
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Turtle plastron bones develop by intramembranous ossification, suggesting that they are derived, like the facial bones, from neural crest cells. We have previously shown that a wave of cells expressing neural crest markers emerges from the neural tube later than in comparably staged chick or mouse embryos, and appears to migrate ventrally to populate the plastron dermis. This second, later wave of HNK1+ cells can also be observed migrating away from cultured neural tubes from St.17 embryos. These late emerging neural crest cells also express PDGFRα, which is typically expressed by cranial neural crest cells. We have examined the expression pattern of plastron mesenchyme cells by antibody staining and gene expression analysis, and their potential for differentiation by in vitro culture. Plastron mesenchyme cells have a gene expression pattern similar to cranial skeletogenic neural crest cells. They also appear to have functional similarities to

cranial neural crest cells, as they differentiate readily in culture to form clusters of collagen I-positive cells. Other types of neural crest derivates (neurons, melanocytes) are not observed. These data support our hypothesis that the plastron of the turtle is formed by a late emerging population of neural crest cells that collect dorsally in the carapace, migrate ventrally to the plastron, and undergo intramembranous ossification.

Program/Abstract # 216
Raising the shield: the origin and loss of periodic patterning in the turtle shell
Moustakas, Jacqueline, University of Helsinki, Helsinki, Finland; Cebra-Thomas, Judith (Millersville University, Millersville, United States); Mitchell, Katherine (Swarthmore College, Swarthmore, United States); Jernvall, Jukka (University of Helsinki, Helsinki, Finland); Gilbert, Scott F. (Swarthmore College, Swarthmore, United States)

The developmental mechanisms responsible for the formation of the turtle shell remain one of the great mysteries in evolutionary biology. The keratinous scutes of the turtle shell are novel epidermal structures, the patterns of which are diagnostic of different taxa. Looking at paracrine factors and their inhibitors, we show that scutes develop from placodal signaling centers that originate in a manner significantly distinct from those of other reptilian and avian ectodermal appendages. Inhibiting Shh and BMP signaling experimentally results in the loss of these signaling centers and scutes. Furthermore, we show that the scuteless soft-shelled turtle lacks these placodal signaling centers. We propose that these signaling centers represent a novel mechanism to develop ectodermal appendages and that the regulation of these centers have allowed for the diversification of turtle shell function.

Program/Abstract # 217
Determining the role of maternal IRF6 in extra-embryonic development
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Defects in trophectoderm differentiation and placental development are associated with a number of common pregnancy complications, including spontaneous abortion, preterm birth and preeclampsia. In mammals, the cell types that compose the placenta are derived from the trophectoderm. Interferon regulatory factor 6 (Irf6), a member of the interferon regulatory factor family of transcription factors, is maternally expressed in zebrafish and *Xenopus*. Genetic studies in zebrafish and *Xenopus* suggest a conserved role for maternal Irf6 in development of primary superficial epithelium (PSE). Inhibition of maternal Irf6 using a putative dominant negative Irf6-DNA binding domain results in embryonic lethality due to improper differentiation of the PSE. The PSE can be likened to the trophectoderm as they are both simple, monolayer epithelium that encompass the developing embryo. We hypothesize that maternal Irf6 is expressed in murine oocytes, and that it is necessary for proper differentiation of the trophectoderm in mice. To determine the presence of maternal Irf6 in oocytes, cross-sections of ovaries collected from wildtype female mice were immuno-stained with an antibody against Irf6. To determine the function of maternal Irf6 in trophectoderm differentiation, conditional gene knock-out was used. Specifically, a conditional Irf6 allele along with an oocyte-specific Cre recombinase was used to delete Irf6 in oocytes. Ovaries collected from females with oocyte-specific deletion of Irf6 were sectioned and stained with hematoxylin and eosin to assess morphological abnormalities in oocyte development. We observed that Irf6 was highly expressed in murine oocytes. Irf6 expression could be detected in the ooplasm of developing oocytes. Irf6 was also expressed in the granulosa cells surrounding the oocyte. Female mice with oocyte specific deletion of Irf6 are born at Mendelian ratios. Preliminary experiments show no morphological abnormalities in oocytes from mice with oocyte-specific deletion of Irf6 suggesting that maternal Irf6 is not required for oocyte development. The role of Irf6 of trophectoderm has yet to be determined. The expression data and conserved role for maternal Irf6 in zebrafish and *Xenopus* suggest that maternal Irf6 has a role in the differentiation of trophectoderm. The findings of this research will provide a novel role for Irf6 in development of extra-embryonic tissue. This is research has clinical relevance because 30% of the population carries a DNA variant that alters IRF6 expression.

Program/Abstract # 218
Is TMED2 essential in the chorion for normal interaction between the allantois and the chorion in mice?
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During vesicular transport between the endoplasmic reticulum and the Golgi, members of the transmembrane emp24 domain (TMED) protein family form hetero-oligomeric complexes that facilitate protein cargo transportation and secretion. In our laboratory, we are studying the function of one member of the TMED protein family, TMED2, in mouse placental labyrinth development. Formation of the mouse labyrinth layer requires proper interactions between two extraembryonic tissues, the allantois and the chorion; and is essential for nutrition, waste, as well as hormone exchange

between fetal and maternal circulation. We have shown that Tmed2 is expressed in both allantois and chorion and is required for normal labyrinth layer formation. We hypothesized that TMED2 is essential in the chorion or allantois for normal interaction between the allantois and chorion- a critical step in placental labyrinth layer development. To test this hypothesis, we have generated an ex-vivo allantois and chorion recombination model. In our model, we recapitulated the early events of labyrinth layer development: chorioallantoic attachment, fusion of the mesothelium and allantois, and chorionic trophoblast differentiation. We used in situ hybridization and immunohistochemistry to confirm the chorioallantoic attachment event and to monitor development of labyrinth layer in the chimeric explants. We will then use combinations of wildtype and Tmed2 null chorion and allantois in these ex-vivo cultures to follow branching morphogenesis in the chorion. Our work will provide insight into the contribution o fplacental-specific vesicular transport by TMED2 to labyrinth layer morphogenesis. Ultimately we will identify novel mechanisms that may be implicated in the prediction and treatment of placental diseases such as EPL and IUGR.

Program/Abstract # 219
Cis-regulatory analysis of Ets1 expression in neural crest reveals important inputs from Sox and Hox factors
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Neural crest cells emigrate from the dorsal neural tube and migrate to diverse sites within the embryo to contribute to the bones of the face, peripheral nervous system, melanocytes and other cell types. To identify regulatory connections that lead to neural crest formation, we have investigated the cis-regulatory elements involved in the expression of neural crest specifier gene Ets1. Ets1 is particularly interesting because it is uniquely expressed in cranial but not trunk neural crest cells. Our previous results revealed Ets1 to be a direct input for expression of the neural crest specifier gene, Sox10, in the cranial neural crest. We find that Ets1 is first expressed in the dorsal neural tube at 5 somites (stage 8) prior to Sox10, and continues to be expressed in migrating neural crest of the head. To identify putative enhancer elements, we isolated a genomic region, conserved between birds and mammals, which drives gene expression in a manner that recapitulates much of the Ets1 expression in the neural crest. Mutation of SoxE putative transcription factor binding sites results in reduction of enhancer activity in the neural crest, thus revealing a critical input for SoxE family members such as Sox9 and Sox10. A putative Hox site also is required for neural crest expression. In contrast, a putative cMyc/E-box sequence appears to act as a repressor binding site. Finally, a putative TFAP2a binding site mutation results in different responses at different times, acting as a repressor binding site early but as an activator later in neural crest development. ChIP analysis and knockdown of putative inputs are in progress to confirm dir

Program/Abstract # 220
Origin of the mammalian neural crest: from mouse, to rabbit, to human
Garcia-Castro, Martin I., Yale University, New Haven, United States

Neural crest (NC) cells are vertebrate-specific, arise early in development, migrate throughout the body, and contribute to a wide variety of derivatives. Aberrant neural crest development results in a large number of human health conditions, including cleft lip/palate, Hirschprung and Waardenburg syndromes, and melanoma and neuroblastoma. Therefore, neural crest development is of great interest to basic biologists and translational researchers alike. Current knowledge of the origin of neural crest cells is abundant in frog, fish, and chick embryos. Previously we identified the specification of NC occurring prior to gastrulation and an essential role for the early marker Pax7 in chick embryos. Despite the wide use of the mouse as a model organism, minimal progress has been gained towards understanding the early specification and induction of the mammalian neural crest. Here we will report a multi-mammalian approach to advance our understanding of early NC development with particular interest to contribute to human NC biology. We have investigated the role of the transcription factor Pax7 in early murine NC development, and we attempted to identify early NC specification. To overcome limitations of the mouse system, and embracing the benefits of multiple comparative samples of mammalian NC development, we have incorporated the rabbit as an alternative mammalian model organism. Here we present for the first time, the expression profile of markers of the NC gene regulatory network in the rabbit and the specification of NC in a mammalian embryo. Finally we undertook complementary studies in humans through embryonic stem cell differentiation and early embryo expression, to establish induction and ontogeny of human NC cells.

Program/Abstract # 221
Hindlimb evolution and the bilateral loss of digits in the bipedal jerboa
Cooper, Kimberly L.; Uygur, Aysu; Tabin, Clifford, Harvard Medical School, Boston, United States

Selective pressure to run or bound over great distances has repeatedly reduced the number of digits in diverse species. In many cases, such as the large hooved animals including deer and horses, these extreme adaptive changes are found in animals that are phylogenetically far removed from model organisms and in taxa that are themselves not amenable to

experimental analysis. The jerboas, in contrast, are a group of bipedal desert-adapted rodents that provide an extraordinary opportunity to address the developmental and genetic basis of limb evolution. Not only do their hindlimbs display the bilateral loss of digits 1 and 5, they are closely related to mice with whom they can be developmentally compared. Moreover they are small mammals that are plentiful in the wild and amenable to rearing in a laboratory setting. We find that the shape of the early hindlimb bud of the three-toed jerboa differs from that of the forelimb and from the mouse hindlimb bud. This narrowing of the footplate correlates with an increased expression of bmp4, an important regulator of cell death and proliferation. At early condensation stages, the proximal aspects of five digital rays are initially specified, but the first and fifth are truncated resulting in only three fully formed digits. Additionally, an increase in programmed cell death in the non-digit tissue surrounding the small truncated condensations suggests excessive sculpting contributes to digit loss. We are extending these studies to identify the genetic changes underlying evolutionary digit loss in the jerboa and are investigating possible mechanistic convergence in the symmetric loss of lateral digits in horses.

Program/Abstract # 222
Functional characterization of Dlx intergenic enhancers in the developing mouse
Esau, Crystal, University of Ottawa, Ottawa, Canada; Poitras, Luc; Yu, Man; Lesage-Pelletier, Cindy; Fazel Darbandi, Siavash; Ekker, Marc (University of Ottawa, Ottawa, Canada)

The Distal-less homeobox (Dlx) genes encode homeodomain transcription factors found in all animals of the phylum Chordata. These genes are involved in early vertebrate development of limbs, sensory organs, branchial arches and the forebrain (telencephalon and diencephalon). The mouse and human genomes each have six Dlx genes organized into convergently transcribed bigene clusters (Dlx1/2, Dlx3/4 and Dlx5/6). In the forebrain, Dlx1/2and Dlx5/6 genes play essential roles in GABAergic neuron proliferation, migration and survival. Each bigene cluster includes a short intergenic region (~3.5-16kb) harboring cis-regulatory elements (CREs) that control expression of the Dlx genes. The Dlx1/2 intergenic region harbors the I12b/I12a CREs, while Dlx5/6 includes I56i/I56ii. In determining the regulatory roles of the CREs on Dlx activity and forebrain development, we have characterized the phenotypic changes that occur in mice with a targeted deletion of I12b or ofI56ii. The effect of a single nucleotide polymorphism (SNP) in the I56i enhancer which is present in patients with autism spectrum disorder was also assessed. Mutant mice with a targeted deletion of I12b, I56ii or I56i SNP are viable, fertile and do not show obvious developmental or behavioral defects. The deletion of each Dlx enhancer studied thus far decreases Dlx expression. An increase in Dlx1 transcript levels may compensate for the reductions in Dlx5and Dlx6 expression. We are determining the functional importance of the I56i enhancer through characterization of the phenotypic changes in I56i null mice. This research will contribute to a better understanding of the role intergenic enhancers play in Dlx forebrain function.

Program/Abstract # 223
Ectodysplasin regulates activator-inhibitor balance in murine tooth development through modulation of Fgf20 signaling
Haara, Otso, Institute of Biotechnology, University of Helsinki, Finland, Helsinki, Finland; Harjunmaa, Enni; Lindfors, Päivi (Helsinki, Finland); Huh, Sung-Ho (St Louis, United States); Fliniaux, Ingrid; Åberg, Thomas; Jernvall, Jukka (Helsinki, Finland); Ornitz, David M. (St Louis, United States); Mikkola, Marja L.; Thesleff, Irma (Helsinki, Finland)

Uncovering the origin and nature of phenotypic variation within species is the first step in understanding variation between species. Mouse models with altered activities of critical signal pathways have highlighted many important genes and signal networks regulating the morphogenesis of complex structures, such as teeth. The detailed analyses of these models have indicated that the balanced actions of a few pathways regulating cell behavior modulate the shape and number of teeth. Currently, however, most mouse models studied have had gross alteration of morphology, while analyses of more subtle modification of morphology are required to link developmental studies to evolutionary change. Here we have analyzed a signaling network involving Ectodysplasin (Eda) and Fibroblast growth factor 20 (Fgf20) that affects tooth morphogenesis subtly. We found that Fgf20 is a major downstream effector of Eda, and affects Eda-regulated characteristics of tooth morphogenesis including number, size and shape of teeth. Fgf20 function ispartly compensated for by other Fgfs, in particular Fgf9 and Fgf4, and is part of an Fgf signaling loop between epithelium and mesenchyme. We showed that removal of Fgf20 in an Eda gain-of-function mouse model results in an Eda loss-of-function phenotype in terms of reduced tooth complexity and third molar appearance. However, the extra anterior molar, a structure lost during rodent evolution 50My ago, was stabilized in these mice.

Program/Abstract # 224
Prenatal administration of dexamethasone during early pregnancy negatively affects placental development and function in mice

Lee, Ji-Yeon, Yonsei University College of Medicine, Seoul, Korea, Republic of; Park, Sung Joo; Kim, Sang Hoon; Kim, Myoung Hee (Yonsei University College of Medicine, Seoul, Korea, Republic of)

Prenatal treatment of dexamethasone, a synthetic stress hormone, leads to low birth weight and affects adult pathophysiology. Since fetal growth and survival is critically dependent on the successful placental development, we aimed to investigate the effects of prenatal dexamethasone exposure on placental growth and function, particularly focusing on issues surrounding the time of stress exposure and in a developmental context. Dexamethasone was administered at a dose of 1 mg/kg (DEX1) or 10 mg/kg (DEX10) intraperitoneally at gestational days 7.5, 8.5, and 9.5 in pregnant mice and placentas were then dissected at gestational days 11.5 and 18.5. Placental size and weight were significantly reduced at day 11.5 in a dose-dependent manner. In contrast, a considerable heterogeneity was shown at day 18.5, especially in DEX10-treated mice. Some placentas were small and malformed, whereas some were enlarged with structural abnormalities in spongiotrophoblasts and labyrinth layers. Although placental overgrowth under such condition seemed to compromise fetal demand for nutrient supply, disorganized cell structure with reduced fetal vasculature observed in large placentas suggests that prenatal stress exposure during the early gestational period negatively affects placental development and efficiency.

Program/Abstract # 225
Live imaging analysis of dorsal aortae formation in the mouse gastrula embryo

Sanchez, Veronica, Rosalind and Morris Goodman Cancer Research Centre, Montreal, Canada; Yamanaka, Yojiro (The Rosalind and Morris Goodman Cancer Research Centre-McGill University, Montreal, PQ, Canada)

The cardiovascular system is the first system to develop in the vertebrate embryo. Mesodermal cells migrated from the posterior primitive streak to form endothelial precursors, called angioblasts, at the proximal region of the embryo. By the end of gastrulation, around E8.5, the extraembryonic yolk sac vasculature develops from the angioblasts; meanwhile, the formation of the paired dorsal aortae (DA) takes place at both sides of the notochord of the embryo. Concomitantly, the embryonic heart develops and the blood circulation begins. Although all endothelial cells in the yolk sac, the DA, and the heart are derived from Flk1 positive angioblasts, it is not known how they are specified and from where they originate. The aim of our study is to identify the origin of DA and endocardial cells in the gastrula embryo using live imaging techniques. In order to visualize angioblasts and endothelial cells, we used the Flk1-GFP mouse line, in which the GFP gene is knocked-in at the endogenous Flk1 allele. Live-imaging analysis of E7.5 gastrula embryos showed the active migration of a small population of Flk1-GFP positive angioblasts from the area adjacent to the amnion to the future DA forming site. The migrating cells occasionally aggregated and initiated luminal formation. They lined and formed a distinct tubular structure at the DA forming site. Interestingly, we identified that there are two distinct levels of Flk1-GFP expression, high and low. Only high Flk1-GFP cells contribute to the embryonic and extraembryonic vasculatures. Further studies are required to understand the identity of the Flk1-GFP low cells.

Program/Abstract # 226
Tracking a rudimentary colon in the vertebrate lineage.

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The acquisition of a colon was as important an event in tetrapod evolution as the fin-to-limb transition; animals were challenged by dehydration in their new terrestrial environment. The development of a colon allowed tetrapods to maintain water homeostasis and prevent desiccation on land. Recent histological, genetic and physiological data point to a rudimentary colon in marine elasmobranchs. A rudimentary colon in elasmobranchs is surprising because elasmobranchs, appearing 450 mya, predate the transition from aquatic to terrestrial life (approximately 370 mya). This suggests several models for colon evolution. First, the rudimentary colon in elasmobranchs may be analogous to the tetrapod colon, having arisen by convergent evolution. A rudimentary colon may have been advantageous to elasmobranchs, allowing them to adapt to different environments with changing salinities. A second model considers that despite their method of osmoregulation, elasmobranchs were pre-adapted for conserving water prior to the tetrapod transition. To address these possibilities, the digestive tracts of representative species from gnathostomes and chondrichthyes are being examined for cell and developmental markers, and histology indicative of a colon. Furthermore, examination of digestive tracts from ray-finned fish will help elucidate whether the terrestrial vertebrate colon evolved from the expansion of the rudimentary colon of elasmobranchs within the vertebrate lineage.

Program/Abstract # 227
Conserved genetic mechanisms for bilaterian gut regionalization

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The through-gut, consisting of separate portals for ingestion and excretion, arose in organisms at the base of the bilaterian tree and is a trait shared by the majority of protostomes and deuterostomes. Despite this shared origin, there are key differences in gut lineage, cell types, and organization of the specialized cell types between protostomes and deuterostomes – including the emergence of several unique gut organs within the vertebrate lineage (e.g. pancreas, gall bladder, and liver). Because cell type specification and organization are controlled by earlier endodermal patterning events, comparing these events between bilaterian lineages can clarify how differences and novelties in through-gut organization arose during evolution. We are interested in the origin of those novel vertebrate gut organs, so we have chosen to investigate endoderm patterning in the hemichordate *Saccoglossus kowalevskii*. *Saccoglossus* is a basal deuterostome with a relatively simple tripartite gut and a body plan patterned along the anterior-posterior (A/P) axis similarly to chordates. Expression of the patterning genes FoxA, GATA4/5/6s, Hox1, Pdx, and Cdx is highly conserved across bilaterians, suggesting an ancient pattern for A/P gut regionalization. Expression of FoxA and Hox1 in the 1st gut partition of *Saccoglossus* is reminiscent of FoxA/Hox1 expression in the vertebrate pharyngeal endoderm. Expression of GATA4/5/6 and Cdx in the 2nd and 3rd gut partitions, respectively, is similar to GATA4/5/6s and Cdx expression in the posterior foregut and intestines, respectively. We hypothesize that the 1st, 2nd, and 3rd gut partitions in *Saccoglossus* may be homologous, respectively, to the vertebrate pharynx, posterior foregut, and intestines.

Program/Abstract # 228
Evolution of spinal cord expression and function of Lbx transcription factors.
Juarez-Morales, Jose-Luis, Syracuse University, Syracuse, United States; Weierud, Frida (Cambridge University, United Kingdom); Lewis, Katharine (Syracuse University, Syracuse, United States)

The patterning and development of the spinal cord is very well conserved across vertebrates. For example, the expression patterns of transcription factors in specific dorsal-ventral regions of the spinal cord are, in the main, highly conserved across the vertebrate lineage. Lbx transcription factors are an exception to this rule as they are expressed differently in zebrafish and mouse spinal cord. Zebrafish have three lbx genes, all of which have distinct spinal cord expression patterns. In contrast, amniotes have two Lbx genes, but only Lbx1 is expressed in the spinal cord. This suggests that there may be some evolutionary plasticity in the genomic networks that specify spinal interneurons in different vertebrates. To understand how Lbx genes have evolved, we previously performed phylogenetic and synteny analyses of Lbx loci in bony vertebrates 1. We demonstrated that teleosts have two lbx1 genes, as a result of the additional genome duplication in the teleost lineage and one lbx2 gene. All other vertebrates studied had at most one Lbx1 and one Lbx2 gene1. To investigate how lbx spinal cord expression patterns have evolved we have examined expression in the shark *Scyliorhinus canicula* (dogfish) and frog *Xenopus tropicalis*. These results suggest that lbx1 spinal cord expression is highly conserved in vertebrates. However, lbx2 is not expressed in the dogfish spinal cord suggesting that spinal cord expression of lbx2 may have evolved in the ray-finned fish lineage. In mouse, Lbx1 is required to specify the inhibitory neurotransmitter phenotypes of dI4 and dI6 spinal interneurons. We are using lbx mutants and morpholinos to determine if the additional lbx spinal cord expression domains in zebrafish correlate with new spinal cord functions. 1 Wotton KR,Weierud FK,Dietrich Sand Lewis KE.(2008). Comparative genomics of Lbx loci reveals conservation of identical Lbxohnologs in bony vertebrates BMC Evol Biol. 8:171.

Program/Abstract # 229
Transcription factors with an ancient function in the specification of immunocytes
Solek, Cynthia M., University of Ottawa, Ottawa, Canada; Oliveri, Paola (University College London, London, United Kingdom); Rast, Jonathan (Sunnybrook Research Institute, Toronto, Canada)

Vertebrate hematopoiesis is the focus of intense study, yet immunocyte development is well characterized in only a few invertebrate groups. The sea urchin embryo provides a simple model for immune cell development in an organism that is phylogenetically allied to vertebrates. Larval immunocytes, including pigment cells and subsets of the blastocoelar cells, emerge from a small population of precursors specified at the blastula stage. A first step in immunocyte specification is the division of this cell field into precursors with distinct blastocoelar and pigment cell gene regulatory programs. We characterized the expression and function of key hematopoietic transcription regulators including SpGatac, an ortholog of vertebrate Gata-1/2/3 and SpScl, an ortholog of Scl/Tal2/Lyl1. Both play critical roles in the specification of immunocytes. Perturbation of SpGatac affects gastrulation, possibly through a secondary effect involving blastocoelar cell migration. SpScl perturbation disrupts proper segregation of pigment and blastocoelar cell precursors by a non–cell-autonomous mechanism. Orthologs of additional transcription regulators that interact with GATA and Scl factors invertebrate hematopoiesis are also expressed in this system, including SpE2A, a homolog of vertebrate E2A/HEB/ITF2 and SpLmo2, orthologous to a cofactor of the Scl-GATA transcription complex. Cis-regulatory analysis of SpGatac indicates that the transcription factor SpGcm suppresses blastocoelar cell fate in pigment cell precursors by repressing SpGatac expression.

These findings provide a comparative basis to understand the evolutionary origins and regulatory biology of vertebrate hematopoiesis in the context of a tractable gene regulatory network model.

Program/Abstract # 230
Role of ADAM metalloproteases in craniofacial development of zebrafish
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The cranial neural crest (CNC) cell is a population of pluripotent cells originating from the dorsal part of the future brain. They migrate ventrally along define pathways and give rise to the majority of the craniofacial structures. Defects in the migration and differentiation of these CNC cells lead to either lethality or a wide range of craniofacial defects. We have identified in the frog *Xenopus laevis* many proteins involved in CNC migration, including 3 members of the ADAM (A Disintegrin and Metalloprotease) family: ADAM9, 13 and 19. The two main functions of these ADAM are to cleave cell surface proteins like Cadherin-11 and to control the expression of genes like Calpain8-a. While the CNC of all vertebrates migrate and give rise to the future face, *Xenopus* CNC display some peculiarities. Frog CNC displays a biphasic migration pattern (first migrating as a cohesive sheet of cells before migrating as single cells) while most other species' CNC migrate as single cells. In order to further our understanding of the role of these ADAM during craniofacial development across species, we propose to study the role of these ADAM proteins in other vertebrate species whose behavior closely resembles the behavior of human CNC. The Zebrafish is an ideal model system to study this question. We present the expression pattern of the ADAM9, 12, 13 and 19 in Zebrafish as well as the analysis of the knock down of the single or combined knock down of these ADAM. The differences and similarities of the morphotypes between zebrafish and *Xenopus* will be presented. The implications on the functional evolution of ADAM during the craniofacial formation in vertebrates will be discussed.

Program/Abstract # 231
Intracellular localization and regulation of matrix metalloproteinase 2 in zebrafish muscle
Fallata, Amina, University of New Brunswick, Fredericton, Canada

Matrix metalloproteinases (MMPs) are zinc-dependent proteases best known for their roles in extracellular matrix remodeling. However, recent evidence has revealed the localization of MMP-2 (Gelatinase A) within rat cardiac myocytes, where it degrades protein components of the sarcomere under conditions of oxidative stress during ischemia/reperfusion injury. Also, human MMP-2 activity is regulated by phosphorylation, which is characteristic of intracellular enzymes. The objectives of my research are to determine if these unexpected intracellular roles of Gelatinase A are evolutionarily conserved in vertebrate muscle, and if so, to establish the zebrafish as a model system for their study. Using confocal and electron microscopy, I have obtained evidence that zebrafish Mmp2 is present in sarcomeres of skeletal muscle. I will use 32P metabolic labeling and immunoprecipitation to determine if Mmp2 is phosphorlated in zebrafish. Ultimately, I plan to investigate the physiological roles of Mmp2 in muscle cell development and physiology.

Program/Abstract # 232
Swim-training changes the spatio-temporal dynamics of skeletogenesis in zebrafish larvae (*Danio rerio*)
Fiaz, Ansa, Wageningen University Experimental Zoology, Wageningen, Netherlands; Léon-Kloosterziel, Karen M.; Gort, Gerrit (Wageningen University, Wageningen, Netherlands); Schulte-Merker, Stefan (Hubrecht Institute-KNAW & UMC Utrecht and Wageningen University, Utrecht, Netherlands); van Leeuwen, Johan L.; Kranenbarg, Sander (Wagening, Netherlands)

Fish larvae experience many environmental challenges during development such as variation in water velocity, food availability and predation. The rapid development of structures involved in feeding, respiration and swimming increases the chance of survival. It has been hypothesized that mechanical loading induced by muscle forces plays a role in prioritizing the development of these structures. Mechanical loading by muscle forces has been shown to affect larval and embryonic bone development in vertebrates, but these investigations were limited to the appendicular skeleton. To explore the role of mechanical load during chondrogenesis and osteogenesis of the cranial, axial and appendicular skeleton, we subjected zebrafish larvae to swim-training, which increases physical exercise levels and presumably also mechanical loads, from 5 until 14 days post fertilization. Here we show that an increased swimming activity accelerated growth, chondrogenesis and osteogenesis during larval development in zebrafish. Interestingly, swim-training accelerated both perichondral and intramembranous ossification. Furthermore, swim-training prioritized the formation of cartilage and bone structures in the head and tail region as well as the formation of elements in the anal and dorsal fins. This suggests that an increased swimming activity prioritized the development of structures which play an important role in swimming and

thereby increasing the chance of survival in an environment where water velocity increases. Our study is the first to show that already during early zebrafish larval development, skeletal tissue in the cranial, axial and appendicular skeleton is competent to respond to swim-training due to increased water velocities. It demonstrates that changes in water flow conditions can result into significant spatio-temporal changes in skeletogenesis.

Program/Abstract # 233
Epigenetic restriction of neural crest emigration by DNMT3B
Hu, Na, Caltech Biology Pasadena, United States

The neural crest is a multipotent stem cell-like population that emigrates from the central nervous system (CNS) to populate diverse derivatives including peripheral ganglia and the craniofacial skeleton. Although there are numerous studies regarding initiation of the process of neural crest emigration from the dorsal neural tube, far less is known about why the emigration process stops. Here, we show that an epigenetic modifier, DNMT3B, influences the timing of neural crest emigration. We find that DNA methyl transferase 3B is expressed in the dorsal neural tube and migratory neural crest cells during stages of neural crest migration and differentiation. Normally, EMT of the cranial neural crest is complete by stage 11. Interestingly, morpholino-mediated knockdown of DNMT3B prolongs the time of emigration from the neural tube, but has no effect on initial neural crest specification. At later stages, this is manifested in extra neural crest cells and their premature differentiation in the trigeminal ganglion. In contrast, DNMT3B morpholino does not alter the numbers of proliferating cells. Consistent with the intriguing possibility is that DNMT3B plays a role in EMT, Snail2 is upregulated after loss of DNMT3B. Since neural crest EMT is prolonged by knock-down of DNMT3B, we hypothesize that DNMT3B plays a role in repressing the process of EMT from the neural tube at the proper time.

Program/Abstract # 234
Investigating the roles of the Argonaute CSR-1 in modulating chromatin and building kinetochores
Wedeles, Christopher; Claycomb, Julie M., University of Toronto, Canada

The CSR-1 pathway (Chromosome Segregation and RNA Interference Deficient) is a small RNA pathway in *C. elegans* that is required for proper chromosome segregation and kinetochore formation. The pathway requires several factors that are also required for RNA interference, including the Argonaute CSR-1. CSR-1 associates with small RNAs, called 22G-RNAs, which are antisense to approximately 4200 germline-expressed protein coding genes. Unlike Argonautes in canonical gene-silencing pathways, CSR-1 does not affect the steady state levels of target mRNAs or proteins. Instead, CSR-1 associates with its target gene loci, which are adjacent to genomic regions that are enriched for the conserved centromeric histone H3 protein, CENP-A/HCP-3. Loss of the CSR-1 pathway leads to the disorganization of kinetochore proteins, including HCP-3. We hypothesize that CSR-1 and the 22G-RNAs are involved in organizing chromatin domains that are essential for chromosome segregation. We have validated several histone modifications that are enriched at CSR-1 targeted chromosomal loci. Interestingly, these modifications are characteristic of euchromatin and transcriptionally active regions of the genome. These observations are consistent with recent findings in which euchromatic histone modifications and transcription within the core centromeric chromatin play key roles in maintaining centromere function in several species, including humans. As such, we are investigating the relationship between CSR-1, the 22G-RNAs, these euchromatic histone modifications and transcription to determine their roles in kinetochore formation throughout animal development.

Program/Abstract # 235
Dissecting the role of D2096.8/NAP-1 in small RNA-mediated chromatin regulation
Francisco, Michelle Ann; Claycomb, Julie M., University of Toronto, Canada;

Small RNA-mediated gene-silencing pathways related to RNAi are critical regulators of gene expression throughout development. Some of the least understood roles for these small RNA pathways are in modulating chromatin and organizing the genome. An essential small RNA pathway in *C. elegans*, the CSR-1 pathway (Chromosome Segregation and RNAi Deficient), is required for the formation of functional kinetochores and for proper chromosome segregation. Small RNAs, called 22G-RNAs, guide the Argonaute CSR-1 to interact with specific genomic loci and influence the centromeric chromatin on which kinetochores are built. We hypothesize that this process involves the recruitment of chromatin-modifying proteins, which assist in modulating centromeric chromatin. From a candidate screen of chromatin-related factors implicated in gene silencing, I identified the gene D2096.8/NAP-1, which encodes a homolog of the human Nucleosome Assembly Protein, NAP-1. Loss of D2096.8/NAP-1 results in a set of phenotypes that are unique to the CSR-1 pathway: mislocalization of germ line P granules, embryonic chromosome segregation defects and RNAi deficiency. A nucleosome assembly protein is an attractive candidate for factors that cooperate with the CSR-1 pathway in regulating centromeric chromatin. Thus, I am conducting a series of experiments to elucidate the roles of D2096.8/NAP-1 in small

RNA-directed chromatin modulation. Proper centromere function is essential for chromosome segregation and its misregulation leads to infertility, developmental defects and cancer. Thus, dissecting the mechanism of small RNA-mediated centromere modulation by D2096.8/NAP-1 and the CSR-1 pathway is likely to provide key insights into fertility and oncogenesis.

Program/Abstract # 236
Investigating the roles of Argonaute proteins in *C. elegans* development
Wu, Monica, Toronto, Canada; Claycomb, Julie M. (Toronto, ON, Canada)

Argonaute (AGO) proteins are the key effector components of RNA interference (RNAi) and related endogenous small RNA pathways. The nematode, *Caenorhabditis elegans*, possesses at least 26 AGO proteins that execute a variety of functions throughout development. Although deletion mutant strains for each of the *C. elegans* AGOs have been generated, the developmental roles of only a handful of these proteins are understood. Recent mRNA expression data collected by our lab demonstrates that many AGOs share similar expression patterns, suggesting that they may function in overlapping roles. I have focused my initial studies on two uncharacterized AGOs: C04F12.1 and T23D8.7. I have found that T23D8.7 mutants have a significantly reduced brood size and a low-penetrance embryonic chromosome segregation defect. I am currently deep sequencing the small RNAs associated with T23D8.7 to gain further insight into its gene regulatory functions in germline and embryonic development. I have established that C04F12.1 localizes to P-granules, consistent with localization studies conducted on other RNAi-related factors, and I am currently working to determine whether there are overlapping functions between C04F12.1 and the closely related essential AGO, CSR-1. Many of the *C. elegans* AGOs are broadly conserved amongst different species of nematodes, implicating them in key, evolutionarily conserved developmental roles. The AGOs that have been characterized thus far function in distinct gene-regulatory roles. Thus, characterization of the remaining AGOs may lead to novel insights in small RNA mediated gene silencing functions throughout development.

Program/Abstract # 237
Investigating Glial cell abnormalities in lpr-1 and let-4 mutants
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Glial cells provide support to neuronal cells by covering their axons and aiding in processes such as oxygen and nutrient transport. In the nematode *Caenorhabditis elegans*, sheath and socket glia form a tubular structure that serves as a channel for phasmid sensory neuron axons to reach the outside environment. lpr-1 and let-4 genes are important to maintain lumen integrity in unicellular tubes in the excretory (renal-like) system. The lipocalin LPR-1 is a secreted protein that is required for luminal connectivity between two unicellular tubes in the worm's excretory system. LET-4 is a transmembrane protein that is required for maintenance of luminal connectivity. The lpr-1 and let-4 mutants that survive to the L4 stage show dye filling defects in the phasmids. This dye filling defect could arise as a result of defects in the phasmid glia cells or the neurons themselves due to the loss of one or both of these genes. Due to the similarities between excretory and phasmid glia tubes, we hypothesize that their development may require some of the same genes. To address this problem, our research is studying the expression of lpr-1 and let-4 genes in the sheath, socket and phasmid cells. Furthermore, different stages will be studied to observe if the defect is present at birth or if it appears later in the developmental process. As part of our methodology, L4 worms expressing let-4::GFP or lpr-1::GFP reporters were bathed in rhodamine-DiI to stain the phasmid neurons and determine if our genes of interests were expressed in them.

Program/Abstract # 238
microRNA regulation of Notch signaling in zebrafish retinal and vascular development
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microRNAs (miRNAs) are small, endogenous, non-coding RNAs that regulate gene expression by binding to target sites within the 3' untranslated regions of mRNAs. Precise regulation of the Notch signaling pathway is essential for normal vertebrate development. Here, we sought to examine the relationship between miR-216a and its target, sorting nexin 5 (snx5),which is a binding partner of mind bomb (mib), a ubiquitin ligase required for efficient activation of Notch signaling by Delta. Loss of function of miR-216a and gain of function of snx5 lead to obvious defects in both retinal and vascular patterning, while snx5 loss of function and miR-216a overexpression yield larval zebrafish with striking defects in vascular development. All of the observed phenotypes are consistent with disruption of Notch signaling. Because snx5 contains conserved membrane binding domains, we hypothesize that snx5 is a vital partner of mib in promoting endocytosis of Delta, which is required for Notch signaling. The regulation of snx5 by miR-216a, as well as new insight

into the role snx5 playsin the Notch pathway, represent intriguing ideas about the precise control of developmental signaling by miRNAs.

Program/Abstract # 239
Identification and characterization of a long-range enhancer element in the dPax2 cone cell specific enhancer sparkling
Evans, Nicole C.; Strom, Amy; Barolo, Scott, University of Michigan, Ann Arbor, United States
Enhancers are cis-regulatory elements that control gene expression and patterning during development. As enhancers are often located at considerable genomic distances from their target genes, distal enhancer promoter interactions are a crucial part of transcriptional regulation, yet surprisingly little is known about how these interactions are facilitated in vivo. To better understand this essential enhancer function we have undertaken a novel in vivo analysis of the EGFR/MAPK- and Notch- regulated dPax2 cone cell-specific sparkling enhancer (spa) and its ability to activate gene expression from both a promoter proximal, and distal position. In this study reporter gene expression is driven by the spa enhancer placed either adjacent to, or at a distance from, a heterologous promoter. Using this approach, we have identified a sequence within spa that is required when the enhancer is placed at a distance from the promoter, but is dispensable when the enhancer is proximal to the promoter. As this DNA sequence appears to convey long-range enhancer activity to the sparkling enhancer we refer to it as the “remote control” element or RCE. Our current work focuses on determining the functional properties and capabilities of the RCE, as well as the identification and characterization of proteins that interact with the RCE and allow it to perform its essential activities. This study will allow us to better understand the mechanisms by which enhancers engage in long-range transcriptional regulation.

Program/Abstract # 240
Multiple enhancers integrate patterning signals to drive rhombomere-specific gene expression in the hindbrain
Gongal, Patricia, Ecole Normale Superieure Insitut de Biologie, Paris, France; Labalette, Charlotte; Le Men, Johan; Bouchoucha, Yassine; Gilardi-Hebenstreit, Pascale; Charnay, Patrick (Paris, France)
Proper patterning of the neural tube along the anterior-posterior (A-P) axis is essential for the correct specification of neuronal fate during development. A complex network of signaling molecules and transcription factors regulating this process has been identified. However, the interactions between diverse signaling networks and the cis-regulatory elements that drive cell type-specific transcription remain poorly understood. Among the key regulators of vertebrate A-P patterning is the transcription factor Krox20, whose activity is required for hindbrain segmentation and the specification of rhombomeres 3 (r3) and 5 (r5). We have identified two enhancers that drive krox20 expression in r5, termed Elements B and C. Using transgenic reporter lines in zebrafish, we have found that the two enhancers drive transcriptional activity in rhombomere 5 with unique temporal and spatial profiles. While Element B initiates expression at the end of gastrulation, it is mainly active at later stages in the ventral part of ther hombomere. In contrast, Element C is active in only a small subset of r5 cells until mid-somitogenesis, when it increasingly drives transcription in the dorsal part of the rhombomere. Although activated in fewer cells within r5, mouse knockout experiments indicate Element C is essential for this rhombomere’s development. We also demonstrate that the two regulatory elements differ in their response to and their requirement for signals known to regulate krox20 expression in r5, including Hox proteins, FGF, Mafb, and vHNF1. Together, our work suggests that multiple enhancers, possibly acting via unique mechanisms in different rhombomere sub-territories, are required to establish segmental gene expression.

Program/Abstract # 241
Identification of a 2.1 Mb region associated with the rumpless phenotype in the *Araucana* chicken breed
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The Araucana chicken breed is characterized by a rumpless phenotype, caused by the caudal-most vertebrae not forming. This trait is inherited in an autosomal dominant fashion. We isolated genomic DNA from 60 Araucana from 6 separate flocks with rumpless, tailed, or partially tailed phenotypes. To identify genomic regions associated with the rumpless phenotype, we conducted a genome wide association study (GWAS) using Illumina 60k SNP (single nucleotide polymorphism) BeadChips. Case/control analysis with 41 rumpless and 14 tailed Araucana was carried out for 56,685 SNPs. Correction for false positive results was accomplished using 100,000 per mutations of the association analysis. The 10 most significant p genome values were obtained for SNPs on chromosome 2. A haplotype block of 2.1 Mb surrounding these and other supporting SNPs was identified in the rumpless *Araucana* population. We hypothesize that the causative mutation for the rumpless phenotype lies within this haplotype block. Evaluation of candidate genes and validation of the mutation responsible for the rumpless Araucana phenotype is underway.

Program/Abstract # 242
Mutational and biochemical analysis of a UBX-responsive regulatory element
Hersh, Brad; Biery, Amy; Sharpnack, William, Allegheny College, Meadville, United States
Identification of direct Hox target genes is crucial to understand how the Hox family establishes anterior-posterior pattern in developing animals. The Hox protein Ultrabithorax (UBX), which is necessary for specification of the *Drosophila* haltere, binds the DNA sequence TAAT in vitro. Co-factors, such as Extradenticle, can increase the DNA sequence specificity of UBX at some target sites, but in many instances the mechanisms for discriminating between target and non-target sequences are poorly characterized. A single conserved UBX binding site is necessary for the function of a cis-regulatory element (CRE) for the Cpr47Ee gene in the developing haltere. We identified additional sequence important for activation of GFP reporter gene expression in the haltere by mutagenesis of conserved sequences flanking the UBX binding site. In addition, we purified full-length UBXIa protein to test its ability to bind mutated CRE sequence, and to determine whether the conserved regions are necessary for binding of UBX. We have also assessed the evolution of this regulatory element by testing homologous CRE sequences from several *Drosophila* species for function in the *D.melanogaster* haltere. To identify specific evolved sequence changes that alter CRE function we generated chimeric elements combining *melanogaster* and *ananassae* sequence. These combined mutational, evolutionary, and biochemical analyses contribute to a more complete understanding of the mechanisms of Hox protein function.

Program/Abstract # 243
Transcriptional repression of Fgf8 by retinoic acid signaling during early mouse embryogenesis
Kumar, Sandeep; Duester, Gregg, Sanford-Burnham Med Research Institute, La Jolla, United States
Retinoic acid (RA) is a signaling molecule required for vertebrate embryogenesis. RA serves as a ligand for RA receptors (RARs) that directly control transcription via RA response elements (RAREs) located near genes. During early embryogenesis, fibroblast growth factor-8 (Fgf8) plays important roles in the caudal progenitor zone, heart, and somitogenesis as the body axis extends from head to tail. RA represses Fgf8 at the anterior end of the caudal progenitor zone thus preventing FGF signaling from extending into the developing trunk. During early heart organogenesis, RA mediates anteroposterior patterning of the heart by repressing Fgf8 in mesoderm lying posterior to the heartfield. These opposing gradients of RA and Fgf8 operate in concert as a switch that controls mesodermal development. A direct role of RA in regulating Fgf8 was suggested by previous studies that identified a conserved RARE upstream of Fgf8. Here, we investigate the mechanism of RA-Fgf8 antagonism. Chromatin immunoprecipitation (ChIP) performed on E8.25 mouse embryos shows that the Fgf8 RARE binds all three RARs *in vivo*, and mutation of this RARE abolishes the binding of RARs in gel shift assays. To test a potential repressive role of RA, we analyzed the ChIP patterns of repressive and activating histone marks (H3K27me3 and H3K4me3, respectively) near the Fgf8 RARE in different regions of E8.25embryos. Chromatin obtained from head/heart and caudal progenitor zone showed enrichment of H3K4me3 compared to H3K27me3 correlating with transcriptionally active Fgf8. In contrast, the trunk showed enrichment of H3K27me3 versus H3K4me3 suggesting Fgf8 repression. Our findings thus provide evidence that RA may directly repress Fgf8 via its RARE.

Program/Abstract # 244
The promoter regulates the dynamics of gene activation in development
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BMP/Dpp signaling gradients have been implicated in a variety of metazoan developmental processes, and much is known about how these gradients produce different spatial patterns of gene expression. However, there is considerably less information about the mechanisms underlying the temporal control of gene expression. Here we employ a novel quantitative imaging assay to measure the dynamics of gene activation during Dpp signaling in the *Drosophila* embryo. Genes containing paused RNA polymerase (Pol II) exhibit more synchronous and rapid induction of gene expression than those lacking Pol II, but not all paused promoters are equivalent. Some mediate faster activation dynamics than others. This differential timing correlates with Pol II pausing stability, and mutations that destabilize paused Pol II cause a delay in gene expression. We conclude that the promoter is a prime determinant of developmental timing.

Program/Abstract # 245
Fhl1 promotes myogenesis of C2C12 in response to Wnt signaling

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Previous studies have shown that Wnt signaling involves in postnatal mammalian myogenesis, in which for example, Wnt4 can activate the canonical beta-catenin pathway to stimulate myogenesis, and R-spondin, a Wnt signaling activator, promotes skeletal myogenesis. On the other hand, soluble freezle inhibits myoblast differentiation. However, despite the influence of Wnt is evident, the downstream mechanism ofWnt signaling in muscle cell differentiation is limited. Here, we report that the Four and a half LIM domains 1 (Fhl1), which induces muscle hypertrophic, can be stimulated by beta-catenin and Licl treatment. On the other hand, knock-down of Fhl1 gene expression in C2C12 cells caused a reduction of myotube formation. By reporter gene expression assays, we demonstrate that either beta-catenin or LiCl significantly activated Fhl1 promoter, which contains 4 conserved Tcf/Lef binding sites. Mutations of 2 of these sites caused a significant decrease in promoter activity by luciferase reporter assay. Thus, we suggest that Wnt signaling induces muscle cell differentiation, at least partly, through Fhl1 activation.

Program/Abstract # 246

Novel enhancers regulate patched in *Drosophila* embryos

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The Hedgehog (Hh) signaling pathway is one of the most conserved pathways in development, and proper Hh signaling is essential for the formation and function of nearly all animals. All targets of the pathway are regulated by Cubitus interruptus (Ci), the *Drosophila* effector of Hh signaling. This transcription factor, conserved invertebrates as the Gli family, functions through binding to DNA in cis-regulatory regions of the genome known as enhancers. These elements are the key to the spatial and temporal regulation of target genes. Relatively few Hh target genes have been identified, but among those that have is patched (ptc), which encodes the Hh receptor and is activated in all Hh-responding cells in both flies and vertebrates. The only known ptc enhancer requires three consensus Ci binding sites to promote expression in the developing wing, but does not respond to Hh signaling in embryos, underscoring that we do not know how Hh signaling directly contributes to embryonic ptc expression. Clarification of this context specific regulation is essential to understand how the Hh signaling pathway promotes normal, healthy development in all animals. Our recent work identifies novel ptc enhancers that suggest a role for non-consensus Ci bindingsites in the proper regulation of ptc inthe embryo.

Program/Abstract # 247

Tlx3 modulates Prrxl1 promoter activity via two distinct mechanisms

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The establishment of the nervous nociceptive circuitry depends on a group of transcription factors, including Prrxl1 (Drg11) and Tlx3, that controls the differentiation and further specification of neuronal cells. It was recently suggested that Tlx3, which highly co-localizes with Prrxl1, is required to maintain Prrxl1 expression. In order to dissect the Tlx3-dependent transcriptional mechanisms that drive Prrxl1 expression, luciferase reporter assays were performed using two Prrxl1 regulatory regions containing different alternative promoters. Tlx3 overexpression induced the activity of the TATA-containing promoter by directly binding to a bipartite DNA motif, as it was demonstrated by DNA-pull down experiments followed by western-blotting. Regarding the other promoter region (lacking a TATA box), Tlx3 overexpression prompted a higher induction of luciferase expression, an effect that was significantly enhanced by Prrxl1. Prrxl1 silencing reduced the inductive effect caused by Tlx3 overexpression, suggesting that Prrxl1/Tlx3 interaction is required to activate the Prrxl1 TATA-less promoter. This hypothesis was confirmed by co-immuno precipitation assays. Moreover, pull-down assays showed that this Prrxl1/Tlx3 induction on Prrxl1 promoter is indirect. The Tlx3-interaction domain on Prrxl1 protein was mapped using different N- and C-terminal truncated Prrxl1 versions. Luciferase reporter assays and co-immuno precipitation experiments demonstrated that the Prrxl1 C-terminal region is important for transcriptional activity while the domain encompassing the 143-180 residues is implicated in Tlx3 interaction. Altogether, our results suggest that Tlx3 strongly modulates the Prrxl1 promoter activity using two distinct mechanisms.

Program/Abstract # 248

Meis gene regulation during embryonic development

Zerucha, Ted; Barrett, Cody; Nelson, Kyle; Wellington, Allen, Appalachian State University, Boone, United States

The Meis genes (named for myeloid ecotropic leukemia virus integration site) are an evolutionarily conserved family of genes and homologues of this family have been found in all animals. Meis proteins function as cofactors, interacting directly with other transcription factors as well as DNA to facilitate transcriptional regulation. Most notably, they appear to act as co-factors of Hox proteins as well as of other homeodomain proteins. The Meis genes are expressed in similar

patterns during development of all vertebrates, however little to nothing is known of the mechanisms directing their embryonic expression. Using a comparative genomics approach, phylogenetic footprinting, we have identified a putative cis-regulatory element associated with the Meis2 gene (meis2.2 in zebrafish). We have named this element m2de1 (for Meis2 downstream element 1). This element is remarkably well conserved in sequence and relative genomic location amongst all vertebrates. It also contains putative binding sites for proteins implicated in the regulation of the Meis genes. In transgenic zebrafish embryos, m2de1 is able to direct the expression of a reporter gene in a manner consistent with endogenous Meis2 expression strongly suggesting that it is a Meis2 cis-regulatory element.

Program/Abstract # 249
Characterization of Sprouty2 cis-acting elements responsive to FGF and BMP signals
Zhang, Ying; Lewandoski, Mark, National Cancer Institute CDBL/Genetics of Vertebrate Development Section, Frederick, United States)

The Fibroblast Growth Factor (FGF) family is one of the first signaling pathways discovered to act during development, but ironically, compared to other signaling pathways, we know little about the downstream mediators and FGF-responsive elements (FREs) in target genes. Therefore we are studying potential FREs in Spry2. We chose this gene because it encodes an important modulator of the FGF signaling and is expressed in most FGF signaling centers during development. We have identified an 8 kb Spry2 upstream region that is FGF-responsive in NIH3T3 cells. We generated a series of transgenic mouselines with this element driving lacZ. β-gal staining reveals that this construct recapitulates endogenous Spry2 expression in nearly all regions from embryonic day E7.0 through E11.5. Furthermore, this expression is FGF-responsive. For example, limb bud Spry2-lacZ activity is absent in mutants lacking the apical ectodermal ridge, a source of FGF activity. Also, primitive streak expression is lost when Fgf4 and Fgf8 are inactivated in that tissue with TCre-activity. Intriguingly, by studying a series of mutants with different Fgf4 and Fgf8 alleles, we found that Spry-lacZ activity is more sensitive to FGF loss than the endogenous Spry2 gene. Thus we have generated the first FGF-reporter mice and are refining our in vitro and in vivo 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.

Program/Abstract # 250
Probing the endogenous HAND2 target gene range using next generation genome-wide approaches in mouse embryos
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Hand2 encodes a bHLH transcription factor with essential functions during limb, cardiac and neural crest derived tissue morphogenesis. In limb buds, the zone of polarizing activity (ZPA) is defined by Shh expression, which controls anterior-posterior (AP) patterning, survival and proliferation of chondrogenic progenitors. We have shown that HAND2 activates Shh expression by directly binding to the far upstream limb-specific ZRS region. While genetic analysis revealed a general role of Hand2 in setting up the AP limb bud axis, the range of direct HAND2 target genes remains largely unknown. Using dual RMCE (dRMCE), we have introduced a 3xFLAG epitope tag into the endogenous HAND2 protein that allows its detection with unprecedented sensitivity and specificity. We used chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) to identify the endogenous HAND2 target regions in mouse embryos. This genome-wide dataset revealed that HAND2 interacts preferentially with genomic landscapes of genes key to development of the limb, heart and branchial arches. In particular, HAND2 interacts with candidate regulatory regions of genes involved in prepatterning of the nascent limb bud, such as Gli3 and Tbx. In line with its role in early limb bud specification, HAND2 interacts with elements of genes important for proximal development and is required for femur morphogenesis. Most interestingly, HAND2 binds to a region within the ZRS that contains point mutations which cause congenital polydactylies in both humans and mice. Currently, we are investigating the effects of these close-by point mutations on the interactions of the endogenous HAND2 protein complexes with the ZRS during the onset of SHH signalling.

Program/Abstract # 251
Prdm1a regulation of the gene network for zebrafish neural crest specification
Powell, Davalyn R.; Hernandez-Lagunas, Laura; Artinger, Kristin, University of Colorado Denver, Aurora, United States

Neural crest cells are multipotent precursor cells that are induced at the neural plate border by a series of complex signaling and genetic interactions. Several transcription factors called neural plate border and neural crest specifiers are necessary for early neural crest development; however, the nature of their interactions and regulation is not well

understood. Here, we have established the genetic interactions between prdm1a, ap2a/c, and foxd3, key regulators of neural plate border and neural crest specification in zebrafish embryos. We have shown that prdm1a acts downstream of Wnt and Notch signaling and forms regulatory feedback loops with foxd3 and tfap2a/c, where perturbation of any of these members of the gene cascade influences expression of the others as well as normal development of the neural crest. Through rescue experiments and chromatin-immuno precipitation (ChIP), we have determined that Prdm1a directly binds to and regulates a putative enhancer for foxd3, an established neural crest specifier, and that foxd3 is a functional direct target of Prdm1a regulation. Based on these and previous data, we predict that Prdm1a is transcriptional activating foxd3 at the neural plateborder. Additional data using dominant-activator and dominant-repressor versions of Prdm1a suggest that Prdm1a functions both as a transcriptional activator and transcriptional repressor during development. By comparing RNA-seq and ChIP-seq data, we will elucidate the nature of Prdm1a regulation of neural crest specification genes at the transcriptional level. Through this work, we have demonstrated that prdm1a is an important regulator in the gene network that is required for proper neural crest formation.

Program/Abstract # 253

A characterization of regulatory linkages in a genetic network for a derived fruit fly trait.

Butts, John C.; McNamee, Connor, University of Dayton, Dayton, United States; Rebeiz, Mark (University of Pittsburgh, Pittsburgh, United States); Williams, Thomas (University of Dayton, Dayton, United States)

Phenotypes are the culmination of spatial and temporal patterns of gene expression of genes comprising a genetic network. These patterns are controlled by cis-regulatory elements (CREs) and genes are connected into networks when a CRE regulating its expression possesses binding sites for network transcription factor proteins - so called regulatory linkages. Gains and losses of linkages are a suspected common route of CRE and network evolution; though, their emergence remains poorly understood as few case have revealed the before and after states in sufficient detail. The male-specific abdominal pigmentation of *Drosophila melanogaster* evolved from a monomorphic ancestral state, a key modification to the pigmentation network being the evolution of sexually dimorphic expression of the Bab transcription factor proteins. These proteins turn off expression of the yellow and tan genes that are required for pigmentation. The research presented here addresses two questions. First, does Bab form direct regulatory linkages with CREs that control the male-specific expression of the *Drosophila melanogaster* yellow and tan genes? Second, when historically were these CREs and the irregulatory linkages gained? To answer these questions we are: systematically mutating CRE sequences to find motifs needed to integrate the repressive effects of Bab, and evaluating the regulatory activities of sequences related to the Drosophila melanogaster CREs. Future studies will explore whether this divergence included the gain of Bab binding sites in dimorphic species or whether these binding sites were ancestral and conserved during trait evolution.

Program/Abstract # 254

Inspecting the regulatory architecture of a toolkit gene locus governing trait development and evolution

Camino, Eric; Francis, Kaitlyn; Velky, Jordan; Williams, Thomas, University of Dayton, Dayton, United States

Complex spatial and temporal patterns of gene expression are crucial to animal development and changes in expression patterns are a common mode of evolutionary innovation. Thus, understanding development requires answering: (1) what are the DNA elements, so called CREs, controlling expression, (2) how the DNA sequences of CREs encode gene regulatory capabilities, (3) whether and how CREs work together to make complex expression patterns, and (4) how CRE sequences identify their gene target(s) of regulation in a 3-dimensional nucleus? These answers will aid studies to reveal the mechanisms of gene expression, and thus animal, evolution. A model to address these questionsis the bab locus of fruit flies. This locus contains the duplicate bab1 and bab2 genes that shape a derived pattern of pigmentation in the species Drosophila melanogaster. The relevant bab expression pattern is controlled by two CREs which we found to interact in a non-additive, or synergistic, way to yield this pattern. Ongoing studies seek to trace: when and how CRE synergism evolved, which CRE sequences encode their synergistic activity, how these CREs interact with the bab gene promoters, and whether synergistic regulation extends to additional gene loci. Ultimately, this work aims to connect how animal form is programmed into 1-dimensional DNA sequence and how this program evolves.

Program/Abstract # 255

Fbxo16 mediated protein degradation regulates neurogenesis in *Xenopus laevis*

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The development of the central nervous system is a dynamic process during which protein levels are regulated temporally and spatially by synthesis and degradation. While much is known about the regulation of gene expression during development, little is known about the control of protein degradation. Studies of cell cycle regulation show that a major mechanism of protein degradation is through F-box ubiquitin ligases, which function in the recognition and recruitment of

specific targets for the Ub/26S proteasome pathway. We performed a genome-wide screen in *X. tropicalis* for targets of REST, the RE-1 Silencing Transcription factor, which silences neuronal genes in neural progenitors and non-neuronal cells to restrict expression to neurons and identified Fbxo16 ubiquitin ligase. We determined that as expected for a neuronal gene regulated by REST, Fbxo16 is expressed in the differentiating neurons in the brain but excluded from the neural progenitor zone. Loss of function analysis using morpholino knock-down and a dominant negative construct showed that Fbxo16 modulates neuron formation by affecting the function of the proneural protein Neurogenin (Ngn). This is complemented bygain-of-function analysis, which shows elevated neurogenesis with increased Fbxo16. We found that the effect of Fbxo16 on neurogenesis is not through cell cycle regulation but a direct consequence of its ability to regulate proteins required for neurogenesis. In fact, our half-life analysis showed that Fbxo16 stabilizes Ngn, which is a short-lived protein. Our findings suggest that Fbxo16 functions to protect Ngn from degradation to allow its accumulation as neural progenitors differentiate, ensuring the activation of its transcriptional targets.

Program/Abstract # 256
Increased levels of hydrogen peroxide induce a HIF-1-dependent remodeling of lipid metabolism in *C. elegans*
Xie, Meng; Roy, Richard, McGill University, Montreal, Canada

Cells have evolved numerous mechanisms to circumvent environmental stresses caused by the environment, many of which are regulated by the AMP-activated kinase (AMPK). Unlike most organisms, C. elegans AMPK null mutants are viable, but die prematurely in the “long-lived” dauer stage due to rapid exhaustion of triglyceride energy stores. Using agenome-wide RNAi approach we found that the disruption of genes that increase hydrogen peroxide levels enhance the survival of AMPK mutants by altering both the abundance and the nature of the fatty acid content in the animal by increasing the HIF-1-dependent expression of several key rate-liming enzymes involved in de novo fatty acid biosynthesis. Our data provide a mechanistic foundation to explain how an optimal level of hydrogen peroxide can provide cellular benefit; a phenomenon described as hormesis, by instructing cells to readjust their lipid biosynthetic capacity through downstream HIF-1 activation, as a means to correct cellular energy deficiencies.

Program/Abstract # 257
AMPK is essential to mediate survival during nutrient stress in *C. elegans*
Demoinet, Emilie; Mantovani, Julie; Roy, Richard, McGill University, Montreal, Canada

During periods of prolonged nutrient stress, many organisms undergo developmental or reproductive diapause, which are reversible states of developmental dormancy. When growth conditions are suboptimal, *Caenorhabditis elegans* can arrest its development and execute a diapause like state.The best characterized of these are the first larval stage (L1) and dauer diapause. The *C. elegans* L1 arrest is a response to an insufficient level of nutrient to initiate postembryonic development; whereby development is suspended and environmental stress resistance is increased without obvious morphological modification. Wild type L1 hatchlings can normally survive up to 2 weeks in the absence of food under these conditions. We have shown that the maximal survival in the L1 diapause requires aak-2, one of the 2 homologues of the alpha subunit of AMP-activated protein kinase (AMPK). AMPK is a metabolic master switch that is activated in response to various nutritional and stress signals. Its main function is to maintain cellular energy homeostasis by up-regulating pathways that produce ATP; while down-regulating energy-consuming anabolic processes. We found that in absence of AMPK (aak-0), larvae lose their capacity to fold proteins and aggregates accumulate in the L1 larvae, leading to their premature death after 5 days in the L1 diapause. Under normal conditions, this accumulation of misfolded protein activates a highly conserved adaptative signaling cascade known as the unfolded protein response (UPR). Based on genetic and cellular biological data, we find that AMPK regulates a new UPR like response involving novels effectors to ensure organism survival during condition of energy stress.

Program/Abstract # 258
Regulation of Pax3 neural expression by the Wnt-Cdx pathway
Sanchez, Oraly; Coutaud, Baptiste; Samani, Taraneh; Tremblay, Isabelle; Souchkova, Ouliana; Pilon, Nicolas, UQAM, Montreal, Canada

Members of the vertebrate Cdx family, Cdx1, Cdx2, and Cdx4 are key regulators of posterior embryo development. Cdx genes are strongly expressed in the posterior neurectoderm at the time of induction of neuralcrest cells (NCC), but their role in these processes is still poorly understood because of functional redundancy and overlapping expression patterns. Here we report that Cdx proteins act downstream of canonical Wnt signal to control the expression of Pax3, a known Wnt-induced gene essential for NCC induction. Pax3 and Cdx genes are co-expressed in the posterior neurectoderm and Pax3 expression is reduced in Cdx1-null embryos. RT-PCR analyses in undifferentiated P19 cells and in NCC-derived N2a cells indicate that Pax3 expression is induced by the Wnt-Cdx pathway. Co-transfection analyses, electrophoretic mobility shift

assays, chromatin immunoprecipitation as well as transgenic studies further indicate that Cdx proteins operate via direct binding to an evolutionary conserved neural crest enhancer of the Pax3 proximal promoter. Taken together, these results suggest a novel neural function for Cdx proteins within the gene regulatory network controlling neural crest development.

Program/Abstract # 259

The transcription factor Sal-like 1 (Sall-1) is a direct transcriptional target of Wnt/beta-catenin signaling and regulates neural patterning along with morphogenesis.

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Amphibian neural development occurs as a two-step process: induction confers an anterior neural fate in undifferentiated ectodermal precursors and transformation posteriorizes the portion that gives rise to the spinal cord and hindbrain. Signaling through the Wnt pathway is necessary and sufficient to induce posterior fates in the neural plate. Despite extensive knowledge about this signaling as a posteriorizing factor in neural development, the mechanism by which Wnt signaling generates discrete domains of neural gene expression along the anterior-posterior axis remains poorly understood. To address this question, we used RNA-Seq to identify direct transcriptional targets in neural tissue by activating Wnt signaling in *Xenopus* neural explants pretreated with the translation inhibitor cycloheximide. Wnt-activated neural tissue resulted in 262 genes expressed greater than two-fold when compared to anterior neural tissue. in situ hybridization analysis of highly expressed transcription factors and RNA-binding proteins showed specific posterior neural expression. Of particular interest, the transcription factor Sal-like 1 (Sall-1) showed specific posterior neural expression suggesting a role in Wnt induce neural patterning. Chromatin immunoprecipitation using abeta-catenin specific antibody revealed an enrichment of binding to a distal enhancer of Sall-1. Sall-1 overexpression posteriorizes the embryo as evidenced by repression of the anterior cement gland and morpholino knockdown results in anteriorization, shown by a loss of Krox-20 expression, a shortened axis anda failure to close the neural tube. Theseexperiments show Sall-1 is a direct transcriptionaltarget of canonical Wnt signaling, is required for proper neural patterning, and links Wnt-induced patterning with morphogenesis of the cells comprising the vertebrate neural axis.

Program/Abstract # 260

SOCS36E attenuates STAT signaling to facilitate proper cell migration in the *Drosophila* ovary

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Cell migration plays a critical role in developing organisms. Thus, precise specification of migratory cell populations is imperative. Border cell migration in *Drosophila* oogenesis provides an exemplary model to study acquisition of a migratory state. The developing egg of Drosophila is composed of an epithelial monolayer surrounding sixteen germline cells. At mid-oogenesis, two epithelial cells at the anterior secrete a paracrine signal, Unpaired (UPD)- the activating ligand of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway. Cells with the highest levels of STAT activation become the migratory border cells; those with lower activation remain in the epithelium as stretch cells. The border cells detach from the stretch cells and migrate to the oocyte, where they are required for egg fertilization and development. Given the importance of this process, STAT signaling is regulated by a meticulous genetic circuit. The transcription factorapontic (apt) functions in a negative feedback loop with STAT, partly through regulation of mir-279. We have determined that suppressor of cytokine signaling (socs) 36e is also required for proper border cell specification and migration. Null mutations in socs36e result in mis-specification of migratory cells and poor movement. Genetic analysis suggests socs36e acts in the STAT regulatory circuit. We believe SOCS36E is needed downstream of APT to attenuate STAT signaling, and thereby to establish a discrete division between the border and stretch cell fates.

Program/Abstract # 262

***Nematostella* reference transcriptome and high throughput gene regulatory network construction**

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Gene Regulatory Networks are a powerful tool to elucidate the important regulator yevents of embryogenesis. We are studying the embryonic Gene Regulatory Network for the cnidarian, *Nematostella vectensis*, because its pivotal position in the tree of life as the outgroup to Bilaterians will allow us, by comparing regulatory network architecture, to address key outstanding questions about the evolution of body axis specification. As a first step in the network construction pipeline, we have built a reference transcriptome from 0hr, 6hr, 12hr, 18hr and 24hr embryos using 100 bp paired end reads from Illumina HiSeq 3rd generation chemistry. With the resulting 238 million high quality reads we assembled the transcriptome into 88,684 transcripts using four distinct denovo and reference-based approaches. The de novo assembly was performed using either Trinity or a combination of digital normalization, Velvet and Oases. These assemblies were compared to each other and also compared to the method of assembly using the reference genome which was done through a separate

collaboration. RNA Spike-In controls from the External RNA Controls Consortium (ERCC) were used to enable quantitative information on transcript abundance. This reference transcriptome will be avaluable resource for others working with Nematostella and is an important first step in our high throughput Gene Regulatory Network construction pipeline which will be widely applicable.

Program/Abstract # 263
Functional characterization of the upstream regulatory regions of XMSR, a gene involved in vascular and neural development
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XMSR, also known as APJ receptor, is a G-protein coupled receptor that functions in the development of the vascular and nervous systems of all vertebrates. Previous studies have shown that the XMSR gene has varying levels of interspecies conservation, both in the coding and regulatory regions. While its role in vascular development is relatively well-studied, the role of XMSR in neural development is not well understood. XMSR is expressed within the ciliary body of the eye. The ciliary body of Xenopus laevis is home to a large population of retinal stem cells, serving as a useful model for stem cell research. This project aims to characterize the transcriptional regulation of XMSR in the retina. We have cloned two kilobases of the upstream region and created a transgenic line to drive expression of GFP. Results from these experiments indicate that this regulatory region drives expression of XMSR in the ciliary body of the retina. We have compared expression of XMSR in these transgenic embryos to wild type embryos. Additionally we have used sequence analysis to identify interspecies conservation of identified upstream transcriptional regulators. Using bioinformatic and transgenic analyses of XMSR, we are conducting promoter analysis to identify the transcription factors and corresponding binding sites responsible for the ciliary body expression and morpholino “knockdown” experiments to determine its role in development.

Program/Abstract # 264
Genomic copy number variation during trophoblast giant cell endoreplication
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The placenta is a mammalian specific organ crucial for fetal development. A key feature of the placenta is a polyploid trophoblast cell type that invades and remodels the uterus to promote flow of blood and nutrients to the fetus. In rodents, these are called trophoblast giant cells (TGCs) and have up to 1,000N DNA content due to endoreplication. Recent work has shown that placental specific defects in TGC endopolyploidy cause impairment to fetal growth, resulting in perinatal death. However, the function of endopolyploidy in TGCs remains unknown. Two hypotheses are that 1) polyploidy acquires the necessary quantity of genes while saving materials and time and 2) polyploidy regulates gene expression by selectively amplifying certain genomic regions. We examined the genomic organization of TGCs using array comparative genomics hybridization (aCGH). We found that certain regions of the genome are preferentially underreplicated (UR domains). To further investigate, we compared our aCGH data to our data on RNA expression and the histone modifications H3K27ac, H3K4me1 and H3K4me3 in cultured TGCs and their progenitors, trophoblast stem cells (TS cells). We found that UR domains anticorrelate with RNA expression and active histone marks in both TGCs and TS cells. As active histone marks anticorrelate with late replicating DNA in other 2N cell types, we are pursuing the hypothesis that replication timing in TS cells causes UR domains in TGCs. Endocycles may be progressing with such speed that they do not have time to replicate late-replicating DNA. This suggests that endocycle speed is crucial to TGC function, supporting the hypothesis that polyploidy is important foracquiring the necessary quantity of genes while saving time.

Program/Abstract # 265
FACS-assisted deep sequencing of the zebrafish neural crest transcriptome
Hultman, Keith; LaBonne, Carole, Northwestern University, Evanston, United States

The neural crest, a vertebrate-specific population of progenitor cells, arises during neurulation and gives rise to a broad range of derivatives including facial cartilage, bone, peripheral neurons and glia, and pigment cells. The stem cell-like attributes of the neural crest, as well as its migratory and invasive behavior, are controlled by a network of signaling pathways, transcription factors and effector genes referred to as the neural crest gene regulatory network (NC-GRN). Misregulation of the NC-GRN in other cells or tissues can result in tumor progression / metastasis and other abnormalities. We have exploited the power of next generation sequencing to identify novel components of the NC-GRN, focusing on the zebrafish neural crest cell transcriptome. Using the Tg(Sox10:GFP) transgenic line, near pure populations of GFP positive neural crest cells were sorted from dissociated embryos at key stages of neural crest development, and used to make cDNA libraries for deep sequencing. This unbiased approach identified novel genes enriched in neural crest. It also found many known neural crest GRN components, including sox10, sox9b, AP2, and snail1b, thus validating the findings and

approach. A similar strategy was used to identify miRNAs enriched or excluded from the neural crest, as these small regulatory RNAs are understudied in the context of this network. We report that many miRNAs enriched in pre-migratory populations of zebrafish neural crest (14 hpf) have also been categorized as inhibitors of metastasis found in invasive cancers. We use the zebrafish model to probe the expression and function of identified novel NC-GRN components.

Program/Abstract # 266
Hearing regeneration: zebrafish as a model for a large-scale mutation screening
Pei, Wuhong; Varshney, Gaurav; Huang, Sunny; Liang, jin; Gildea, Derek; Wolfsberg, Tyra; Burgess, Shawn, NHGRI, NIH, Bethesda, United States

Hearing loss, primarily caused by a loss of sensory hair cells in the inner ear, affects an estimated one-third of the US population between the ages of 65 and 75 and close to one-half of those older than 75. There is also an increasing trend of young people experiencing hearing loss, due to excessive noise exposure, usually listening to music too loudly. In humans and other mammals, hearing loss is an irreversible process, since the hair cells do not regenerate, the lost hair cells are not replaced. Zebrafish is a good model for studying hearing regeneration, because of the conservation in gene function, the similarity in inner ear structure, and mostly importantly, the ability to regenerate its inner ear hair cells. In our study, we performed a gene expression profiling analysis on adult zebrafish recovering from a noise-induced inner ear hair cell loss, and identified approximately 2000 genes involved in the hair cell regeneration process. We created a zebrafish mutation library by retroviral insertions in the zebrafish genome and established large-scale genotyping methods to genotype mutants using real-time PCR or high-resolution melting analysis. Mutation screening and phenotype examination are ongoing to assess the contribution of each from thetranscriptional profiling to both hearing development and hair cell regeneration. Functional studies, pathway analysis and environmental perturbations will be carried out to illustrate the gene-gene and gene-environment interaction networks underlying inner ear hair cell regeneration in zebrafish, with an ultimate goal to shed a light on the prevention and treatment of hearing loss in humans.

Program/Abstract # 267
Extracellular regulation of FGF signaling in the early *Xenopus* embryo
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Fibroblast growth factors play an important role in development and homeostasis, and their activity needs to be precisely regulated to ensure proper signaling. We previously presented the secreted serine protease xHtrA1 as positive regulator of FGF signaling in the extracellular space (Hou et al., (2007) Developmental Cell 13:226-41). xHtrA1 through cleaving proteoglycans release cell-surface bound FGF ligands and stimulate long-range FGF signaling during establishment of the embryonic body plan. It is obvious that, if not tightly controlled, the activity of xHtrA1 would lead to an unlimited amplification and propagation of FGF signals. We have isolated a full-length cDNA clone encoding a secreted serine protease inhibitor (xSPI) that may act as a negative regulator of xHtrA1/FGF signals.xSPI shows distinct expression in the early embryo and promotes anterior development in mRNA-injected *Xenopus* embryos. xSPImRNA induced enlargement of head structures, suppression of mesoderm and reduction of neuronal differentiation. These effects are reminiscent of those caused by knockdown of xHtrA1 or inhibition of FGF signaling. In contrast, down regulation of xSPI by antisense morpholino oligonucleotides caused microcephaly, a phenotype that is also induced by misexpression of xHtrA1 or components of the FGF-MAPK pathway. Moreover, xSPI immunoprecipitates with xHtrA1, suppresses xHtrA1-induced FGF signaling, and prevents xHtrA1 from degrading Syndecan-4 in mRNA-injected embryos. Together, the data suggest that xSPI via suppression of xHtrA1 proteolytic activity and stabilization of cell surface proteoglycans may add another layer to the extracellular regulation of FGF signals.

Program/Abstract # 269
The relationship between centrosomal PKA and Hedgehog signaling
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The Sonic Hedgehog (Shh) pathway is essential for embryonic development as well as tissue homeostasis in the adult organism. Loss or gain of Shh function can result in developmental abnormalities and cancers such as holoprosencephaly and basal cell carcinoma, respectively. In vertebrates, Shh signaling requires the primary cilium, a microtubule-based structure present on almost all cells in the body. Protein Kinase A (PKA) is a cAMP-dependent protein kinase that serves as a negative regulator of Shh signaling, and loss of PKA function results in full activation of the pathway. Studies have shown that PKA localizes to the centrosome, which serves as the basal body of the primary cilium in interphase cells. Biochemical experiments suggest that this association between PKA and the centrosome is mediated through interactions with the pericentriolar proteins pericentrin and AKAP9. PKA is localized to the basal body of the primary cilium in the presence and absence of Shh ligand, but it is unclear if centrosomal localization of PKA is required for its regulatory role

in the Shh pathway. A strong allele of Pericentrin, which greatly reduces the amount ofcentrosomal pericentrin, causes lethality at e14. Pericentrin mutants at this stage show incompletely penetrant polydactyly, suggesting a mild gain of Shh signaling. Shh-dependent dorsal-ventral patterning is only mildly affected in the pericentrin mutant, and PKA appears to localize to the pericentriolar region in pericentrin mutants, suggesting that additional proteins mediate PKA localization to the centrosome. Akap9 null mutants are viable and male sterile, and do not exhibit defects in Shh signaling. We are currently analyzing the phenotypes of Pericentrin and Akap9 double mutants to test whether these proteins have overlapping functions in the localization of PKA to the centrosome. Through this study we will establish whether the centrosomal localization of PKA is important for Shh pathway output and thus mouse embryonic development.

Program/Abstract # 270

The role of hedgehog signaling pathway in the development of the mouse patellar tendon

Liu, Chia-Feng; Aschbacher-Smith, Lindsey (Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States); Butler, David (University of Cincinnati, Cincinnati, OH, United States); Wylie, Christopher (Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States)

Tendon can be divided into two anatomical areas: midsubstance and insertion site. Using a mouse reporter line, we found Gli1 was expressed in the tendon-to-bone insertion site but not in the midsubstance. GLI1 is a downstream effector of hedgehog (Hh) signaling pathway. Thus, its expression in the insertion site suggests Hh pathway may be involved in the differentiation of insertion site. To test this hypothesis, we first activated Hh pathway by expressing constitutively active smoothened (SmoM2), the Hh signal transducer, using CRE recombinase driven by the promoter of scleraxis(ScxCre), a transcription factor expressed in developing tenocytes. SmoM2 animals died shortly after birth due to respiratory problems. However, we observed that several insertion site markers were expressed ectopically in the midsubstance during the fetal period. We confirmed these findings in organ cultures *in vitro*. Next, we targeted the Smo gene in the tenocyte population using ScxCre. There was a reduced development of the tendon-to-bone insertion site in mutant. These data suggest that differentiation of the insertion site is controlled by Hh signaling in the mouse. Our studies provide new insight into the role of Hh signaling during the development of tendon.

Program/Abstract # 271

***Drosophila* G-protein-coupled receptor kinase 2 regulates cAMP-dependent Hedgehog signaling**

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G-protein-coupled receptor kinases (GRKs) play a conservedrole in Hedgehog (Hh) signaling. In several systems, GRKs are required forefficient Hh target gene expression. Their principal target appears to be Smoothened (Smo), the intracellular signal generating component of the pathway and a member of the G-protein-coupled receptor (GPCR) protein family. In *Drosophila*, a GRK called Gprk2 is needed for internalization and down regulation of activated Smo, consistent with thetypical role of these kinases in negatively regulating GPCRs. However, Hh target gene activation is strongly impaired in gprk2 mutant flies, indicating that Gprk2 must also positively regulate Hh signaling at some level. To investigate its function in signaling, we analyzed several different readouts of Hh pathway activity in animals or cells lackingGprk2. Surprisingly, although target gene expression was impaired, Smo-dependent activation of downstream components of the signaling pathway was increased in the absence of Gprk2. This suggests that Gprk2 does indeed play a role in terminating Smo signaling. However, loss of Gprk2 resulted in a decrease in cellular cAMP concentrations to a level that was limiting for Hh target gene activation. Normal expression of target genes was restored in gprk2 mutants by stimulating cAMP production or activating the cAMP-dependent Protein kinase A (PKA). Our results suggest that direct regulation of Smo by Gprk2 is not absolutely required for Hh target gene expression. Gprk2 is important for normal cAMP regulation, and thus has an indirect effect on the activity of PKA-regulated components of the Hh pathway, including Smo itself.

Program/Abstract # 272

Do Mek1 and Mek2 regulate distinct functions during mouse development?

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The mammalian genome contains two ERK/MAP kinase kinase genes, Mek1 and Mek2, encoding dual-specificity kinases responsible for ERK/MAP kinase activation. Mek1-/-embryo die of placental defects at E10.5, while Mek2-/- and Mek1+/- micesurvive with a normal lifespan. However most of Mek1+/- Mek2+/-embryos die during gestation of underdevelopment of the placenta indicatingthat both Mek genes contribute to placental development. Spatio-temporal expression profile, or difference in MEK1 and MEK2 properties, could explain the specific role of each Mek genes in placental development. To

verify the functional specificity of Mek1 and Mek2, we generated Mek1 knockin allele in which the Mek2 cDNA is under control of Mek1 regulatory sequences. Mek1 Mek2/Mek2 homozygous knockin mice are viable, fertile and show no apparent phenotype suggesting that both proteins are redundant and that functional difference is most likely mediated by the expression profile. Scaffold proteins, like MP1, can modulate proteinkinase activity via the formation of a MEK1-ERK1/2 complex. MP1 could thereforecontribute to the difference in function between MEK1 and MEK2. To clarify therole of MP1 during development, knockout mice were generated. Interestingly, embryos Mp1-/- die between days 6 and 7 ofgestation, suggesting that MP1 is not restricted to the signaling pathway via MEK1. The analysis of Mp1-/ - phenotype indicates a defectin the extraembryonic ectoderm but the molecular causes remain to bedetermined. Phenotypic analysis of Mp1-/- mutants andthe study *of* knock-in Mek1Mek2/Mek2 will enableto provide preliminary results regarding the functional redundancy or specificity between MEK1 and MEK2. (Supportedby CIHR)

Program/Abstract # 273

Role of ERK/MAPK pathway in syncytiotrophoblast formation during the establishment the blood-placental barrier

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The mammalian genome contains two ERK/MAP kinase kinase genes, Mek1 and Mek2, encoding dual-specificity kinases responsible for ERK/MAP kinase activation. Loss of Mek1 function in mouse causes embryonic lethality, while Mek2 mutant mice survive with a normal lifespan. The Mek1 mutation causes placental defects affecting morphogenesis and vascularization. Placental vascularization starts by the invasion of the labyrinth by the layer II of syncytiotrophoblasts (SynT-II). This process guides the migration of the endothelial cells arising from the allantoïc tissue. Immunohistochemical analyses demonstrate that the ERK/MAP kinase pathway is strongly activated in SynT. We have also shown that Mek1+/-Mek2+/- mice die during gestation due to labyrinth malformations. Thus, even though Mek1 plays a predominant role in placenta formation, Mek2 is also involved in this process. In Mek1+/-Mek2+/- mutants, the vascularization of the labyrinth is reduced and defects in SynT-II formation lead to the accumulation of Multinucleated Trophoblasts Giant cells (MTG). Mek1 deletion in SynT-II is not sufficient to compromise embryo survival. However, the deletion of both Mek1 allelesin allantoïs-derived tissues in Mek1+/-Mek2+/- placenta increases the penetrance and the expressivity of the MTG placental phenotype. Moreover, by using genetic and histopathological approaches, we have recently demonstrated that the MTGs result from the fusion between SynT-I and SynT-II. We have also shown that the normal development of the SynT-I into a thin layer of multinucleated cells depend on the presence of the SynT-II. Finally, Gcm1 and Pparg, ERK/MAPK targets, are deregulated inmutant placentas and can contribute to the placental phenotype. (Supported by CIHR)

Program/Abstract # 274

A mass spectrometry-based approach to identify new interaction partners of the tyrosine phosphatase DEP-1

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The vulva of the nematode *C. elegans* serves as an excellent model to study evolutionary conserved signaling pathways like the RAS/MAPK, DELTA/NOTCH, and WNT-pathways. Vulval development is induced by the activation of the RAS/MAPK pathway, which specifies the 1° fate of the vulval precursorcell (VPC) P6.p. Subsequently, several negative regulators of the RAS/MAPKsignaling pathway, such as LIP-1 and the density enhanced phosphatase DEP-1 areup-regulated in the neighboring VPCs P5.p and P7.p to inhibit the activation ofthe RAS/MAPK pathway, allowing NOTCH to specify the 2° fate in these VPCs. In this process, the class III receptor protein tyrosine phosphatase DEP-1 negativelyregulates LET-23 EGFR signaling. Despite its importance as a tumor suppressor in many epithelial tissues, thephysiological functions of mammalian Dep-1/Scc1 during normal development and tumorigenesis, as well as its physiological substrates are poorly characterized. In orderto identify new interaction partners of *C.elegans* DEP-1, we performed a mass spectrometry-based approach. DifferentGST-tagged versions of DEP-1 were expressed in E. coli, affinity purified, and incubated with total *C. elegans* protein extracts. Proteinsthat bound to DEP-1::GST were then identified by LC-MS/MS. Interactions of themost promising candidates were verified invitro by performing different pull-down experiments. In addition, the biological functions of the novel identified DEP-1 interacting partners during vulval development are currently analyzed invivo by performing RNAi experiments and genetic interaction analyses.

Program/Abstract # 275

Prioritized differentiation of stressed placental and embryonic stem cells

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As the mouse embryo implants into the uterusit is made of undifferentiated stem cells that will make the embryo, and extraembryonic yolk sac and placenta. Before implantation pluripotent stem cells all divide. But within a day of

implantation subpopulations of embryonic and placental stem cells (ESC and TSC, respectively) normally lose potency and differentiate to produce the first essential functions. This is defined by studies of gene expression and null mutant lethals. We study stress responsesof embryos/stem cells. At lower stresses anti-apoptotic and anabolic-to-catabolic shift responses conserve energy, but there is no differentiation. At higher exposures cell growth is diminished and differentiation induced. Since fewer cells produce more essential differentiated product/cell we call this compensatory differentiation. ESC and TSC increase early and suppress later essential lineages, a stress response called “prioritized” differentiation. Stress-activated protein kinase (SAPK) does not mediate stress-induced loss of nuclear potency factors, but mediates increases in nuclear differentiation factors. SAPK is also the mediator of prioritized differentiation, increasing early, and suppressing later lineages in a stress dose-dependent manner proportional to the amount of SAPK activated. AMP-activated protein kinase (AMPK) mediates stress-induced loss of potency factor proteins. AMPK rescues potency mRNA, thus enabling reversibility. Prioritized differentiation gives an understanding of how embryos/stem cells adapt to stress and will produce biomarkers of stressed reproduction, drug and chemical toxicity tests, and insights into changes that affect pre- and post-natal dysfunctions.

Program/Abstract # 276

The f-box protein atrogin enhances foxo in *Drosophila melanogaster*

Connors, Colleen; Staveley, Brian, Memorial University of Newfoundland, St. John's, Canada

Muscle atrophy can occur as the result of a wide range of conditions including diabetes, AIDS, sepsis and food deprivation. Atrogin encodes an F-box protein that is the substrate recognition component of an SCF ubiquitin ligase complex. It has been shown that atrogin acts in muscle degradation and is highly expressed during skeletal muscle atrophy in *Homo sapiens*, *Mus musculus*,and Salmo salar among others.Specifically, atrogin can target proteins for degradation that are essential for muscle synthesis such as myoD and eIF3f. However, not all target proteins of atrogin are targeted for proteolysis. Foxo, a transcription factor and member of the insulin receptor signalling pathway, is ubiquitinated by atrogin where the ubiquitin chain prevents the negative regulation of foxo by akt. Therefore, foxo localizes in the nucleus, and a positive feedback loop between atrogin and foxo is activated, as atrogin is a target gene of foxo. An atrogin candidate had been identified in *Drosophila melanogaster* and is well conserved between arthropods and mammals. Analysis shows that overexpression of the gene can enhance phenotypes when co-expressed with foxo in the eye. Also, atrogin overexpression can increase survivorship during nutritional stress, whereas a reduction of atrogin impairs the ability to endure starvation. Taken together these findings may suggest a conserved role for atrogin in the regulation of foxo. In addition, overexpression has been shown to cause a rapid degeneration of climbingability, and reduced longevity under standard conditions. The potential for atrogin in degenerative phenotypes in *Drosophila* is being investigated. Funding by an NSERC CGS to C.B. Connors and an NSERC Discovery Grant to B.E. Staveley.

Program/Abstract # 277

JNK phosphorylation of hnRNP K is required for axon outgrowth during nervous system development in *Xenopus laevis*

Hutchins, Erica J.; Szaro, Ben G., State University of New York, Albany, United States

The RNA-binding protein hnRNP K is required for axon outgrowth. Its suppression in *Xenopus* embryos causes defects in the translation of mRNAs of multiple cytoskeletal genes. Studies in cell lines have established that hnRNP K shuttles between the nucleus and the cytoplasm to bind and regulate the fates of its target RNAs, from splicing to export and translation. At each step, hnRNP K is regulated through post-translational modifications that alter its nucleic acid and protein interactions, and its subcellular localization. How this happens in developing neurons to coordinate cytoskeletal gene expression with the extracellular signals directing axon outgrowth is unknown. We have identified a JNK phosphorylation site within hnRNP K that is essential for its function during neuronal development. Treatment with SP600125, a pharmacological inhibitor of JNK, prevented formation of axons in primary neuronal cultures; a phospho-mimetic mutation of the JNK site on hnRNP K successfully rescued axon outgrowth in the presence of SP600125, implicating hnRNP K as a major substrate on which JNK acts to affect axonogenesis. We propose a mechanism whereby JNK controls translation of hnRNP K’s target mRNAs, and by extension axon outgrowth, at the point of translation initiation through prevention of 80S ribosome assembly. JNK has long been implicated in the intracellular signaling pathways that mediate effects of several receptors on axon outgrowth, although a mechanism of its action had not previously been described. These data suggest a role for hnRNP K as a central regulatory component linking extracellular signals that regulate axon outgrowth directly with the expression of key axonal structural components. Funded by NSF IOS 951043.

Program/Abstract # 278

Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is crucial for the regeneration of *Xenopus* optic axons

Szaro, Ben G.; Liu, Yuanyuan; Yu, Hurong, State University of NY at Albany, United States

Neurons express unique structural proteins that organize the cytoskeleton into an axon. During axon outgrowth, expression of these proteins is tightly coordinated to meet ever-shifting demands for structural materials. For example, in *Xenopus* optic nerve regeneration, changes in neurofilament protein expression result from a complex interplay between transcriptional and post-transcriptional gene regulatory mechanisms. In developing *Xenopus* neurons, the RNA-binding protein, hnRNP K, plays an essential role in the trafficking and translation of not only neurofilament mRNAs, but also the mRNAs of additional cytoskeletal proteins involved in organizing the axonal cytoskeleton, and which collectively are required for axonogenesis. To test whether hnRNP K plays a similar role in the post-transcriptional control of these genes during *Xenopus* optic axon regeneration, we used intravitreal injection of antisense Vivo-Morpholino oligonucleotide to suppress hnRNP K expression. In uninjured eye, knockdown was restricted to the retinal ganglion cell (RGC) layer and induced neither an axotomy response nor axon degeneration. After crush injury, hnRNP K knockdown prevented regrowth of axons beyond the lesion site. The injured RGCs nonetheless responded by increasing expression of several growth-associated RNAs, but those that were regulated by hnRNP K exhibited defects in nuclear export and failed to be loaded onto polysomes for translation. Thus, hnRNP K is an essential component of a novel post-transcriptional regulatory pathway that is essential for successful CNS axon regeneration. Funded by NSF IOS 951043 and an AHA Predoctoral Fellowship to YL.

Program/Abstract # 279

Buffy rescues and debcl enhances a-synuclein induced phenotypes in *Drosophila*

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To more fully understand the biological basis of Parkinson disease (PD), we study aspects of the disease in the very well understood model organism, Drosophila melanogaster.In brief, the directed expression of α-synuclein,the first gene identified to contribute to inherited forms of PD, to the dopaminergic neurons of flies has provided a robust and well-studied Drosophila model of PD complete with the loss of neurons and accompanying motor defects. In contrast to the complexity found in mammals, only two Bcl-2 family member genes have been found in Drosophila: the pro-cell survival Buffy and, debcl, the sole anti-cell survival homologue. In the α-synuclein-induced Drosophila model of PD, we have altered the expression of Buffy and debcl in the dopamine producing neurons and, in complementary experiments, in the developing neuron-rich eye. When these two genes were overexpressed in the dopamine producing neurons, debcl enhanced the α-synuclein-induced loss of climbing ability over time while Buffy acted t orescue this phenotype. In an analogous manner, when over expressed in the developing eye, Buffy suppressed and debcl enhanced the severity of the α-synuclein-induced disruption of the ommatidial array. Taken together, these experiments suggest a potentially protective role for Buffy and a potentially detrimental one for debclin α-synuclein-induced protein toxicity and possibly in Parkinson disease.

Program/Abstract # 280

The role of calcium signaling and voltage-gated calcium channels in neurotransmitter phenotype specification

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There has been a significant amount of research analyzing the ‘hard-wired’ aspects of nervous system development, such as the role of transcriptional regulation.However, recent literature points to another, relatively novel, mechanism forneuro transmitter phenotype specification: spontaneous electrical activity in the form of calcium transients. Calcium plays a critical role in neuronal development and its activity seems to play an essential role in neuronal phenotype specification, particularly neurotransmitter phenotype. Calcium ionfluctuations occur at early stages of neuronal development and alterations in this activity in *Xenopus laevis* have been shown to modify the ratio of excitatory and inhibitory neurons. Our overarching hypothesis is that voltage-gated calcium channels mediate the spontaneous activity found in embryonic neurons. By imaging changes in intracellular calcium concentrations using confocal microscopy, we have correlated expression of voltage-gatedcalcium channels (VGCCs) with specific patterns of activity on a single-celllevel. Eight VGCC a1 subunits are expressed in neural tissues during development, and of them, CaV2.1and CaV2.2 are associated with high frequency activity. Pharmacological blockade of VGCCs disrupts neurotransmitter phenotype specification in cell cultures dissected from *Xenopus* neural ectoderm, leading to an increase in the number of cells synthesizing glutamate and a decrease in the number of cells synthesizing GABA. These studies provide strong evidence that calcium entry through VGCCs plays an important role in neuronal phenotype specification.

Program/Abstract # 281

Identification and functional characterization of Nrdp1 as a potential new regulator of planar cell polarity signaling

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Planar cellpolarity (PCP) signaling coordinates the polarized structure, orientation and movement of cells within a tissue. Activation of PCP begins with asymmetricWnt/Fz signaling at the cell membrane, which is regulated by the transmembrane protein Van Gogh-like (Vangl). Vangl is a core PCP signaling protein and, in contrast to Wnt/Fz, is specific to PCP. While the role for Vangl in PCP signaling is well established, the molecular mechanisms underlying Vanglactivity remain unclear. To address this question, we performed a membrane yeast two-hybrid (MYTH) screen to identify novel Vangl-interacting proteins.Among our hits, we identified the E3 ubiquitin ligase Neuregulin receptor degredation protein (Nrdp1). Zebrafish Nrdp1is maternally and ubiquitously expressed early in development, but by 28 hours post fertilization (hpf) it becomes highly expressed in the central nervous system. Remarkably, Nrdp1 protein localizes to neuronal membranes, axons andcilia. To assay the function of Nrdp1 we used targeted zinc finger nucleases to generate zebrafish mutants with a two base pair deletion in nrdp1. This mutation, within the highly conserved RING domain of Nrdp1, results in a premature stop codon. Homozygous mutants are morphologically normal and viable until at least 7 days postfertilization. However, strong maternal nrdp1expression may rescue early embryonic development. We are currently generating maternal-zygotic mutant embryos that have a complete loss of nrdp1. Future experiments will elucidate the function of Nrdp1 in PCP signaling ciliogenesis and neuronal development.

Program/Abstract # 282

The adhesion GPCR Gpr125 modulates Dishevelled distribution and planar cell polarity signaling

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During embryogenesis, gastrulation establishes the three germ layers and the animal body plan. Vertebrate gastrulation relies on polarized cell behaviors to drive convergence and extension (C&E) movements that narrow embryonic tissues mediolaterally and elongate them anteroposteriorly 1. Although planar cell polarity (PCP) signaling is a key regulator of C&E movements, how it polarizes cells during gastrulation is not well understood 2, 3. Here, we identified the adhesion G protein-coupled receptor Gpr125 asa novel modulator of PCP signaling. Excess Gpr125 impaired C&E movements and the underlying cell polarity of wild-type gastrulae. Reduced Gpr125 function exacerbated the C&E and facial branchiomotor neuron migration defects of PCP mutants. Intriguingly, Gpr125 recruited Dishevelled (Dvl), the signal transducer of PCP signaling 2, 3, to the cell membrane and promoted Dvl clustering into discrete membrane subdomains and reciprocally, Dvl promoted the clustering of Gpr125into such membrane subdomains. Pull-down assays suggested Gpr125 and Dvl influence their mutual subcellular distribution via direct interaction. We hypothesize that Gpr125 modulates PCP signaling and polarized cell behaviors in part by promoting formation of PCP protein supramolecular membrane complexes.

Program/Abstract # 283

***Drosophila* Maf1 controls body size and developmental timing by modulating tRNAiMet synthesis and systemic insulin signaling**

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How growth and size are controlled during animal development is an important question in biology. Several families of conserved cell-cell signaling pathways regulate organ size by controlling cell growth, proliferation and survival. In addition, environmental factors such as nutrients, oxygen and temperature influence tissue and organismal growth during development. The conserved Target-of-Rapamycin (TOR) kinase is perhaps the best-understood nutrient-dependent regulator of cell metabolism and growth in animals. The key effectors underlying this growth are, however, unclear. Here we show that Maf1,a repressor of RNA Polymerase III-dependent tRNA transcription, is an important mediator of nutrient-dependent growth in *Drosophila*. We find nutrients promote tRNA synthesis during larval development by inhibiting Maf1. Genetic inhibition of Maf1 accelerates development and increases body size. These phenotypes are due to a non cell-autonomous effect of Maf1 inhibition in the larval fat body, the main larval endocrine organ. Inhibiting Maf1 in the fat body increases growth by promoting the expression of brain-derived insulin-like peptides and consequently enhanced systemic insulin signaling. Remarkably, the effects of Maf1 inhibition were reproduced in fliescarrying one extra copy of the initiator methionine tRNA, tRNAiMet. These findings suggest the stimulation of tRNAiMet synthesis via inhibition of dMaf1 is limiting for nutrition-dependent growth during development.

Program/Abstract # 284

Investigation of novel hypomorphic alleles of akt in *Drosophila melanogaster*

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The development of an organism requires a balance between cell growth and cell survival. A key regulator of cell growth is the protein kinase akt, a centralcomponent of the insulin receptor signalling pathway. Up-regulation of akt leads to an increase in growth, while reduced akt activity decreases growth, and may be lethal. Several novel akt hypomorphic alleles were generated through the mobilization of a P-element inserted within the upstream control region of the gene in *Drosophila*. Characterization of the hypomorphs showed variability in development and overall growth. The time required for the hypomorphic mutants to reach eclosion is delayed when compared to controls. Consistent with previously analyzed hypomorphic alleles, analysis of both mutant eyes and somatic clones of the eye reveal that altered akt activity leads to a reduction in cell number and size. A downstream target of akt, the transcription factor foxo, is key to the control of cell cycle and apoptosis. The hypomorphic reduction of akt activity results in a reduction in organismal size which is the probable result of a slight increase in the activity of the foxo transcription factor. A series of *Drosophila* lines containing both a hypomorphic allele of akt and a null mutation of foxo were created via recombination. These double mutants exhibit novel and unique phenotypes unlike either of the contributing mutants. Biometric analysis of these double mutants may lead to a better understanding of the control of cell growth and survival downstream of akt. Support contributed by an NSERC PGS-D3 to J.D. Slade and an NSERC Discovery Grant to B.E. Staveley.

Program/Abstract # 285

Regulation of vesicle endocytosis and acidification by Rabconnectin-3a in zebrafish neural crest migration

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Neural crest (NC) cells undergo epithelial-to-mesenchymaltransitions (EMT), directed migration and specification into a wide variety ofcell lineages, each of which must be precisely controlled spatially andtemporally. All threeprocesses require Wnt signaling and dynamic regulation of cell-cell adhesionmolecules, such as Cadherins (Cads). There is growing evidence that a keyfeature of this regulation is a tight control of endocytosis and lysosomaldegradation of Wnt-receptor complexes and Cad molecules from the plasmamembrane of migrating NCs. A microarrayscreen performed with early neural plate-stage zebrafish embryos deficient inNC cells identified rabconnectin-3a (rbc3a) as an unexpected gene with early premigratoryNC expression. rbc3a knockdown disruptsmigration of a subset of NC cells, which remain stuck in the dorsal midline andlater produce pigment and express melanocyte lineage markers exclusively. Lossof rbc3a leads to aggregation ofaberrantly acidified early endosomes in these unmigrated NC cells but not lateendosomes or lysosomes, suggesting a regulatory role in endosome maturation andacidification. Acidification of endocyticvesicles is required for proper receptor-ligand dissociation as well as the recyclingand lysosomal degradation of target membrane-bound molecules. Loss of eitherWnt signaling or Ncad expression phenocopies many aspects of rbc3a loss-of-function and our data suggestthat Rbc3a mediates endosomeacidification and trafficking of both processes to coordinate NC cell EMT, migration,and specification.

Program/Abstract # 286

Modulation of smooth muscle contraction in the zebrafish intestine by the high molecular weight Caldesmon isoform

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In smooth muscle, the high molecular weight caldesmon isoform (h-CaD) tethers the actin and myosin filaments and modulates their interaction. Several studies have implicated h-CaD in both the regulation of contractile force and the maintenance of resting tone in smooth muscle; however its function *in vivo* has not been established. To examine the role of h-CaD in smooth muscle contraction, we have developed a zebrafish model which permits in vivo imaging and evaluation of smooth muscle function. In this work, we have cloned cDNA for the zebrafish homologue of human smooth muscle caldesmon and have established its regulatory role on contraction in vivo. Using an antisense knockdown approach in combination with transgenic overexpression of critical h-CaD protein domains, we were able to specifically disrupt h-CaD function allowing us to observe its contribution during smooth muscle contraction. A significant increase in contractile force, as determined *in vivo* using an intestinal propulsion assay, was observed in zebrafish larvae with disrupted h-CaD function. Additionally, knockdown of h-CaD in larvae deficient in enteric nerve signaling resulted in a partial rescue of intestinal propulsion. Our findings provide for the first time evidence of h-CaD’s modulatory role in vivo during smooth muscle contraction and suggest its importance in endogenous actomyosin interactions in the absence of

neuronal signaling to smooth muscle. Our study demonstrates the importance of h-CaD on force development in smooth muscle and the need for further investigation of its potential role in intestinal motility disorders.

Program/Abstract # 287
The integrity of the hippocampus in SIV-infected infant Primates
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Pediatric HIV infection remains a global health crisis with a worldwide infection rate of 2.5 million (WHO, Geneva Switzerland, 2009). Children are much more susceptible to HIV-1 neurological impairments than adults, which is exacerbated by co-infections. A main and obvious obstacle in pediatric HIV research is sample access. The proposed studies will take advantage of ongoing pediatric SIV pathogenesis and vaccine studies to maximize the use of nonhuman primate resources. This expands the original pediatric SIV-related immunology studies to include quantitative neuropathology studies. Newborn rhesus macaques (Macacca mulatta) that received oral inoculation with arepeated-exposure of SIVmac251 (n=3) or vehicle (control n=3) were recruited for this study. After a 6-18 week survival time, the animals were sacrificed and the brains prepared for quantitative histopathological analysis. Here we report the total neuronal population of CA1, CA2, and CA3 are significantly reduced after two months of SIV infection using design-based stereology. This project assesses the impact of early HIV infection on the brain towards the long-term goal of evaluating treatment paradigms designed to protect the integrity of the developing brain from combined viral and bacterial infections through an interdisciplinary approach.

Program/Abstract # 288
Temporal polar and anterior cingulate cortical thinning in psychopath offenders
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Psychopathy is characterized by a lack of empathy and the formation of transient and exploitive interpersonal relationships. The goal of this study was to determine whether brain regions implicated in emotional processing and behavioral regulation showed structural alterations in psychopath offenders. Using a surface-based whole brain analysis to detect associations between gray matter thickness and the Hare total score were identified in entire cortex analyses in 97 violent offenders (29 offenders with and 68 without psychopathy defined by the PCL-R scale. We found that psychopathy was associated with highly significant (FDR=0.01) thinning of the cortical surface within the left dorsal anterior cingulate and temporal pole cortices. Given recent evidences of gray matter changes during neurodevelopment, these findings may indicate impairment in cortical maturation in important brain areas implicated empathic and emotional processing in offenders with psychopathy. Keyword: psychopathy, MRI, thickness, offenders

Program/Abstract # 289
Identification of MPPED1 as a protein interacting with human FOXP2 R553H mutant protein associated with speech and language disorder
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The genetic linkage study of the KE family shows that a missense R553H mutation of FOXP2 is the cause of a severe speech and language disorder in the affected members. Clinical neuroimaging studies indicate that the caudate nucleus of striatum and the inferior frontal gyrus are structurally and functionally abnormal in the patient's brain. FOXP2 is therefore the first gene identified to be involved in speech and language. Our study is aimed to study the biological function of FOXP2 in developmental and functional of the cortical basal ganglia circuits in the brain. FOXP2 was nuclear protein, but FOXP2R553H mutant protein was expressed in the cytosol. To identify the proteins that might interact with FOXP2R553H mutant protein, we preformed the yeast two-hybrid system to screen for interacting proteins. We screened 1.31 x 109 clones of human fetal brain cDNA library. One of the interacting proteins that we identified was Metallophosphoesterase Domain Containing Gene 1 (MPPED1), a cyclic nucleotide phosphodiesterase that was localized in cytosol. Co-immunoprecipitation assay confirmed that FOXP2R553H mutant protein, but not wild type FOXP2 protein interacted with MPPED1 in transfected HEK293T cells. Double immunostaining further demonstrated co-localization of FOXP2R553H mutant protein and MPPED1 in cytosol of transfected neural ST14A cells. These findings suggest that FOXP2R553H mutant protein may have gain-of-function by interacting with MPPED1 protein that can hydrolyze cAMP. The potential interference of MPPED1 activity by FOXP2R553H mutant protein may be involved in the pathophysiology of KE language disorder. Supported by National Science Council grant NSC97-2321-B-010-006, NSC98-2321-B-010-002, NSC99-2321-B-010-002, NSC100-2321-B-010-002.

Program/Abstract # 290

A molecular and genetic approach to identifying a clinical rabbit craniosynostotic model and its relevance to craniofacial development

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Craniosynostosis, the premature fusion of the sutures of the cranial vault, clinically manifests with a range of severity from subclinical phenotypes to severe cases, exhibiting increased intracranial pressure, severely altered head shape, and premature death. Much of what is known about craniosynostosis is derived from studies in animal models and human mutations in transforming growth factor beta receptors (TgfβRs), fibroblast growth factor receptors (FGFRs), Twist1, and Msx2; these mutations, however, only account for 15 % of known craniosynostosis cases in humans. We have previously described a colony of rabbits with an autosomal dominant heritable pattern of coronal suture synostosis. We undertook a molecular analysis todetermine whether TgfβR1, TgfβR2, FGFR1, FGFR2 and / or Twist1 were the loci ofthe causative mutation within our model system using single nucleotide polymorphisms (SNPs); SNPs were identified within our colony through cDNA and genomic DNA cloning and sequencing. These SNPs were assayed for segregation with disease phenotype in 22craniosynostotic animals. SNP analysis within the TgfβR1, TgfβR2, FGFR1, FGFR2, and Twist1 genes indicated that none of these loci are linked to the craniosynostotic phenotype as no allelic combination showed any specific correlation with disease phenotype. As no SNP arrays exist for rabbits, we sought a molecular /bioinformatics method to create a SNP map in rabbits with the ultimate goal of mapping the defect in our rabbit colony. Utilizing recent advancements in next generation sequencing, we used the recently published restriction site associated DNA sequencing technique (RADsequencing) to identify SNPs in our rabbit genome compared to wild type animals maintained separately from our breeding colony. This analysis described over 60,000 SNPs within our animals that can be mapped to the low-density rabbit genome currently available. Subsequent bioinformatics analysis identified approximately 6,000 SNPs that map to the craniosynostotic colony, an important first step in identifying genes that segregate with the craniosynostosis phenotype for future mapping purposes. Since we have already ruled out five genes known to be involved in cranio synostosis, this well-established clinical model provides a unique opportunity to decipher the molecular pathways functioning in craniofacial development.

Program/Abstract # 291

Effect of low molecular weight chitosan oligosaccharides reduces pulmonary fibrosis in a Bleomycin mouse model

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In human, idiopathic pulmonary fibrosis is characterized by alveolar epithelial cell injury and lose its functions. This disease leads to impairment of gas exchange and pulmonary function because of the loss of lung elasticity and alveolar surface area. Bleomycin is limited because it produces dose-dependent pulmonary toxicity which induces interstitial pulmonary fibrosis. Low molecular weight chitosan oligosaccharides (LM-COS) has effects of anti-inflammatory. LM-COS (<1.0 kDa), consisting of glucosamine, are capable of inhibition both antigen-stimulated degranulation and cytokine generation in rat basophilic leukaemia RBL-2H3 cells. The protective effect of LM-COS against ovalbumin (OVA)-induced lung inflammation in a mouse asthma model was also examined. The aim of this experiment is to ascertain a result for lung fibrosis treatment in chitosan treated mice. 7 to 9 weeks old Male C57B1/6 mice weighing 22-24g are used in the study. And Bleomycin sulphate is dissolved in 0.9% saline a concentration of 1 U/ml. Bleomycin solution loads into micropipette and a dose of 2U/kg in 25ml is injected into mice, twice weekly for 4-8 weeks. Then, those mice are treated saline, bleomycin and LM-COS. From this experiment, we observe variation of body weight, groups' histology, TUNEL assay for the identification of apoptotic cells, BAL fluid analysis. LM-COS has an effect on significant reduction in the levels of protein in the lung tissue and BALF. The levels of IL-4, IL-13, and TNF-α mRNA and protein in protein in BALF induced by oral administration of LM-COS are significantly decreased, compared to those in the control group. These findings might suggest a potential role for LM-COS in the treatment lung fibrosis.

Program/Abstract # 292

Identification of KLF13 interacting partners in the heart

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Congenital heart defects (CHD) is the largest class of birth defects in humans and are a major cause of infant mortality and morbidity. Unfortunately, identifying the aberrant molecular and genetic pathways underlying CHD is still a challenging puzzle. We have previously demonstrated that the zinc-finger kruppel-like transcription factor KLF13, expressed predominantly in the atria, binds evolutionarily conserved regulatory elements known as CACC-box on cardiac promoters. Importantly, our targeted gene disruption of KLF13 was shown to be associated with cardiac outflow tract development.

Moreover, our previous studies have shown that KLF13 acts as a cardiac transcriptional activator, and both functionally and physically interacts with the cardiac master regulator GATA-4. In the current study, we examined KLF13's mechanism of action by investigating its transcriptional activity and its partnering profile with other transcription factors on target genes; we found that KLF13 functionally and physically interacts with several critical cardiac regulators such as Tbx5 and Nkx2.5. Structure-function studies identified distinct functional domains important for KLF13 DNA-binding and protein-protein interaction. Lastly, some mutant proteins associated with congenital heart defects were found to have impaired interaction with KLF13. These experiments provide novel insight into cardiac transcription and KLF13 mechanisms of action and suggest that KLF13 may be a genetic modifier of human congenital heart diseases.

Program/Abstract # 293
Essential role for KLF13 in heart development
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KLF13 is a member of the Krüppel-like transcription factors that are important regulators of cell proliferation and differentiation. Several KLF members are expressed in the heart in a spatial and temporal specific manner. KLF13 is highly enriched in the developing heart where it is found in both myocardial and endocardial cells. In myocytes, it interacts with GATA4 and regulates the A- and B-type natriuretic peptide genes, NPPA and NPPB. In xenopus, knock down of KLF13 causes developmental heart defects which indicate an important role for KLF13 in heart morphogenesis. To test whether this role is evolutionary conserved in the mammalian heart, we deleted the KLF13 gene in transgenic mice using homologous recombination. Mice lacking both KLF13 alleles are born at reduced frequency; variable cardiac phenotypes are observed in these knockouts mainly endocardial cushion defects including "Goose-neck" deformity and atrioventricular (AV) valvular abnormalities. Epithelial-mesenchymal transformation (EMT) seems to be affected in these mice and they have reduced proliferation in the AV cushion. Surviving KLF13 null mice have several structural cardiac anomalies. NPPB mRNA levels are decreased by 50% and expression of several cardiac genes is altered. Our data uncover a role for a new class of transcription factors in heart formation and point to KLF13 as a potential congenital heart disease causing gene.

Program/Abstract # 294
Akt mediates acute alcohol inotropic effects on the heart
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Cardiovascular disease is among the major causes for increased morbidity and mortality rates. High alcohol consumption may lead to cardiomyopathy, cardiac arrhythmias, and a suite of other disorders. Previous studies in our lab have linked the AKT/PI3K pathway to cardiovascular disease. In this study we investigate the role of AKT gene expression (RT-PCR) that contributes to changes in cardiac contraction (Ionoptix imaging) with acute alcohol retrograde coronary perfusion of the heart. Our results demonstrate that AKT expression is diminished with Low alcohol (LA) exposure; however, high alcohol (HA) treatment has increased expression over LA exposure. LA increased cellular and sarcomeric contraction associated with increase in the velocity of contraction. Relaxation and calcium sequestration was also improved by LA. Inhibition of PI3K negated the contractile effects of LA, but enhanced the relaxation ones. HA decreased the strength of contraction and increased speed of relaxation and calcium sequestration. Our data suggest that LA improves cardiac function through a reduction of the Akt pathway; furthermore, HA has detrimental inotropic effect through an elevation of the Akt gene expression. Also, PI3K/Akt pathway seems to regulate the lusitropic effects of both LA and HA.

Program/Abstract # 295
A *Xenopus*-based system to study the biochemical and genetic etiology of Fetal Alcohol Spectrum Disorder
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Exposure of human embryos to ethanol results in a high incidence of Fetal Alcohol Spectrum Disorder (FASD). Children with FASD can exhibit facial dysmorphology, microcephaly, short stature, central nervous system, neurodevelopmental abnormalities, behavioral and psychological anomalies. We used *Xenopus* embryos to study the mechanism underlaying the developmental malformations in alcohol (ethanol)-treated embryos. Ethanol and its detoxification compete with the biosynthesis of retinoic acid resulting in abnormally low levels. The strongest effect of this competition takes place close to the onset of gastrulation centering on the retinaldehyde dehydrogenase 2 (Raldh2; Aldh1a2) activity in Spemann's organizer. There is great difficulty in predicting the phenotype of children exposed to alcohol based solely on mother size, amount and frequency of alcohol ingested, developmental stage of exposure and other environmental factors. Also

dizygotic twin studies have shown that they can exhibit different severities of alcohol-induced developmental defects suggesting that genetic variation influences FASD severity. The Raldh2 gene in the human population shows the existence of variants that differ by an amino acid, which in turn might affect the enzymatic activity. Here, we use *Xenopus* embryos to determine the genetic/enzymatic predisposition to FASD by analyzing the different Raldh2 isoforms in the presence of ethanol in vivo. We use our retinoic acid signaling rescue assay and compare between the different human Raldh2 alleles to determine which alleles modify the enzymatic activity such that it increases or decreases the risk for the embryo to develop FASD as a result of ethanol exposure. The results of these studies will increase our knowledge on the effects of ethanol on embryonic development, will demonstrate the feasibility of the approach and will establish the experimental conditions to screen for genetic predisposition to FASD.

Program/Abstract # 296

Human BMP receptor mutations causing fibrodysplasia ossificans progressiva lead to ligand-independent receptor activation in zebrafish embryos

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The human dominant disease fibrodysplasia ossificans progressiva (FOP) is caused by mutations in the Alk2 BMP receptor. Most patients with classic FOP carry the mutation Arg206His, changing the tertiary structure of the intracellular Alk2 GS domain and causing hyperactivation. Two mutations of Gly328 (Gly328Trp/Glu) cause a more severe FOP phenotype, but it is unclear how these mutations cause disease. We used the zebrafish embryo to investigate the activity of these codon 328 FOP mutations. In zebrafish, BMP signaling plays an important role in patterning embryonic dorsal-ventral axial tissues. mRNA misexpression studies are therefore an excellent tool to study the effect of these mutations on BMP signaling in vivo. Alk2 mRNA containing either Arg206His (cFOP), or a variant mutation (Gly328Trp, Gly328Glu) was injected into wild type (WT) or BMP7 deficient embryos at the one-cell stage. Phenotypes were assessed at 24 hpf for ventralization or dorsalization. Early and mid-gastrula embryos were analyzed for phospho-Smad1/5 expression and for markers of ventral and dorsal tissues, respectively. Mutations in the G328 codon cause ventralization of WT embryos and rescue BMP7 deficient embryos, but to a lesser extent than the cFOP mutation. Phospho-Smad1/5 expression is increased in all injected embryos, but more profoundly in the cFOP group. In summary, although in silico modeling has not demonstrated changes in the tertiary structure of the G328 mutant Alk2 receptors, evidence from this study suggests that G328 mutants also confer increased receptor activation and ligand-independent signaling via Smad phosphorylation. Future studies will investigate the mechanism through which alk2 G328 mutations cause hyperactivation.

Program/Abstract # 297

GATA4 A new biomarker for Rhabdomyosarcoma ?

Nemer, Georges; Haidar, Wiam; Saab, Raya, American University of Beirut, Beirut, Lebanon; Nemer, Mona (University of Ottawa, Ottawa, Canada)

Background: We have previously shown that GATA4, a zinc finger cardiac-enriched transcription factor, is expressed in subsets of undifferentiated skeletal muscle cells where it acts as a negative regulator of myogenesis. GATA4 is expressed in the widely used c2c12 mouse myoblast cell line where a rapid decrease of the endogenous GATA4 binding activity occurs after switching cells to a differentiation-promoting medium. These findings identify GATA4 as a novel regulator of skeletal myogenesis and suggest that GATA4 and MyoD play pivotal and opposing roles during the premyogenic-myogenic transition. Objective: We aim at establishing a role for GATA4 in rhabdomyosarcoma the skeletal-related form of cancer, which is the most prevalent soft tissue sarcoma in children. The ultimate goal being to show that modulation of GATA4 might open the way towards designing an efficient therapeutical regimen to treat the disease. Results: We have shown that GATA4 is expressed in both embryonal (JR-1) and alveolar (Rh30 and Rh41) rhabdomyosarcoma cell lines. In addition serum withdrawal did affect the expression profile of GATA4 in the cells but not as much as in the C2C12 model. We characterized a new microRNA that could regulate GATA4 expression in both Rhabdomyosarcoma cells lines. Finally we showed that GATA4 is differentially expressed in rhabdomyosarcoma biopsies collected from different patients. Conclusion: The results showed for the first time an expression of GATA4 in Rhabdomyosarcoma and hypothetically linked this expression to the inability of the cells to differentiate into skeletal muscle. This goes along with our previous results on C2C12 whereby over expression of GATA4 blocks their differentiation into myotubes. We hope to establish GATA4 as a novel biomarker for Rhabdomyosarcoma and link its expression profile and intensity to the severity of the phenotype and response to treatment.

Program/Abstract # 298

The Gene Expression Database (GXD): a resource of mouse gene expression data for developmental biologists

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The Gene Expression Database (GXD) is a free resource of mouse developmental expression information, covering all developmental stages anddata from wild-type and mutant mice. GXD integrates data from different assaytypes, including RNA *in situ* hybridization, immunohistochemistry, knock-in reporter studies, Northern and Western blots, and RT-PCR. Data are acquired through curation of the literature, via electronic data submissions from conventional laboratories, and by collaborations with large-scale data providers. All these data are carefully reviewed by GXD curators and annotated using standardized text annotations. Detailed entries include the genes analyzed, the probes/antibodies used, the strain and genotype of the specimen, and the developmental stages and anatomical structures in which expression was reported to be present or absent. Wherever possible, database records are linked to images of expression data. GXD currently contains over 223,300 images and more more than 1.1 million annotated expression results from over 57,300 assays for more than 13,000 genes, including expression data from over 1600 mouse mutants. As part of the larger Mouse Genome Informatics (MGI) resource, GXD combines its expression data with other genetic, functional, phenotypic, and disease-oriented data. Therefore, users can search for expression data and images in many different ways, using a variety ofbiologically and biomedically relevant parameters. In this way, GXD facilitates progress towards understanding the molecular basis of development and human disease. GXD is freely available through the MGI web site (www.informatics.jax.org), or directly at www.informatics.jax.org/expression.shtml. Supported by NICHD grant HD062499.

Program/Abstract # 299

The chromatin-remodelling factor CHD7 controls multiple developmental programmes during development of the cerebellum

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The CHD7 gene encodes a chomodomain helicase DNA-binding factor implicated in the epigenetic fine-tuning of developmental gene expression. Mutations in CHD7 result in CHARGE syndrome. In addition to the core features of CHARGE syndrome (Coloboma, Heart defects, Atresia of the choanae, Retardation of growth and development, GU abnormalities and Ear abnormalities), neurodevelopmental defects such ashypoplasia of the cerebellar vermis have been reported. To understand the developmental basis of these defects we analysed cerebellar development in Chd7-deficient mouse models. We found that the establishment of normal levels of Fgf8 expression in the isthmus organiser is sensitive to the Chd7 gene dosage. Despite a reduction in Fgf8 expression and signalling in Chd7+/- embryos, these animals do not present with vermishypoplasia, suggesting that the reduction in FGF signalling in these mutants may not be sufficient to cause cerebellar defects. In agreement with this hypothesis, Chd7+/-; Fgf8+/- mutants exhibit severe vermis hypoplasia and the loss of posterior midbrain tissue. Gene expression analyses implicate CHD7 as a regulator of several genes that function during mid-hind brain development, including Otx2 and Gbx2. The analysis of Chd7 conditional mutants in which Chd7 is deleted throughout the mid-hindbrain region or in upper rhombic lip-derived cells of the cerebellum reveals multiple roles for Chd7 during the development of the midbrain and cerebellum. The functions of CHD7 during these later stages of development are currently under investigation and the results of these studies will be presented.

Program/Abstract # 300

The inhibition of Tenascin-C, well-known for its role in regeneration and development, through RNAi in breast cancer

Young Hoon, Park; Seul Ki Na, Lee; Hyo Sang, Park; Yun Jeong, Choi; Su A, Kang; Dae Young, Kim; You Jin, Hwang, Gachon University, Incheon, Republic of Korea

Tenascin-C (TNC) is a kind of aglyco protein which belongs to tenascin family that carries various functions such as cell communication, development, regeneration and etc. Besides recent studies about TNC on breast cancer indicate that TNC induces metastasis to lungs and other organs, thus stating TNC one of the most important factor in breast cancer. Our goal of this study is to inhibit the progression of breast cancer through the blockage of TNC production by RNA interference (RNAi) of TNC producing gene. We cultured breast carcinoma cell line in order to investigate TNC expression and insert microRNA (miRNA) to suppress TNC producing gene. We examined quantity of proteins and expression of genes through western blot and electrophoresis. The result seemed to show a possibility in controlling cancer growth as well as cancer metastasis by suppressing TNC production. We believe our study is distinguishable to other studies in that we found TNC suppression as an useful way to treat breast cancer.

Program/Abstract # 301

Dual roles for canonical and non-canonical Wnt signaling in craniofacial development and patterning.

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Craniofacial development is a complex process that requires signaling from the pharyngeal arch epithelia to regulate the patterning of the skeletal precursor cells, neural crest cells (NCC), into distinct dorsal and ventral elements. While the Wnt signaling pathway has been implicated in multiple processes of NCC development the exact nature of Wnt signaling in craniofacial patterning has not been fully characterized. Using transgenic zebrafish lines to overexpress a dominant negative Tcf3, (Tg(hsp70I:tcf3-GFP), and ectopically express the canonical Wnt inhibitor dickkopf1 (dkk1), (Tg(hsp70i:dkk1-GFP), we have repressed Wnt signaling in a temporal manner. Loss of Wnt signaling in dntcf3 embryos results in reduction of ventral cartilage elements, proliferation defects, and loss of dorsal-ventral (DV) patterning genes such as hand2, dlx3b, dlx5a, and nkx3.2. This closely resembles the DV patterning defects seen following loss of Bmp signaling. In addition, hs-dkk1 embryos present a unique craniofacial phenotype – clefting of the mandible at the ventral midline and stacking defects that resemble non-canonical Wnt mutants. Dkk1 is expressed exclusively in the pharyngeal arch endoderm and transplantation of hs-dkk1+ endoderm into control embryos is sufficient to phenocopy mandibular clefting. Therefore, Wnt signaling regulates mandibular development through both canonical and non-canonical signaling pathways – we propose that canonical signaling interacts with Bmp signals from the ectoderm to control DV patterning, while non-canonical Wnt signaling from the endoderm regulates mandibular growth and morphogenesis.

Program/Abstract # 302

The levels of Sox21 alter its function in neurogenesis

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Neurogenesis, the progression from neural progenitor to committed neuron, is a tightly regulated process that is fundamental for development ofthe central nervous system (CNS). Members of the SoxB transcription factor family play critical roles in this process. Whereas SoxB1 proteins, which act as transcriptional activators, 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. However our loss of function assays demonstrated that the decrease in Sox21 reduced neuron formation while progenitors remained unaffected. Our gain and loss of function analyses together suggest that Sox21 plays more than one role in neurogenesis, where a threshold level is required for differentiation of neurons from progenitors but a high concentration of Sox21 inhibits neurogenesis and instead promotes proliferation. Thus like other Sox proteins, Sox21 functions in a dose dependent manner. 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.

Program/Abstract # 303

Relationship between Calcium activity, neurotransmitter phenotype, and expression of the transcription factor Ptf1a in the developing *Xenopus laevis* retina

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To establish a mature visual system, retinal neurons must acquire the appropriate neurotransmitter fate. While the mechanisms regulating retinal cell type identity are well studied, the processes by which retinal cells acquire a particular neurotransmitter phenotype are less well known. The role of transcription factors in retinal cell specification has been extensively studied, but there has been far less focus on the role of activity, particularly the role of early calcium activity. In this study, we employ *Xenopus laevis* embryos as a model to investigate the possible correlations among calcium activity, transcription factor expression, and neurotransmitter phenotype at the individual cell level. Specifically, we hypothesize that early calcium activity in the developing retina modulates transcription factor expression thereby inducing a specific neurotransmitter phenotype. We predict that specific patterns of calcium activity will correlate with the coexpression of neurotransmitter phenotype markers and transcription factors. To test this hypothesis, we are utilizing *in vivo* calcium imaging to analyze the calcium activity of individual retinal cells from primary retinal cell culture from early developmental stages of *Xenopus* embryos. *In situ* hybridization is then performed using neurotransmitter phenotype markers for GABA and glutamate. Additionally, we are assessing calcium activity for cells expressing Ptf1a, a

transcription factor associated with a GABAergic phenotype. More broadly, we are examining the different patterns of calcium activity displayed by developing retinal cellsand the relationship to phenotype.

Program/Abstract # 304
Lineage commitment and differentiation of renal progenitor cells
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Maintenance and differentiation of stem cell/progenitor state are central concept in cancer and embryo development. Pax2/8 genes are found to be necessary and sufficient in lineage commitment and cell differentiation in different stages of kidney development. Previous studies from our lab clearly establish that embryos deficient for Pax2 and its paralog Pax8 fail to specify the first renal cells in the embryo. Conversely, ectopic expression of Pax2 in mesodermal progenitor cells was sufficient to induce mesodermal-epithelial transition, tubulogenesis and specify the renal fate (Bouchard et al., Genes Dev, 2002). Hence, we hypothesize that Pax2 acts as a master control gene regulating the transition from stem cell/ progenitors to a more differentiated state by integrating upstream signals and establishing a lineage-restricted transcriptional program. Although the crucial importanceof Pax2/8 in renal lineage commitment from progenitor cells is well established the molecular and cellular mechanisms responsible for this transition are poorly understood. The aim of this project is to study the molecular signals that activate Pax2/8 expression to initiate cell lineage specification from progenitor cells. To examine it we recently developed a primary culture system in which we purifyrenal primordium cells expressing a Pax2-GFP transgene from E9.25 embryos.These cells in culture gradually turn off GFP expression, acquiring a progenitor-like state. We then tried to follow renal lineage specification from these progenitors by reactivation of GFP through addition of candidate Pax2 inducing factors. We were able to identify some of the early regulators of nephric lineage activation. Our long-term goal is to shed some light on the exact mechanisms of cellular transition from a stem cell/progenitorstate to a lineage-restricted committed state. This will impact our understanding of cellular mechanisms that have direct consequences not only for the renal developmental diseases but also for the study of embryogenesis.

Program/Abstract # 305
Kruppel-like factor 5 is required for villus morphogenesis and terminal differentiation of the intestinal epithelium
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Kruppel-like factor 5 (Klf5) is a transcription factor demonstrated to regulate cell proliferation, migration, and differentiation in a variety of cell types. We created a Klf5 floxed allele and crossed it into mice harboring Cre-recombinase under control of the Shh promoter resulting in embryos deficient in Klf5 throughout the gut endoderm. KLF5 immunohistochemistry confirmed that the Klf5 alleles were efficiently floxed. At E14.5, the forming intestine of Klf5Δ/Δ embryos was morphologically indistinguishable from controls, epithelial cells were proliferating, and expressed the early endoderm markers SOX9 and FOXA1. Between E15.5-E16.5 formation of the crypt-villus axis begins and the first signs of malformation were observed in Klf5Δ/Δ embryos. In mutants, villi failed to form and expression of SOX9 and FOXA1 persisted throughout most of the intestinal epithelium. At later developmental stages, a marked paucity of normal cytodifferentiation was observed. The microvilli comprising the brush border were disorganized and there was a dramatic reduction in the number of goblet and enteroendocrine cells. Microarray analysis and qPCR of fetal intestines revealed misregulation of transcription factors known to drive intestine epithelial terminal differentiation including Elf3, Ascl2, and Atoh1. Notably, reductions in the expression of signaling molecules associated with villus morphogenes is were not detected: PdgfA, Shh, Ptc1, Bmp2, andBmp4. In contrast, the intestinal mesenchyme of embryos with Klf5 deficient epithelia differentiated normally. These observations indicate that KLF5 plays a critical role in the signaling cascade leading to crypt-villus axis formation that precedes epithelial differentiation.

Program/Abstract # 306
KIF17 controls the ciliary localization of GLI2 and GLI3
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Primary cilia are essential for Hedgehog (HH) signal transduction during vertebrate embryogenesis. The HH transcriptional effectors GLI2 and GLI3 traffic through primary cilia, and these cellular organelles are required for proper processing of GLI proteins. However, the mechanisms that control ciliary trafficking of the GLI proteins are largely unknown. Kinesin-2 motorproteins, namely KIF3A, KIF3B, and KIF17, mediate anterograde trafficking of proteins through primary cilia, making them presumptive candidates for regulating anterograde transport of GLI2 and GLI3. However, since KIF3A and KIF3B function in both anterograde cilia transport, as well as cilia formation, teasing out a HH-specific function is difficult. Unlike KIF3Aand KIF3B, KIF17 function appears to be restricted to anterograde

trafficking of cargo proteins and does not affect primary cilia formation. Here we show that expression of dominant negative (DN) versions of KIF17 perturbs GLI2 and GLI3ciliary localization. Surprisingly, expression of DNKIF17 in cell signaling assays and in chick neural tubes does not alter HH-dependent cell signaling orventral neural tube patterning. These data suggest that ciliary accumulation of GLI proteins is not essential for HH signaling.

Program/Abstract # 307

Negative regulation of Epidermal Growth Factor Receptor signalling in the *Drosophila* ovary

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During oogenesis the follicular epithelium is patterned to form the structures of the eggshell, including two dorsal anterior appendages separated by a dorsal midline domain. The primordia that form these structures are marked by differential expression of the Broad complex. Expression of this fate marker is controlled by dorsally localized activation of Epidermal Growth Factor Receptor (EGFR) in the follicle cells by its secreted ligand Gurken (Grk) from the oocyte. We are studying how alterations of EGFR activity lead to changes in the patterning of this tissue. For example, when the negative regulator Sprouty (Sty) is lost, dorsal fates are expanded ventrally as predicted from the expected increase in EGFR activity. Unexpectedly, these domains are also shortened along the anterior posterior (AP) axis. Here we look at two other negative regulators, D-cbl, which functions as an E3 ubiquitin ligase for EGFR, and Gap1, which promotes the inactivation of Ras. We show that loss of D-cbl in follicle cells results in expansion of dorsal fates onto the ventral side of the follicular epithelium. Additionally, these clones display a shortening of dorsal fates along the AP axis. We also show that loss of Gap1 results in the expansion of dorsal fates ventrally but fails to display any shortening of dorsal fates along the AP axis, suggesting that the function of Gap1 is distinct from that of D-cbl and Sty. We also looked at the regulation of a downstream factor, Capicua, as a readout of EGFR activity. Interestingly, the pattern of EGFR-mediated changes in Capicua localization is expanded in D-cbl and Gap1 mutant clones along both the AP and DV axes. This AP expansion is therefore inconsistent with the observed decrease in dorsal fates.

Program/Abstract # 308

The Tbox-20 transcription factors Midline and H15 function as localized negative regulators of epidermal growth factor receptor signaling output

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We use the *Drosophila* ovarian follicular epithelium to study signaling pathways and patterning during development. This epithelium secretes the egg shell, including the two dorsal appendages derived from the dorsal anterior primordia. These primordia can be recognized through the high expression of the Broad-Complex (Broad) transcription factor, and are separated by a region of cells at the dorsal midline that do not express Broad. Outside of this region, all follicle cells express basal Broad. Dorsally-localized EGF signalling coming from the underlying oocyte initiates this spatial pattern along the dorsal-ventral axis, but the signals that pattern the anterior-posterior (AP) axis are less well understood. To better understand AP patterning, we characterized a new locus called F27 required for this process. In F27 mutant epithelia, the posterior limit of the high Broad expression domain is extended, suggesting that dorsal anterior fates have been determined more posteriorly. We also show that wild type posterior follicle cells are refractory to ectopic EGFR activity, but that F27 mutant epithelia respond to ectopic activation of the EGFR signalling cascade by adopting dorsal anterior fates. We mapped the F27 mutation to the midline (mid) gene, which encodes a Tbox transcription factor. Mid is expressed in the posterior follicular epithelium, and ectopic expression of Mid in the dorsal anterior domain represses dorsal fates. We found that the Mid paralog H15 participates in this process as well. Our data show that these factors regulate posterior fate in the follicular epithelium, and are acting as novel negative regulators of EGFR signalling output.

Program/Abstract # 309

Cdx1 and Cdx2 have context dependent functional specificity in the intestine

Grainger, Stephanie L.; Hryniuk, Alexa; Lohnes, David,University of Ottawa, Ottawa, Canada)

The caudal related homeodomain transcription factors Cdx1 and Cdx2 are expressed in the developing endoderm, persisting into adulthood. Cdx1-/-mutants are viable and fertile and display no overt phenotype in the intestine, while conditional mutation methods have revealed that Cdx2 is required for patterning the intestinal epithelium and specification of the colon. During adult homeostasis of the intestinal tract, Cdx1 appears to functionally overlap with Cdx2 in the more distal regions of the intestinal tract, as Cdx1/2 double mutants have a stronger anterior transformation of the distal colon than Cdx2 mutants; however, Cdx1 seems to be dispensable during small intestinal patterning and differentiation. Cdx1 and Cdx2 are differentially expressed in the intestinal epithelium. While both are expressed in a posterior-high gradient,

Cdx1 peaks in the distal colon and Cdx2 in the proximal cecum. Moreover, Cdx1 expression is graded along the crypt-villus axis, with more abundant protein found in the crypts, which house stem cells, and diminishing towards the differentiated cells in the villi. Cdx2 is expressed uniformly along this crypt-villusaxis, but is differentially phosphorylated. The functional consequences and mechanisms of regulation of these different expression domains of the Cdx proteins remain unknown. Herein, we demonstrate that Cdx2 driven by Cdx1 regulatory elements cannot functionally compensate for loss of endogenous Cdx2. Furthermore, we show evidence that Cdx2 cannot functionally compensate for Cdx1 in the context ofintestinal homeostasis in the distal colon. This study provides novel in vivo evidence that Cdx1 and Cdx2 have context dependent functional specificity.

Program/Abstract # 310
Cdx and FGF interactions establish a molecular switch for posterior nervous system specification
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During development, neural cells can respond to the same signaling factors in remarkably different ways depending on their molecular context or competence. What determines a cell's competence and its specific response to signaling inputs remains unknown. We have investigated issues of neural competence in a simple binary-decision system: the specification of caudal neural plate cells to hindbrain or spinal cord fates. Hindbrain and spinal cord specification depend on the signaling activity of FGFs and the caudally restricted transcriptional activity of Cdx. We have examined how molecular context, presence or absence of Cdx, allows prospective hindbrain cells (Cdx negative) and prospective spinal cord cells (Cdx positive) to respond in distinctive ways to FGF signaling by activating particular developmental programs in a cell-specific fashion. Here we show that a network of FGF and Cdx interactions specify and pattern the spinal cord. Using loss and gain of function strategies, we show that induction of spinal cord fates requires Cdx independently of FGF. However, subsequent maintenance of spinalcord fates by Cdx requires FGF activity. Similarly, induction of patterning genes (hox) expression only requires Cdx, but their maintenance requires Cdx and FGF activities. The maintenance of spinal cord specification and patterning information involves the maintenance of Cdx auto-regulation by FGF. We propose that Cdx and FGF interactions establish a molecular switch that stabilizes the induction of spinal cord fates and promotes the patterning of posterior nervous system.

Program/Abstract # 311
Interneuron specification in zebrafish spinal cord
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Most of the neurons in the vertebrate Central Nervous System (CNS) are interneurons and interneurons function in almost all neuronal circuits and behaviors. However, we still know very little about how specific interneurons with particular physiological and functional characteristics develop and form functional neuronal circuitry. All of the evidence so far suggests that the properties of distinct spinal cord interneurons are determined by the transcription factors that the cells express as they become post-mitotic and start to differentiate. However, in many cases, it is still unclear which transcription factors specify particular interneuron properties. We also do not know the full complement of transcription factor sexpressed by any population of spinal interneurons in any vertebrate. To address these critical gaps in our knowledge we are using and developing several techniques. For example, we are Fluorescence Activated Cell-Sorting (FACS) and expression profiling specific spinal cord interneuron populations using transgenic zebrafish lines where these cell types are labeled with fluorescent proteins. This enables us to identify all of the transcriptionfactors expressed by these different interneuron populations. Furthermore, we are comparing and contrasting expression profiles of interneurons with similar and different axon trajectories and/or neurotransmitter phenotypes to identify transcriptions factors that are good candidates for specifying these functional properties. We will then test the functions of these transcription factors using mutant lines, morpholino oligonucleotides, and dominant-negative constructs. One interneuron population that we are particularly interested in is V0v spinalcord interneurons. These interneurons are located in the ventral spinal cord and are part of the CNS circuitry that controls locomotion. They can be identified by their expression of the transcription factor Evx1, which, within the spinal cord, is uniquely expressed by these interneurons. We are currently identifying all of the transcription factors expressed by V0v interneurons and inparticular we are examining the functions of different transcription factors inspecifying V0v axonal and neurotransmitter phenotypes.

Program/Abstract # 312
Specific requirement of floor plate Shh in spinal cord development
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Sonic Hedgehog (Shh) is a secreted protein that plays a critical role in ventral CNS development. In the spinal cord, there are two tissue sources of Shh: the notochord (ShhNOTO) and floor plate (ShhFP). Prior studies showed a critical role for Shh in the patterned expression of bHLH and HD proteins; however, the requirement of each source has not been clearly defined. To address this, we employed cre-loxp mediated recombination to selectively inactivate ShhFP while preserving ShhNOTO (ShhΔFP). Using double labeled ShhΔFP/ΔFP mutant tissue at E10.5, we found fewer Nkx2.2+ and Olig2+ cells marking the p3 and pMN domains, respectively, but progenitor domain patterns remain largely intact. At E11 during the transition from neurogenesis to gliogenesis, the notochord is initially in contact with the neural tube but then separates, leaving ShhFP as the only intrinsic source. Strikingly, ShhΔFP/ΔFP mutants at E12.5 show a complete loss of Olig2+ cells. We examined the consequences of this on oligodendrocyte (OL) development at E15.5 and E18.5 and found a decrease in both OL precursor cells (OPC) and OL marker protein. Shh controls target gene expression by promoting Gli activators and inhibiting Gli repressor functions. To address the genetic mechanism of how ShhFP controls OPC protein marker expression, we generated mice mutant to both ShhFP and Gli3, the sole Gli repressor. Significantly, double mutants show a partial recovery of Olig2+ OPCs, showing that ShhFP is specifically required to suppress the formation of Gli3 repressors and allow Olig2 expression. Taken together, our results demonstrate a specific requirement of ShhFP for OL specification and differentiation.

Program/Abstract # 313
PTCH1, PTCH2, and HHIP1 feedback antagonism is required for Hedgehog-dependent vertebrate neural patterning
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Hedgehog (HH) signaling plays critical roles in both invertebrate and vertebrate embryogenesis. Sonic Hedgehog (SHH) ligand specifies distinct ventral neuronal identities within the developing vertebrate neural tube. Precise mechanisms exist to limit the range of HH signaling during development, including feedback upregulation of the HH receptor, PTCH1, and an additional HH-binding protein, HHIP1. While feedback antagonism of PTCH1 or HHIP1 alone is dispensable for normal neural patterning, combined loss of PTCH1 and HHIP1 inhibition produces a dramatic expansion of the HH-responsive domain. This suggests that restraining the HH response during vertebrate embryogenesis is governed by overlapping activities of multiple cell surface HH pathway antagonists. To determine whether additional cell surface antagonists control HH signaling during ventral neural patterning, we have examined the vertebrate-specific protein, PTCH2. The contribution of PTCH2 to HH pathway antagonism during ventral neural patterning is unknown. Using cell-based functional assays and chick in ovo neural tube electroporations, we show that PTCH2 can function as a HH-pathway antagonist. Intriguingly, PTCH2 localizes to the primary cilium, an organelle required for HH pathway function. Although neural patterning is normal in the absence of PTCH2, combined loss of PTCH2 and PTCH1-feedback upregulation increases the range of HH signaling in the developing mouse neural tube. Strikingly, combined loss of PTCH1, PTCH2, and HHIP1 feedback antagonism results in complete ventralization of the neural tube, revealing an essential role for these vital antagonists during vertebrate embryogenesis.

Program/Abstract # 314
Endodermal requirement for Prdm1 in mouse craniofacial development
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Cranial neural crest cells (CNCCs) populate the pharyngeal arches (PAs) during development and subsequent interactions with the endoderm and ectoderm of the PAs is critical for formation of the adult craniofacial skeleton. Prdm1 (Blimp1) is a zinc finger containing transcription factor with a known role in zebrafish neural crest and craniofacial development. Loss of prdm1a in Danio rerio leads to a loss of posterior arch derived ceratobranchials 2-5 and functions downstream of FGF and RA signaling. To address tissue specific requirements of Prdm1 in mammalian craniofacial development, we are utilizing tissue specific Cre- mediated recombination to knockout Prmd1 in the mouse model system. Unlike in zebrafish where prdm1ais expressed in all tissues of the arches, mouse Prdm1 is expressed in the pharyngeal endoderm and ectoderm and Prdm1fl/fl; Foxa2-Cre endoderm deletion leads to morphological defects in the craniofacial region. Gene expression analysis at E10.5 shows increased Fgf8 mRNA expression in the cleft between mandibular and maxillary arch 1, along with decreased expression of Nkx3.2 and Eya, suggesting a defect in the formation of the temporal mandibular joint (TMJ). In the posterior arches, we observe decreased Eya1 and Tbx1 throughout the arches and we hypothesize that Prdm1 expression in the endoderm is necessary for appropriate patterning of CNCCs and their derivatives. Taken together, our

data suggest that Prdm1 may play arole in modulating endodermal derived signals required for TMJ formation in proximal arch 1 and posterior endodermally derived structures.

Program/Abstract # 315
Reciprocal repression of Six1/Eya1 and Irx1 in the pre-placodal ectoderm, the embryonic precursor of cranial sensory organs
Sullivan, Charles H., Grinnell Col Dept of Biol, Grinnell, United States; Neilson, Karen M.; Moody, Sally A. (George Washington University Medical Center, Washington, DC, United States)

Irx1, an Iroquois family member, plays a key role in regulating expression of neural determination bHLH genes in the neural plate. It also is expressed in the posterior region of the pre-placodal ectoderm (PPE), a precursor of cranial sensory ganglia and the otocyst, but its role in placode neurogenesis has not been explored. We studied the relationship between Irx1 and two other transcription factors (Six1 and Eya1) that are required for placode formation and maintain sensory precursors in an immature state. Over-expression of Six1, either singly or in combination with Eya1, repressed Irx1 expression in the PPE at neurula stages and later in the otocyst. Use of a repressive construct (Six1EnR) demonstrated that Six1 acts as a transcriptional repressor of Irx1. Interestingly, two mutant Six1 constructs that are identical to mutations in the human BO/BOR syndrome, which is characterized by deafness, are as effective in repressing Irx1 as is wild type Six1, indicating that the defect is not at this stage of development. Irx1 expression in the PPE lineage reciprocally reduced Six1 and Eya1, suggesting they need to be down-regulated for neural differentiation to proceed. Increased Irx1 expression also caused a down-regulation in Fgf8 in the anterior neural ridge suggesting a role for this signaling pathway in PPE formation. Because both the PPE and cranial neural crest are derived from a common precursor zone, we tested whether Irx1 down-regulation of Six1/Eya1 expanded the neural crest. Irx1 expanded FoxD3 expression but repressed Sox9 expression in both the premigratory region and branchial arches. Increased Irx1 also repressed the expression of Sox9 in the otic placode and resulted in abnormal otocyst development.

Program/Abstract # 316
Examining the role of *C. elegans* forkhead genes in neuron development
Nelms, Brian, Fisk University, Nashville, United States; Smith, Erica; Ridgeway, Naccolaine (Fisk University, Nashville, TN, United States)

The nematode worm *C. elegans* is an attractive system for studying regulation of neuronal fate specification and maintenance. The number of neurons (limited to just over 300) and transparent body allow for manageable observations and manipulations of individual neurons, while the fundamental molecular biology is comparable to higher organisms including humans. Using *C. elegans* as our model system, we hope to dissect the roles of one set of well-conserved genes, the forkhead genes, many of which play important roles throughout development in invertebrates and vertebrates alike. Among the *C. elegans* forkhead genes we are interested in, fkh-8, fkh-9, and fkh-10 seem to be expressed in dopaminergic neurons. fkh-8 is most closely related to the mammalian FoxJ family, some members of which are also expressed in dopamine-signaling neurons of the brain. We plan to further investigate the neuronal function of the FKH-8, FKH-9, and FKH-10 transcription factors, also examining the potential for functional redundancy among these factors in *C. elegans*, potentially giving insight into the neuronal role of their mammalian counterparts.

Program/Abstract # 317
Foxa genes in the development of the intervertebral disk
Maier, Jennifer; Lo, Yinting; Harfe, Brian, University of Florida, Gainesville, United States

Disk degeneration is a cause of chronic back pain; for which there are few effective treatments. Little is known about the development of the intervertebral disk (IVD); knowledge which could lead to better treatments for degeneration, and associated back pain. The IVD is composed of the gel-like nucleus pulposus (NP), the collagenous annulus fibrosus (AF), and cartilaginous endplates. To study the development of the IVD, we used a Foxa1; Foxa2 knockout mouse. Foxa genes are in the forkhead box family of transcription factors and are required for the development of many organs and for post-natal life. They are expressed in the notochord, a structure that becomes the NP. Foxa1 is a null allele, Foxa2 is a floxed conditional under the control of the tamoxifen-inducible ShhcreERT2 allele, allowing us to remove Foxa2 in tissues that Shh is expressed. Giving tamoxifen to dams at E7.5 removed FOXA2 from the notochord by E9.5. Histology of Foxa1-/-; Foxa2c/c; ShhcreERT2/+ embryos showed a severely deformed NP and vertebral abnormalities. Double mutants had aberrant notochord to NP transition, shown by fate-mapping with a LacZ reporter allele. They also had dramatically increased cell death in the tail of the embryo. *In situ* hybridization for notochord, neural tube and sclerotome markers have been analyzed. Hedgehog signaling appears to be disturbed in double mutants, and dorso-ventral patterning of the neural

tube was aberrant. Our data indicate that Foxa1 and Foxa2 are required for the proper formation of the IVD, perhaps by activating the Hedgehog pathway, which is known to be required for IVD development.

Program/Abstract # 318

Irx3 and Irx5 homeobox genes link the anteroposterior and proximodistal axes prior to hindlimb formation

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Development of organs and appendages requires coordination of pattern formation and growth in three dimensions. The anteroposterior (AP) and proximodistal (PD) axes of the embryonic limb are linked when sonic hedgehog (Shh) from the zone of polarizing activity (ZPA) and fibroblast growth factors (Fgfs) from the apical ectodermal ridge (AER) form a positive feedback loop in the developing limb bud. It is unknown whether the AP and PD axes are coordinated before the feedback loop formation. Here we show that both axes are regulated by Iroquois homeobox (Irx) genes Irx3 and Irx5 prior to the establishment of the ZPA and the AER. Analysis of Irx3/5 double knockout (DKO) mutants suggested that Irx3/5 are required early for the formation of Shh-independent elements during hindlimb development. Marker analysis and qRT-PCR data suggested that Irx3/5 promote Gli3 expression in the initiating hindlimb bud to prepattern the AP axis and restrict ZPA-Shh activation posteriorly. Using conditional knockout mutants, we demonstrate that the early function of Irx3/5 in the hindlimb field is essential to specify distal structures. Interestingly, Irx3/5 genetically interact with Gli3 to promote expression of the PD outgrowth signal Fgf8, further indicating their function in limb development along the PD axis. Therefore, AP and PD limb axes are coordinated by these transcription factors prior to establishment of signalling centres.

Program/Abstract # 319

Hyaluronic Acid Synthase 2 expression in the limb mesenchyme is regulated by Shh and plays an essential role in joint pattern formation

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Sonic hedgehog (Shh) signal generated from the zone of polarizing activity plays an essential role in growth and patterning of the autopods. However, major gaps remain in understanding the molecular mechanism by which Shh activity regulates limb development. Through analysis of early limb bud transcriptome, we identified a posteriorly-enriched gene, Hyaluronan Acid Synthase 2 (Has2), as a potential effector of Shh signaling during early mouse limb development. BothRNA in situ hybridization and chromatin immunoprecipitation experiments indicate that Has2 is transcriptionally regulated by Gli3 transcription factor. After analyzing Has2 conditional mutant (Has2cko) mice, we found that Has2 itself is dispensable for A-P digit patterning. However, Has2cko mice displayed a profound phalange patterning defect in which joints have been shifted perpendicularly. Associated with the joint patterning defect is misexpression of joint markers such as Gdf5, Wnt4, Chrd. Further analysis indicates that p-Erk1/2 and cleaved-caspase 3 are ectopically distributed in the center of mutant digits, suggesting that Has2 may prevent p-Erk-induced apoptosis in condensed chondrocytes. Our results reveal Has2 as a downstream target of Shh signaling and an essential regulator in early joint patterning.

Program/Abstract # 320

Characterizing the role of Pitx1, Tbx4 and Tbx5 genes in regulation of limb growth, patterning and identity

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The genetic programs involved in limb development direct the growth and patterning of complex and heterogeneous anatomical structures. While a conserved, generic limb development program underpins the development of all limbs, recent research regarding specification of limb identity has focused on three transcription factor genes with limb-specific expression patterns: Pitx1, Tbx4 and Tbx5. Tbx5 is expressed in forelimbs (FL), while Tbx4 and Pitx1 are expressed in hindlimbs (HL). Tbx5 is necessary for FL bud growth, as Tbx5 -/- mice fail to develop FL at all. Pitx1, meanwhile, has a profound role in HL development: Pitx1 -/- mice develop HL with FL-like anatomical features. Pitx1 expression also partly controls Tbx4 expression: gain-of-function experiments in mouse show that Tbx4 has a more ambiguous role than Tbx5 or Pitx1, directing both growth and patterning. Tbx4 can replace Tbx5 in FL as a growth promoter, rescuing growth in the Tbx5 -/- mutant FL without affecting patterning. Expressing Tbx4 in the Pitx1 -/- mouse HL, however, rescues several HL patterning characteristics, an effect that cannot be replicated by Tbx5. This ambiguous activity has been attributed to the fact that, while Tbx4 and Tbx5 share a transcriptional activation domain that may control growth, Tbx4 has aC-terminal repressor domain that is not present in Tbx5. We are currently analyzing the targets of each of these transcription factors

using expression profiling (RNA-Seq) and ChIP-Seq. These analyses will allow us to assess the degree of divergence between Tbx5, Tbx4 and Pitx1-controlled gene regulatory networks, ultimately defining the gene regulatory networks responsible for growth vs. patterning.

Program/Abstract # 321
Identification of Pitx2c N-terminal domain interacting proteins
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Establishment of asymmetric organogenesis requires organization of left-right axis formation during embryogenesis. One of the factors essential for this process is a bicoid-related homeodomain transcription factor, Pitx2c. Pitx2c is asymmetrically expressed in the embryo on the left side of the lateral plate mesoderm and continues to be expressed on the left side of future asymmetric organs such as the heart and gut. Previous work using loss of function and gain of function of Pitx2c showed that it has an important function in left-right patterning and that the N-terminal domain of Pitx2c is critical for interactions responsible for this function. To better understand the role of the Pitx2c N-terminus, we are identifying its interaction partners. Two yeast-two hybrid library screens using the chick Pitx2c N-terminus against a mouse adult cDNA library and the mouse Pitx2c N-terminus against a mouse E11 cDNA library were performed. Thirty-six candidate interaction partners were identified two or more times including proteins required for ubiquitination and translation. From the resulting proteins, we are confirming both their interaction with Pitx2cN using a yeast two-hybrid swap-vector approach and their co-expression with Pitx2cN by in situ hybridization and immunohistochemistry. Further functional analysis will be performed for candidates that meet both criteria.

Program/Abstract # 322
Dynamic CREB activity coordinates the formation and patterning of mammalian somites
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The segmented body plan of vertebrates is pre-figured by a developmental process known as somitogenesis. Somites form rhythmically from the presomitic mesoderm (PSM), which is temporally linked to a “Segmentation clock”; a molecular oscillator that triggers waves of Notch1 activity (NICD1) throughout the PSM. Once the clock crosses a determination front (Wavefront) set up by the FGF/Wnt morphogen gradients, the clock synchronizes a group of competent PSM cells to bud off and form a somite. Much remains to be understood of how the clock and wavefront are interlinked. We have serendipitously found that the transcription factor CREB plays a novel rolein mammalian somitogenesis. We show that activated CREB cycles in unison with NICD1 in the anterior PSM. Further, the oscillatory activities of CREB are governed by Wnt3a signaling. To directly test CREB function in somitogenesis, we employed a gene inactivation strategy to express a dominant negative agent to CREB in the mouse PSM. In these mutants, progression of somitogenesis is delayed and somites that do form are abnormal and fused, leading to skeleta dysplasia in neonates. To decipher the downstream mediators of CREB, we performed transcriptional profiling comparing control versus mutant PSM and identified differentially expressed genes. Surprisingly, several of them are linked to the Wnt and Notch signaling pathways. For example, Dll-1, a Notch ligand, display reduced expression and contains a putative CREB binding site in its promoter. We are currently testing the *in vivo* occupancy of CREB at this site. We propose that CREB function is regulated by the Wnt3a wavefront and in turn CREBcontrols the Notch1 clock to direct somite patterning.

Program/Abstract # 323
A role for long-chain polyunsaturated fatty acid metabolism in zebrafish dorsoventral patterning and BMP receptor-regulated Smad activity
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In recent years it has become apparent that a better understanding of the cell biology of lipids is necessary to explain many aspects of both development and physiology. Here we show that a fatty acid activating enzyme, long-chain acyl CoA synthetase 4a (Acsl4a), is essential for proper patterning of the zebrafish dorsoventral axis. While there are numerous vertebrate long-chain acyl-CoA synthetase (ACSL) enzymes, ACSL4 is unique in that it has a strong preference for long-chain polyunsaturated fatty acids (LC-PUFA). Embryos are dorsalized following Acsl4a disruption, whereas overexpression of Acsl4a results in ventralization. A gradient of bone morphogenetic protein (BMP) plays a central role in establishing the dorsoventral axis. Using epistasis analysis, we discovered that Acsl4a’s effect on dorsoventral patterning is independent of extracellular BMP ligands or BMP receptor activity. BMP receptor-regulated Smad transcription factors can be negatively regulated by MAPK phosphorylation on a central linker region, which promotes the ubiquitination and

degradation of Smad proteins. We show that overexpression of Smads lacking target residues for MAPK phosphorylation completely rescues the dorsalized phenotype of Acsl4a depletion. Our results reveal a critical role for LC-PUFA metabolism in BMP receptor-regulated Smad activity and, as a consequence, dorsoventral patterning. This is the first report linking LC-PUFA metabolism and BMP signal regulation. BMP receptor-regulated Smads also play numerous key roles in embryonic development and bone homeostasis and our findings may reveal ways that LC-PUFAs influence these processes.

Program/Abstract # 324
Foxh1-Groucho transcriptional switching and spatiotemporal regulation of Nodal expression during early embryonic development
Halstead, Angela M.; Wright, Chris, Vanderbilt University, Nashville, United States

Correct patterning during embryogenesis is greatly dependent upon proper regulation of the TGFβ-related gene Nodal, which encodes a ligand essential for germ layer formation (mesendoderm induction) and establishment of left-right (L-R) asymmetry in all vertebrates. The regulation of Nodal during these processes has been rigorously studied since its discovery, but how Nodal and other members of a "Nodal gene cassette" (Nodal, inducer; Lefty, feedback antagonist; and Pitx2, effector) are dynamically regulated at the transcriptional level remains unclear. It has recently been proposed that in *Xenopus laevis* the forkhead box protein Foxh1 functions as a transcriptional switch, positively or negatively regulating Nodal transcription via interactions with the signal transducer phospho-Smad2 (pSmad2), or the co-repressor Groucho 4 (Grg4), respectively. Much is known on how Foxh1/pSmad2 interactions affect Nodal transcription, but little is known about the Foxh1/Grg4 interaction in repressing Nodal signaling, and whether or not this transcriptional switch mechanism is conserved in higher vertebrates. We are deriving a mouse line in which this interaction is disrupted specifically by a single amino acid alteration in the Grg4 binding domain of Foxh1. Defects resulting from the disruption will be examined during gastrulation and L-R asymmetry specification to gain insight into how this interaction affects Nodal signaling and the cell and tissue responses that are associated with differentiation or morphogenesis. I will also present ideas on how the new mouse lines will allow investigation of occupancy and epigenetic effects at Nodal and other target gene loci.

Program/Abstract # 325
A sub-circuit of the sea urchin GRN integrates spatial information to pattern the embryonic skeleton
McIntyre, Daniel C.; McClay, David, Duke University, Durham, United States

The Gene Regulatory Network (GRN) driving the first 30 hours of development in the sea urchin effectively explains how most tissues in the embryo are specified. Yet the current GRN is not sufficient to explain the complex morphology of the developing embryo. In particular, pattering of the embryonic skeleton is not explained, even though the GRN describing how the mesenchyme cells which produce it are specified is extremely well understood. It is known that signals including VEGF and FGF, coming from a restricted set of ectodermal cells communicate this patterning information to the mesenchyme cells. However, these signals are final messengers, the end result of a process which identifies the site of skeleton formation from a thin band of ectodermal cells neighboring the endoderm- the border ectoderm (BE). To do this, the cells in the BE must utilize a GRN capable of integrating several spatial inputs in order to locate and refine the expression patterns of those signals. In this report we demonstrate the existence of a unique sub-circuit of the ectoderm GRN which is activated by endodermal Wnt signaling. This sub-circuit then uses positional information provided by TGFB signaling along the secondary embryonic axis to define the expression of a series of transcription factors that together limit signaling molecule expression in the ectoderm. Ongoing research using mathematical modeling will show how the negative regulatory logic used by this network is capable of creating the sharp and limited expression of Vegf observed in the embryo. This research shows how a complex3-dimensional structure is patterned during embryogenesis and may provide aparadigm for understanding other, more complex, developmental problems.

Program/Abstract # 326
Gata3 regulates branching morphogenesis and differentiation of the developing prostate.
Nguyen, Alana, McGill University, Montreal, Canada; Beland, Melanie (University of Quebec at Montreal, Montreal, Canada); Bouchard, Maxime (McGill University, Montreal, Canada)

Organ development involves the precise spatial-temporal expression of regulatory transcriptional programs, which are necessary for cell fate specification and tissue morphogenesis. Early in prostate development, we showed that deletion of Gata3 in theurogenital sinus epithelium leads to budding defects associated with a reduction in basal-specific p63 transcription factor expression. To bypass the embryonic lethality of Gata3 deletion, we used Nkx3-1Cre to mediate excision of the floxed Gata3 locus, showing that Gata3 is necessary for branching morphogenesis. The defect in branching is coupled with an increase in cell division accompanied by atypical prostatic hyperplasia. We showed that these

hyperplastic lesions lose stratification of the luminal and basal layers and increase in CK5+/CK8+/CK18+ cell population. Interestingly, the lack of stratification is coupled with a loss of apical-basolateral polarity, which leads to formation of ectopic microlumens. Together, these results demonstrate that Gata3 controls a transcriptional program in the prostate buds important for lineage specification and stratification of the prostate epithelium.

Program/Abstract # 327
Investigating the role of the transcription factor Gata3 during post-natal prostate development
Shafer, Maxwell, McGill University, Montreal, Canada; Nguyen, Alana; Bouchard, Maxime (McGill University, Montreal, PQ, Canada)
The prostate develops at the base of the bladder in the mass of tissue known as the urogenital sinus (UGS). Epithelial cells branch from the UGS and form morphologically distinct ducts composed of differentiated basal, luminal and neuroendocrine cell types. This process, and the concomitant differentiation of the luminal and basal cells of the prostate, may be mediated by controlled cell division and differentiation during both organogenesis and the development of prostate cancer. The aim of this project is to investigate the role and targets of Gata3 during prostate development, and in particular, its function in prostate stem cell homeostasis and cellular differentiation. Preliminary and current results obtained in the laboratory have established that the loss of Gata3 in early prostate development leads to prostate dysplasia, cellular hyperplasia of the basal and luminal compartments, and defects in cell lineage specification. On the basis of these results, we hypothesize that Gata3 regulates key effectors of stem/progenitor cell homeostasis, division and differentiation. Standard molecular and biochemical techniques such as immunocytochemistry, in situ hybridization and confocal microscopy will be used to investigate the expression and function of Gata3 and its downstream target genes in the first two weeks of post-natal prostate development in the mouse. We will take advantage of the Cre-lox technology, which allows prostate specific gene knockouts, to modify the expression of Gata3 and its target genes during development in prostate tissue. Tissue transplantation will be used to introduce genetically modified prostate stem cells (gene knock outs) into wildtype mice to investigate the function of Gata3 and its targets in the development of prostate tissue. This research may lead to the discovery of therapeutic targets for the treatment and/or prevention of prostate cancer and contribute to our understanding of the embryonic development of an important regulatory organ.

Program/Abstract # 328
Novel shadow enhancers regulate HoxB gene expression during heart and gut development
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During vertebrate development, the heart is one of the first functioning organs to form. In bird and mammal embryos it develops from two distinct mesodermal populations defined as the first and second heart field (FHF and SHF, respectively). The left ventricle is derived from the FHF while the right ventricle and outflow tract are derived from the SHF. Work in both invertebrates and vertebrates has defined many of the essential regulators for cardiac specification and differentiation, however the genes and the coordination of the signaling pathways that orchestrate the patterning within these fields are poorly understood. Recently, members of the HoxA and HoxB clusters have been shown to be differentially expressed and important in patterning the murine heart. However the mechanisms that regulate these Hox activities are unknown. In regulatory analyses of the HoxB cluster we have identified an enhancer region that directs dynamic expression in the SHF in addition to expression throughout the adjacent endoderm. In light of the key role of retinoic acid (RA) in heart development, we explored links between this region and retinoid signaling. The enhancer displays a robust response to exogenous RA and we identified a retinoic acid response element (RARE) in the region. Deletion analysis of the enhancer suggests that it integrates multifactorial inputs that may involve members of the GATA transcription family in addition to the retinoid receptors. In a BAC transgenic assay, the region appears to function as a shadow enhancer to control elements present near the Hoxb1 gene to ensure correct expression of HoxB genes during cardiac development.

Program/Abstract # 329
The role of Hoxa3 in the developing 3rd pharyngeal pouch endoderm and its derivatives, early and late
Chojnowski, Jena L.;Masuda, Kyoko; Trau, Heidi; Manley, Nancy, University of Georgia, Athens, United States
Homeobox (Hox) genes encode an evolutionarily conserved family of transcription factors involved in numerous developmental pathways. Mouse Hoxa3 is expressed in the pharyngeal region, including the endoderm and the neural crest derived mesenchymal cells (NCCs). It is required for the development of the third and fourth pharyngeal pouch-derived organs, including the thymus and the parathyroids. Global deletion of Hoxa3 early in development, before pharyngeal pouch development, prevents both thymus and parathyroid initial organogenesis. The loss of Hoxa3 in the NCCs prevents proper migration of the thymus and parathyroid by blocking their separation from the pharynx and each other but it has no

strong affects on organogenesis. To determine the role of Hoxa3 specifically in the 3rd pharyngeal pouch endoderm and its derivatives, we used tissue-specific recombinase transgenes to knock out Hoxa3 in the endoderm, and the developing thymus and parathyroid. The loss of Hoxa3 in the endoderm before the separation of the primordium leads to a smaller, ectopic thymus and the loss of the parathyroids by E18.5. Neither the endodermal knockout nor the NCC deletion phenotype recapitulates the global Hoxa3 loss which leads to the conclusion that Hoxa3 expression in either tissue is sufficient for the correct specification of the 3rd pouch endodermal derivatives. Also, Hoxa3 has a limited role in thymus and parathyroid development after their respective genetic developmental pathways have been turned on. Hoxa3's major contribution to the 3rd pouch endodermal derivatives occurs earlier in development in a variety of tissues (at least the NCCs and the endoderm) before the shared primordium begins its dichotomous journey.

Program/Abstract # 330

The E protein E2a/TCF3 plays an essential role in Nodal signaling transduction

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The requirement for Nodal signaling in establishing mesendodermal cell fates is deeply conserved. We have undertaken a ChIP-Seq approach in human embryonicstem cells and *Xenopus tropicalis* embryos to identify genes and sequence motifs targeted by the Nodal signaling transcription factors Smad2, Smad3, Smad4, and FoxH1. We found that in addition to canonical Smad and FoxH1 motifs, these transcription factors also bind at high frequency to a novel motif that we have termed Smad Complex-Associated (SCA). The SCA motif is bound efficiently by the E proteins E2a/TCF3 and Heb/TCF12, which co-immuno precipitate FoxH1 andSmad2. For this reason, we investigated whether these E proteins might play a role in Nodal signaling invivo. In E2a-depleted *Xenopus tropicalis* embryos, early mesendoderm genes are down regulated and gastrulation movements fail to occur. E2a-depleted embryos are also unable to respond to ectopic Nodal signals. However, E2a-depleted embryos also express increased levels of the extracellular Nodal antagonist lefty, which may account for some of the E2a morphant effect. We therefore conclude that E2a is required for mesendoderm formation and gastrulation in *Xenopus tropicalis*, and are continuing to investigate the mechanism underlying E2a modulation of Nodal signaling at the levels of both transcription factor interaction and regulation of lefty expression.

Program/Abstract # 331

A functional assay for paternal genome activation during early *Arabidopsis* embryogenesis

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In animals, early embryo development is primarily controlled by maternally deposited RNAs and proteins. The point at which control of development is transferred to the zygotic genome is called the Maternal to Zygotic Transition (MZT). Whether delayed genome activation also occurs in plant embryogenesis is controversial: recent studies profiling transcriptional activation of the paternal genome during early *Arabidopsis* embryogenesis have argued for and against immediate activation for the majority of genes. We have taken a purely functional approach to study activation of the paternal genome during the first few days of embryogenesis in *Arabidopsis*. Our approach does not determine transcriptional activation per se. Instead, we examine the ability of wt paternal alleles to complement maternally inherited embryo defective alleles with mutant phenotypes expressed before the globular stage, when large scalepaternal genome activation has been proposed to occur. In our analysis thus far, we have observed that wt alleles for the majority of genes tested cannot immediately complement maternal mutant alleles, i.e. mutant phenotypes are observed during the first few days of embryogenesis, after which the percentageof mutant phenotypes decreases to almost zero. However, for a significant proportion of embryo defective mutants, wehave observed that the wt paternal allele can immediately complement a mutant maternal allele. Our results suggest a general trend where paternal genome activation during early embryogenesis is gradual, with significant exceptions. Thus, each gene must be evaluated on an individual basis.

Program/Abstract # 332

Effect of high glucose concentration on the expression of matrix metalloproteinase 9 and its inhibitor TIMP-1 during blastocyst development in vitro.

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The extracellular matrix metalloproteinase 9 (MMP9) is expressed in mouse primary trophoblast *in vivo* and *in vitro* and is secreted by the mouse blastocyst during implantation and has been involved in the invasion of endometrium. High concentrations of glucose induce chemical changes of functional macromolecules (oxidation, formation of AGEs), inducing oxidative stress and metabolic alterations that lead to changes in gene expression. Exposure to high

concentrations of glucose produced in different cell types changes in the synthesis and degradation of the ECM, because it induces the formation of reactive oxygen species (ROS) and advanced glycation end products (AGEs) that alter the expression of matrix metalloproteinases, therefore the effect of glucose on the expression of MMP-9 and TIMP-1 in cultured blastocysts were evaluated and compared with the action of H2O2. Gestation day fourth blastocysts were cultured in HAM-F10 and glucose 25 mM, orH2O2 10 μM were added in different schedules, glucose 6 mM was used as a control. mRNAs of Mmp9 and Timp1 were measured using real time RT-PCR, MMP9 levels were analyzed by zymography in SDS-PAGE-gels co-polymerized with gelatin. Both, glucose 25 mM or H2O210 μM induce higher levels of MMP9 protein and its mRNA, together with 85% lower concentration of Timp1 mRNA. In presence of high glucose or H2O2 aggregates of biggest giant trophoblast cells were observed. High glucose favored the development of trophoblast giant cells, which invasiveness could be increased, since higher expression of MMP9, and lower expression of its inhibitor were observed. The increased expression of MMP9 may be due to oxidative stress caused by glucose. Supported by PAPIT, DGAPA, UNAM, grant IN230611.

Program/Abstract # 333

Effect of high glucose concentration on reactive oxygen species and the expression of urokinase plasminogen activator and its inhibitor PAI-1 in cultured mouse blastocyst.

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During embryo implantation, the blastocyst penetrates the uterine wall by an invasive process, involving proteases that degrade the extracellular matrix (ECM), including urokinase plasminogen activator (PLAU), that catalyzes the formation of plasmin, starting a proteolytic cascade that contributes to the breakdown of ECM, this enzyme is regulated by the specific plasminogen activator inhibitor type 1(PAI-1). High concentrations of glucose induce chemical changes of functional macromolecules (oxidation, formation of AGEs), inducing oxidative stress andmetabolic alterations that lead to changes in gene expression. Reactive oxygenspecies (ROS) may regulate the activity of transcription factors such as nuclear factor κβ (NF-κβ). Promoter region of PLAU contains an NF-κβ binding element, which is sensitive to redox changes, therefore the effect of glucose on ROS levels and expression of PLAU and PAI-1 in cultured blastocysts were evaluated and compared with the action of H2O2. Gestation day fourth blastocysts were cultured in HAM-F10 and glucose 25 mM, or H2O210 μM were added indifferent schedules, glucose 6 mM was used as a control, ROS, Plauand Pai1 mRNA and PLAU activity were evaluated using 2'-7'-dichloro dihydroflurescein, real time RT-PCR and an amidolyticassay. Glucose 25 mM induces an increase of ROS, higher levels of Plau and Pai1 mRNA, higher activity of PLAU and groups of biggest giant trophoblast cells. H2O2 induce similar changes, except that, Pai1 mRNA was decreased. ROSS induced by high glucose favored formation of giant cells and PLAU expression, but the induction of PAI1 by high glucose can be dependent on another mechanism, probably mediated by AGEs. Supported by PAPIT, DGAPA, UNAM, grant IN230611.

Program/Abstract # 334

The role of Lkb1 in the control of cell polarity and epithelial morphogenesis in the pre-implantation mouse embryo

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The establishment of cell polarity and the orchestration of epithelial morphogenesis are critical processes in the formation of the first lineages of the mouse embryo: epiblast (EPI), primitive endoderm (PE) and trophectoderm (TE). Lkb1, a serine-threonine kinase and tumor suppressor, is involved in the establishmentof cell and epithelial polarity from worms to humans. Whereas null zygotic Lkb1 mutants die at E9.0 with no obvious polarity defects, we hypothesize that the maternal supply of Lkb1 in the oocytehas masked important roles it plays in the pre-implantation embryo. We generated maternal-zygotic (MZ) Lkb1 mutants by conditional excision with an oocyte-specific Cre line (Zp3-Cre), and analyzed cell morphology, polarity and lineage in peri-implantation embryos. Time-lapse imaging showed numerous extruded cells from the different cell layers of MZLkb1 embryos, which continued to divide, often formed trophoblast-like vesicles, and occasionally migrated away from the embryo. Cell lineage markers confirmed that these cells could have TE, PE orEPI identities. Furthermore, cell polarity markers showed that the extruded cells were not correctly polarized. Besides extruded cells, we also observed some ICM cells occasionally nestled in the Cdx2-positive TE epithelium, and increased spreading and flattening of the PE epithelium lining the blastocoel. Significantly, we found that embryos lacking maternal Lkb1, but having paternal Lkb1, exhibited similar phenotypes but to a lesser frequency and severity. Together, these results suggest

that maternally-supplied Lkb1 is important for the maintenance of epithelial integrity and correct cell positioning within the pre-implantation embryo.

Program/Abstract # 335
Identification of ectodermal cells during early mouse embryonic development and EpiSC differentiation
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The ectoderm is one of the three germ layers in early mouse embryos. It has the capacity to develop into both epidermis and the central nervous system. Because there are no specific markers for this lineage, and because accessing this layer is difficult in early embryos, the ectoderm is not well understood in mouse embryonic development. Embryonic stem (ES) cells and epiblast stem cells (EpiSCs) are widely used as *in vitro* models to study mechanisms of early embryonic development, but direct evidence for the existence of the ectodermal cells during pluripotent stem cell differentiation is still lacking. In this work, we defined the ectoderm stage during EpiSC monolayer differentiation. Cells at this stage have the potential to differentiate into epidermal cells (with BMP4) or neural cells (without BMP4). Microarray and Q-PCR analysis on these cells showed some specific genes that were highly expressed in these ectodermal cells. We also studied the differentiation potential of cells in the epiblast tissue at different stages (E6.5, E7.0, E7.5) by explant culture in medium with or without BMP4. Using these techniques, we have identified a group of cells in E7.0 mouse embryos that show ectodermal potential. We will compare *in vivo* and *in vitro* data to further identify ectoderm-specific cell-surface markers, and to study the mechanisms involved in commitment of these cells. This work will not only improve our understanding of ectoderm development *in vivo*, but also improve differentiation towards ectodermal tissues for regenerative medicine.

Program/Abstract # 336
Essential roles for Aurora A in mouse embryonic and extraembryonic development
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Aurora A is a mitoticserine/threonine kinase, involved in centrosome maturation, spindle assembly and chromosome segregation during the cell division cycle. Ablation of Aurora A in mice results in mitotic arrest and pre-implantation lethality. Here, we report the effects of Aurora A ablation on embryo patterning at early post-implantation stages by tissue-specific ablation of Aurora A. Conditional knockout of Aurora A in the epiblast or visceral endoderm layers of the conceptus leads to apoptosis and embryo growth inhibition, causing lethality and resorption by E9.5. The effects on embryo patterning depend on the tissue affected by the mutation. Embryos with anepiblast ablation of Aurora A areable to properly establish the anteroposterior axis but do not proceed through the gastrulation. Mutation of Aurora Ain the visceral endoderm lead to defects in the establishment of the anteroposterior axis likely by limiting the shift of anterior visceral endoderm (AVE) to the anterior side of the epiblast. These results show that Aurora A isessential for embryonic and extraembryonic development in mouse postimplantation embryos and suggests that abnormal development of mutant embryos is linked to abnormal growth brought about by a paucity of epiblast orvisceral endoderm cells.

Program/Abstract # 337
Sp5l is a novel transcription factor involved in the development of left-right asymmetry in zebrafish
Inglis, Rachael, University of Cambridge, Cambridge, United Kingdom; Cutty, Stephen; Soong, Daniel (King's College London, London, United Kingdom); Tay, Hwee Goon; Amack, Jeffrey (SUNY Upstate Medical University, Syracuse, NY, United States); Wardle, Fiona (King's College London, London, United Kingdom)

The zebrafish gene sp5l (sp5-like) encodes a zinc finger transcription factor of the Sp1 family. In a chromatin immuno precipitation study, we identified sp5l as a potential regulatory target of Ntla (No tail a), a highly conserved T-box transcription factor homologous to mouse Brachyury. Accordingly, we have found that sp5l expression is misregulated in ntla mutant embryos, where it is lost from the dorsal forerunner cells (DFCs). The DFCs develop in to Kupffer's vesicle, the ciliated organ of asymmetry in zebrafish. Kupffer's vesicle fails to develop normally in ntla mutant embryos, and they consequently exhibit randomised left-right patterning. We performed functional characterisation of sp5l, and have shown that it is also required for the normal development of Kupffer's vesicle and the proper establishment of left-right asymmetry, suggesting that it may mediate the role of ntla in this process. Furthermore, sp5l is expressed in the otic vesicle, another ciliated organ. Knockdown of sp5l expression also results in abnormalotic vesicle cilia, suggesting a role for sp5l in ciliogenesis.

Program/Abstract # 338
Vacuolar Type (H)-ATPase in zebrafish left-right asymmetric development
Gokey, Jason; Amack, Jeffrey, SUNY Upstate Medical University, Syracuse, United States
Left-right (LR) asymmetry is an interesting and complex aspect of the vertebrate body plan. The Vacuolar Type ATPase, or V-ATPase, is a multi-subunit proton pump that maintains organelle and cellular pH by pumping protons into the organelle lumen and out of the cellular cytoplasm. Small molecule screens have implicated the V-ATPase in vertebrate LR development, however the underlying mechanisms remain unclear. We are using zebrafish to characterize the role(s) of the V-ATPase during LR development. To interfere with V-ATPase function, we treated zebrafish embryos with a small molecule inhibitor of the V-ATPase, concanamycin, or antisense morpholinos that reduce expression of aspecific V-ATPase subunit or an accessory protein. Each of these treatments caused LR patterning defects, such as heart looping defects, and disrupted formation of Kupffer's vesicle (KV). Motile cilia that project into the lumenof KV generate an asymmetric fluid flow that is required for normal LR development. Interfering with V-ATPase activity significantly decreased the length and number of these cilia and reduced the size of the KV lumen. KVdefects in morpholino-depleted embryos were corrected by ectopic expression of V-ATPase components. Interestingly, over-expression of a V-ATPase accessory protein also disrupted KV, suggesting tight control of V-ATPase activity is critical for normal KV formation. These results indicate V-ATPase function regulates KV formation, a critical step in LR development. Next, we will elucidate mechanisms by which the V-ATPase regulates KV development and embryo LR asymmetry by examining potential connections between V-ATPase activity and the Notch, FGF and Wnt signaling pathways.

Program/Abstract # 339
The role of the adherens junction protein aN-catenin in cranial ganglia formation
Hooper, Rachel; Taneyhill, Lisa, University of Maryland, College Park, United States
Neural crest cells are atransient, multipotent cell population that arises during neurulation and are crucial to normal vertebrate development. After undergoing anepithelial-to-mesenchymal transition (EMT), these cells migrate to their final destinations in the developing embryo and differentiate into a variety ofstructures throughout the adult body. Importantly, improper neural crest cell development has been implicated in human congenital and hereditary malformations, diseases and cancers, thus making the study of neural crest cells absolutely vital. We have previously shown that αN-catenin, a neuralsubtype of an adherens junction protein that is expressed later in many non-neural crest neuronal derivatives, plays an important role in controllingneural crest cell EMT and migration. Although down-regulation of αN-catenin is critical for initial stages of neural crest cell migration, the potential functional role of αN-catenin in later neural crest cell migration and differentiation is not known. To address this question, we investigated the spatio-temporal distribution of αN-catenin at later stages of chick development and examined effects on neural crest cell movement and contribution to cranial ganglia after αN-catenin perturbation.Our data reveal that αN-catenin is re-expressed by migratory cranial neural crest cells contributing to the trigeminal ganglia. Over expression or knockdown of αN-catenin reduces or expands the migratory neural crest cell domain, respectively, leading to a disruption in trigeminal ganglia formation that may be partially due to effects on placode cells. Collectively, our results revealan important later function for αN-catenin in cranial neural crest cell migration and differentiation.

Program/Abstract # 340
FOXA2 regulates cell behaviors to induce median hinge point in the neural plate
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During neural tube closure, dynamic cell behaviors at specialized regions (hinge points/HP) of the neural plate help fold it into a neural tube. The molecular mechanisms regulating HP formation are poorly understood. We have demonstrated that spatial and cell-cycle dependent temporal modulation of BMP signaling regulates median HP (MHP) formation by interacting with the apicobasal polarity pathway. These interactions stabilize epithelial organization, and thuscyclic BMP attenuation alters neuronal apicobasal polarity sufficiently to give the neural plate the flexibility to roll up and close into a neural tube. However, the question of how the BMP signal itself is dynamically modulated remains unanswered. In this study, we have used *in vivo* gene misexpression, high-resolution imaging and biochemical analyses to identify the winged helix transcription factor FOXA2 as a potential modulator of both BMP signaling and the apicobasal polarity pathway. In addition, we demonstrate that FOXA2 biochemically interacts with another subfamily (TGFß/Nodal) of Transforming Growth Factor ß ligands, known to regulate cell polarity during epithelial to mesenchymal transformation. Interestingly, increased TGFß signaling in the midbrain mimics the effects of canonical BMP blockade and FOXA2 misexpression, and is sufficient to induce MHP formation. We propose that FOXA2 regulates apicobasal cell polarity and MHP formation by dynamically regulating cross-talk between BMP and TGF-β/Nodal signals cascades.

Program/Abstract # 341

Coordination between canonical and non-canonical Wnt signaling patterns the neuroectoderm along the anterior-posterior axis of the sea urchin embryo

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The mechanisms that specify and pattern the neuroectoderm along the anterior-posterior (AP) axis regulate one of the most important events to occur during the early development of deuterostome embryos. However, these mechanisms are incompletely understood. The anterior neuroectoderm (ANE) of the deuterostome sea urchin embryo has regulatory properties and factors that are remarkably similar to those in the early vertebrate ANE (forebrain/eyefield), which is initially patterned along the AP axis by Wnt signaling. We tested the functions of several Wnt pathway members and our results show the early sea urchin embryo integrates information from several different signaling pathways to pattern the neuroectoderm, including the Wnt/β-catenin, Wnt/Fzl5/8-JNK, Fzl1/2/7 and PKC pathways. Together, through the Wnt1 and Wnt8 ligands, these pathways provide precise spatio-temporal control of aposterior-to-anterior wave of re-specification that restricts the initial, ubiquitous, maternally specified ANE regulatory state to the most anterior blastomeres. Moreover, we show that the Wnt receptor antagonist, Dkk1, protects the ANE fate of these cells through a negative feedback mechanism during the later stages of ANE patterning. Our data indicate that these Wnt pathways converge on the same cell fate specification process, suggesting they function as integrated components of a Wnt signaling network. Our findings also provide strong support for the idea that the sea urchin ANE regulatory state and the patterning mechanisms that position and define its borders were present in the common echinoderm/vertebrate ancestor and still operate to specify anterior neural identity in deuterostome embryos.

Program/Abstract # 343

Multiple Wnt signaling phenotypes in Porcupine homolog mutant mouse embryos

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In mammals, the X-chromosomal Porcupine homolog (Porcn) gene is required for the acylation and secretion/function of all Wnt ligands tested to date. Porcn thus represents a bottleneck in the secretion of all 19 mammalian Wnt ligands. We have generated a mouse line carrying a floxed allele for Porcn as a tool to ablate Wnt sources and investigated embryonic requirements for Wnt ligands. Zygotic Porcn mutants fail to gastrulate and phenocopy Wnt3 mutants, indicating a key role for zygotic Wnts in initiating gastrulation. Similarly, maternal-zygotic Porcn mutants display no defects prior to gastrulation, questioning the relevance of Wnt signaling in pre-implantation development. Heterozygous female embryos exhibit parent-of-origin-specific phenotypes due to imprinted X chromosome inactivation in extra-embryonic tissues; maternal allele mutants display chorio-allantoic fusion defects consistent with Wnt7b mutants. In contrast, paternal allele deletion leads to lethality between E11.5 and P3. Rare surviving females present with skin, hair growth and bone defects, similar to human Focal DermalHypoplasia (FDH) patients carrying mutations in PORCN. In summary, we have generated a tool to ablate Wnt ligand secretion, allowing the identification of Wnt sources and functional redundancy of Wnt ligands in the mouse. Based on our studies, Porcn is required for gastrulation, but not during pre-implantation development. Further, Porcn heterozygous females recapitulate phenotypes of Wnt7b and human PORCN mutations, depending on which allele is affected. The Porcn floxed allele will allow for a better understanding of Wnt post-translational modification as well as ligand redundancy in development and disease.

Program/Abstract # 344

Retinoic acid is required for head development and is involved in syndromes with craniofacial malformations

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Retinoic acid is a central signaling molecule regulating several important processes during early embryogenesis. This same molecule is also known to have teratogenic effects on head development when abnormally expressed. Despite this negative role on head formation, several syndromes exhibiting craniofacial malformations, such as DiGeorge/Velocardiofacial, Vitamin A Deficiency and Fetal Alcohol syndromes, suggest a requirement for retinoic acid for normal head development. In order to study the role of retinoic acid in head formation we used the frog *Xenopus laevis* as a model system, which allowed us to focus on early events inhead formation while manipulating retinoic acid levels. Inhibition of retinoic acid biosynthesis in Wnt8-induced secondary axes resulted in a significant reduction of head formation. By manipulating retinoic acid levels at different developmental stages and different regions of the embryo, we were able to map the requirement for retinoic acid to early gastrula stages, corresponding to the activity of retinoicacid in the Spemann's organizer. Treatment with high doses of retinoic acid signaling inhibitors resulted in severe gastrulation defects. Lower

doses led to inhibition in cell movements while a percentage of the embryos showed abnormal tissue separation. These observations suggest that, in addition to its potential teratogenic effect on head development, retinoic acid has also a positive regulatory role in head induction during early gastrula. This novel activity ofretinoic acid signaling is in agreement with the proposed reduction of this signal in syndromes exhibiting craniofacial malformations.

Program/Abstract # 345
The role of maternal Dpp/BMP pathway in the early *Drosophila* embryo.
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Dorsoventral (DV) patterning in *Drosophila melanogaster* is regulated by the Toll pathway by modulating Cactus/IkB protein degradation, and thus nuclear Dorsal/NFkB protein levels in the early embryo. In addition to the Toll pathway, through previous genetic and biochemical studies we have shown that the maternal Dpp/BMP pathway regulates nuclear Dorsal levels by controlling Toll-independent degradation of Cactus. The maternal Dpp pathway requires Calpain A, a calcium-activatedcysteine protease, to target Cactus degradation and thus to regulate Dorsal levels. Using a Real-time qPCR approach we have shown that the maternal Dpp pathway regulates mRNA levels of CalpainA and Casein Kinase II in the earlyembryo. These results suggest that maternal Dpp functions through a novel mechanism, since the effects of Dpp blockage are observed before the onset of zygotic transcription. This raises the possibility that the maternal Dpp/BMP pathway acts through post-transcriptional mechanisms to regulate stability or degradation of mRNAs. Accordingly, null germline clones for the tkv type I receptor, mad or medea SMADs generate DV patterning defects similar to those reported for blockage of the tkvreceptor. In order to define the functional role of maternal Dpp on mRNA levels we have undertaken a qPCR approach. Recent data reveals that we are able to detect variations in mRNA levels in the pre-blastoderm embryo upon blockage of maternal Dpp signals. The results obtained up to now suggest that Dpp regulates maternal mRNA levels in the embryo. We are currently exploring whether some of these maternal mRNAs participate with Dpp to regulate discrete nuclear Dorsal levels and thus DV patterning. This work is supported by CNPq, Pronex, FAPERJ and INCT-EM.

Program/Abstract # 346
Split top: A maternal regulator of dorsal-ventral patterning and cell migration in zebrafish
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Maternal factors are required for many early developmental processes including fertilization, egg activation, and formation of the body axes during embryonic development of the zebrafish, Danio rerio. Our lab has performed a recessive maternal-effect mutagenesis screen and identified a number of mutants with defects in early developmental processes, including early morphogenesis and body axis formation. One such mutant, Split top exhibits abnormal dorsal-ventral patterning, displaying a dorsalization of the embryonic axis. Clutches of embryos from Split top mutant mothers are characterized by the five classic dorsalized phenotypic classes, as well as some additional defects. The mutant embryos show an expansion of dorsal markers and a corresponding reduction in ventral markers during gastrulation indicative of dorsalization. The additional defects appear to be the result of altered morphogenesis, including defects in epiboly, the process by which the blastoderm cells migrate over and surround the yolk. Split top mutant embryos also appear to be defective in the cell movement process of convergence and extension. We mapped the Split top mutation to chromosome 17, within a 1 MB interval and efforts are currently underway to positionally clone the affected gene.

Program/Abstract # 347
Oocyte asymmetry and the animal-vegetal axis in zebrafish
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The vertebrate animal-vegetal axis is established during oogenesis. Oocyte polarity is pre-requisite for determining the prospective embryonic axes and setting aside the germ cell determinants in non-mammalian vertebrates. The Balbiani body is an evolutionarily conserved oocyte asymmetry present in early oocytes of allanimals examined, including humans. However, bucky ball is the only gene known to be essential to establish oocyte polarity in vertebrates. Lack of maternal Buckyball causes failure to specify oocyte asymmetry and the embryonic axes through an unknown pathway and mechanism. To investigate how Bucky ball regulates asymmetries in oocytes we are testing the hypothesis that buc specifies the oocyte axis upstream or at the level of Balbiani body assembly. We have identified candidate components of the Buc pathway through yeast genetic and affinity purification approaches. To identify regions of Buc protein with potential functional significance we have mapped the regions of the Buc protein that mediate interactions with its binding

partners. Our transgenic gain of function and interference approaches with Bucky ball and its interacting proteins provide insight into Buc regulation and have identified potential roles for RNA binding proteins in oocyte polarity and follicle cell fates.

Program/Abstract # 348
A novel, maternally expressed gene, SMCR7L1, is important for *Xenopus* early development
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Maternal factors, such as mRNAs, that 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, SMCR7L1, 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 SMCR7L1 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 SMCR7L1 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.

Program/Abstract # 349
DV and AP axial patterning are coordinated by an identical patterning clock
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The coordinated timing of embryonic patterning along the body axes is a crucial step in establishing the body plan in vertebrates. Our laboratory has shown that BMP signaling patterns dorsoventral (DV) tissues progressively temporally along the anteroposterior (AP) axis. To examine if DV patterning by BMP signaling along the AP axis is coordinated with AP patterning by the same patterning clock or independently of AP patterning, we altered AP patterning by inhibition or activation of FGF, Wnt, and RA signaling in combination with temporal inhibition of BMP signaling. We found that the anteriorized or posteriorized tissues were patterned at the same temporal interval by BMP signaling as the normally positioned tissues, indicating that DV patterning and AP patterning are temporally coordinated along the AP axis. We then examined the molecular mechanism coordinating DV and AP patterning. Phosphorylation of Smad1 by MAPK and GSK3 inhibits the activity of the BM Preceptor phosphorylated form of Smad1, P-Smad1Cter, in *Xenopus* embryos (Fuentealba et al., 2007). This regulation is postulated to coordinate DV and AP patterning of the ectoderm by BMP, FGF/MAPK and Wnt/GSK3 signaling. To investigate if a similar mechanism regulates P-Smad1/5Cter function in zebrafish, we examined the localization pattern of P-Smad1/5MAPKand P-Smad1/5GSK3. The localization of P-Smad1/5MAPK was maintained in the ventral marginal zone, where FGF/MAPK and BMP/Smad1/5 signaling coexist during gastrulation, whereas P-Smad1/5GSK3 was not.We also found that FGF/MAPK could affect the timing of patterning of DV tissues along the AP axis during gastrulation. These data provide novel insight into the spatiotemporal coordination of DV and AP patterning.

Program/Abstract # 350
Characterization of the presomitic mesoderm progenitor cell and its niche
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Diseases of the mesodermal tissues of the body including wound healing, arthritis, osteoporosis and muscular wasting diseases, affect millions of people across the globe. If existing barriers to the efficient derivation of mesodermal tissues from readily available, patient-matched stem cells could be overcome, these diseases could be treated with stem cell-based regenerative medicine. Unfortunately, reproducible protocols for the differentiation of muscle, cartilage, and connective tissues, or more specifically, those mesodermal derivatives produced solely from pre-somitic mesoderm (PSM), have yet to emerge. While it is possible to obtain some cell types of the PSM from human ESC, these efficiencies (commonly <1%) must be vastly improved before regenerative procedures for these tissues will be feasible. Previous studies have indicated the presence of an endogenous murine PSM progenitor (PSM-Pr) cell, located at the junction of the primitive streak and node, responsible for colonizing the presomitic mesoderm and eventually giving rise to the skeletal muscle, cartilage, and connective tissue of the embryo. The primary focus of the work described here is to identify the murine PSM-Pr and the surrounding cell types that comprise and maintain this stem cell niche. In this project we will (1) identify all cell types located in the border region, and (2) evaluate the role of key signaling pathways in the maintenance of the PSM-Pr and the PSM-Pr niche. Successful completion of these goals will provide unprecedented insight into the generation of somitic mesoderm and enable novel approaches to the generation of therapeutically relevant mesodermal cell typesfor clinical use.

Program/Abstract # 351
Roles of Noggin, a BMP antagonist, in development of craniofacial skeletal elements
Matsui, Maiko; Klingensmith, John, Duke University, Durham, United States

The mammalian craniofacial skeleton consists of more than twenty small bones and cartilages that are formed precisely during development to create functional structures - the face and the head. However there exist known deformities in the mammalian craniofacial skeletal structures. For example, cleft palate and cleft lip are the most common craniofacial defects in human newborns. Micrognathia, which is characterized bymandibular hypoplasia, is another example of common congenital malformation. Yet, the causes of these defects remain incompletely understood. It is critical to study mechanisms of proper craniofacial development. Previously, mice lacking the Bone Morphogenetic Protein (BMP) antagonist Noggin exhibit a whole spectrum of craniofacial defects including fully penetrant primary and secondary cleft palate and micrognathia. Noggin is expressed in multiple domains during the important period of craniofacial development. Different domains of Noggin expressed may cooperate to tune appropriate BMP signaling gradient within these domains as well as surrounding tissues for proper morphogenesis. In this study, we employed tissue specific inactivation of Noggin to address the roles of Noggin in neural crest cells (NCCs) for craniofacial skeletogenesis. Interestingly, our data indicate that Noggin in NCCs plays an important role in NCC-derived tissues as well as in the nonNCC-derived tissues. We also tested if Noggin in NCCs has anon-autonomous role against BMPs in the neighboring mesoderm-derived tissues by overactivating BMP signaling only in NCCs. All together, our results suggest that Noggin in NCCs plays a critical role to regulate patterning and growth of both NCC- and mesoderm-derived craniofacial skeletogenesis.

Program/Abstract # 352
Effects of Methoxychlor (MXC) on expression of SOX9/WNT4 genes in development of the male reproductive system
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Methoxychlor (MXC) is an organochlorine pesticide that alternated DDT because of high toxic reason. MXC, one of estrogenic endocrine disrupting chemicals (EDCs), has been proved to have an adverse effect on developing male reproductive system according to recent researches. MXC has an anti-androgenic effect that reduces testosterone binding sites of androgen receptors. Therefore we studied how MXC can affect embryonic male reproductive system to verify the effect of MXC in a pregnancy period. The chemical was applied 100 mg/kg dose by gavage daily to pregnant mouse and the control group was treated with just sesame oil in same dose of MXC. After embryonic sex differentiation was finished, we took out the embryos to identify the differences between MXC and control groups. We measured SOX9 and WNT4 gene expression with reverse transcription polymerase chain reaction (RT-PCR). In case of SOX9 gene, this begins the cascade reaction of testis morphogenesis. The other, WNT4, is expressed in both male and female although after sex determination, this is involved in just ovary morphogenesis. Exploiting some of embryos, androgen receptors in testis was quantified by western blot and others were detected with immunohistochemistry to analyze the morphological differences in reproductive system in both groups. Through this study, we confirmed the difference of gene expression between in both groups so as the androgen receptors either. Therefore we could understand how embryonic reproductive system got affected by MXC.

Program/Abstract # 353
Retinoic acid role in forelimb initiation is mediated by repression of axial FGF signaling
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Prior to limb budding, retinoic acid (RA) is required to initiate the forelimb fieldin both mouse and zebrafish. Loss of the forelimb bud during RA deficiency hasbeen proposed to be secondary to enlargement of the adjacent heart which expands due to ectopic cardiac FGF signaling. Previous studies on Raldh2-/- embryos completely lacking RA synthesis demonstrated absent forelimbs and enlargement of the heart, associated with ectopic expression of Fgf8 in the heart and caudal progenitor zone resulting in expansion of FGF signaling throughout the trunk. Here, we show that Rdh10 mutants exhibit stunted forelimbs (rather than complete loss) and enlarged hearts. Rdh10 mutants exhibit loss of heart RA activity but maintain caudal progenitor zone RA activity. Accordingly, Fgf8 expression in the heart is expanded posteriorly, while Fgf8 in the caudal progenitor zone appears normal. Thus, Rdh10 mutants differ from Raldh2 mutants in that FGF signaling does not expand anteriorly from the caudal progenitor zone, which we suggest is permissive for forelimb initiation, albeit

delayed and stunted as monitored by expression of Tbx5, the earliest known forelimb marker. Rdh10 mutant hearts exhibit ectopic expression of not only Fgf8 but also Isl1, an FGF8-target gene. Elimination of Isl1 function prevented heart enlargement in RA-deficient embryos, but did not rescue forelimb initiation. Our results show that defects inforelimb initiation during RA deficiency are not due to heart enlargement, but are likely caused by excess FGF signaling in the developing trunk (derived from either the heart or caudal progenitor zone) that prevents initiation of Tbx5 expression in lateral plate mesoderm where the forelimb field normally arises.

Program/Abstract # 354

Spot, a new mouse model for Hirschsprung disease and Waardenburg-Shah syndrome

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Hirschsprung disease (HSCR), or aganglionic megacolon, is a human developmental disorder of the enteric nervous system (ENS). HSCR has an incidence of 1 in 5000 live births and is characterized by a lack of ENS ganglions in the colon, leading to a neuromuscular defect that prevents intestinal motility. ENS ganglions are entirely derived from neural crest cells (NCC). This transient, multipotent cell population gives rise to diverse cell lineages during vertebrate development by undergoing extensive migration, proliferation and differentiation. In HSCR patients, defects in any of these key developmental processes result in a failure of NCC to completely colonize the intestines. HSCR can also occur in association with other features, as in Waardenburg-Shah syndrome (WS) where it is observed in combination with pigmentation abnormalities and sensorineural deafness. The genetics of HSCR is complex and almost 80% of its heritability is still unexplained. A random insertional transgene mutation screen was performed in our lab to identify new genes/loci important for NCC development in the mouse. This screen yielded a mouse line named “Spot” that displays pigmentation abnormalities and, in F2 litters, a phenotype reminiscent of WS that includes aganglionic megacolon as well as balance problems indicative of inner ear defects. Gut colonization by fluorescently-labeled enteric NCC is severely delayed in Spot embryos. We are now examining the inner ear of Spot animals, the sensory epithelia of which are partly derived from NCC. A genomic library screen recently placed the Spot transgene insertion site on mouse chromosome 10D2, in a region syntenic to human chromosome 12q15 and devoid of any previously known HSCR-associated locus.

Program/Abstract # 355

cAMP promotes retinal midline crossing by regulating Nrp1 expression

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The projection of axons to their targets is a key step in establishing neural circuits. Axons navigate through a complex environment by interpreting attractive and repellent cues including Semaphorins (Semas). We previously showed that axons modulate their response to repellents via a G protein coupled pathway. To examine the role of G protein signaling in axon guidance *in vivo*, we used GAL4/UAS to drive the expression of highly selective dominant negative (DN) heterotrimeric G proteins in retinal neurons of embryonic zebrafish. Among these, we expressed a DN construct targeting GαS. Retinal ganglion cell (RGC) axons normally cross at the ventral midline and project to the contralateral tectum. dNGαS expressing RGCs misproject to the ipsilateral tectum. GαS is expressed in RGCs as axons approach and cross the midline. Constitutively active GαS mRNA rescues RGC misprojections in dnGαS transgenics. Thus GαS, canonically known for activating cAMP, is required for reliable midline crossing of RGC axons. dnGαS induced RGC misprojections phenocopy morphantsfor adenylyl cyclase 1 (AC1), AC8, or the Sema co-receptor Nrp1a. AC8 morpholino (MO) and dnGαS synergize to produce ipsilateral misprojections. A likely common downstream effector forthe two is cAMP. We hypothesized that reduced cAMP leads to a decrease in Nrp1 expression. AC8 and Nrp1a MOs induce a synergistic increase in RGC misprojections. QPCR reveals that AC8 MO or dnGαS decrease levels of both Nrp1a and Nrp1b mRNAs. We propose that reduced cAMP levels in dnGαSor AC8 MO embryos cause axon guidance errors by inhibiting Nrp1 expression, thus making axons insensitive to Sema signaling near the midline that promotes midline crossing.

Program/Abstract # 356

Tetraspanin18 restricts neural crest migration by stabilizing epithelial Cadherin6B

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Unlike typical neuroepithelial cells in the developing central nervous system, neural crest cells (NCCs) undergo an epithelial tomesenchymal transition (EMT), detach from the neural tube, and migrate to give rise to diverse structures, such as the peripheral nervous system. Regulation of neural crest EMT is incompletely understood. Tetraspanin18 (Tspan18) is a member of the tetraspanin family of transmembrane proteins that have been implicated in cell signaling, motility and

adhesion. Tspan18 is expressed in chick premigratory NCCs, but is downregulated prior to migration, suggesting that Tspan18 may negatively regulate neural crest EMT. Indeed, when NCCs express Tspan18 past the stage it is normally down regulated, migration is blocked. NCCs that fail to migrate maintain epithelial Cadherin6B (Cad6B) protein despite temporally normal down regulation of Cad6B mRNA, suggesting Tspan18 promotes cell adhesion by stabilizing Cad6B protein to antagonize EMT. In contrast, Tspan18 knockdown leads to premature loss of Cad6B protein, and as a result, some embryos exhibit enhanced migration. Curiously, Tspan18 knockdown also represses Cad6B transcription. This effect on Cad6B mRNA is independent of the Cad6B repressor Snail2, but correlates with increased nuclear b-catenin, perhaps as a consequence of its release from Cad6B-dependent adherens junctions. Finally, consistent with the fact that the NCC transcription factor FoxD3 regulates NCC adhesion and promotes migration, FoxD3 represses Tspan18 expression. Taken together, these data suggest that Tspan18, as a read-out ofFoxD3, plays an important role in antagonizing neural crest EMT by promoting adherens junctions. Supported by NIH F31 GM087951 and a U of MN Grant inAid.

Program/Abstract # 357

Paladin is an antiphosphatase that modulates neural crest formation and migration

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Cells in the vertebrate dorsal neural tube face a dilemma: delaminate and become neural crest, or remain epithelial and become roof plate. Because dorsal neural tube cells co-express neural crest and roof plate transcription factors, neural crest gene expression does not guarantee eventual migration as a neural crest cell. In order to investigate the importance of differential protein activity in determining when and where neural crest regulatory factors are active, we characterized the function of the putative phosphoregulatory protein Paladin (Pald). Pald contains two phosphatase active site motifs and is expressed in neural crest precursors and migratory neuralcrest cells throughout chick neural crest development. Pald knockdown delays expression of neural crest transcription factors Snail-2 and Sox10, but has no effect on FoxD3, indicating Pald differentially regulates genes previously thought to be co-regulated in the neural crest gene regulatory network. Moreover, Pald is not required for proper temporal expression of Cadherin6B or RhoB, although Pald is essential for timely migration away from the neural tube, suggesting Pald regulates specific features of migration downstream of delamination. Finally, because mutation of critical, catalytic cysteine residues within Pald's predicted phosphatase active site motifs does not abolish Pald function in the neural crest, we conclude that Pald is an antiphosphatase. Altogether, our data indicate that Pald modulates the activity of specific regulatory factors by preventing their dephosphorylation in neuralcrest cells, providing an additional layer of regulation during early neuralcrest development. Funding: Minnesota Medical Foundation, F32DE019973, K22DE015309

Program/Abstract # 358

Loss-of-function analysis of RAC1 function in development of the zebrafish olfactory bulb

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Development of the vertebrate brain requires that axons and dendrites grow and elaborate into specific projections, resulting in characteristic cell shapes that allow synaptic partners to appropriately connect. Through experiments in model organisms, much is known about the extracellular molecular cues, and their receptors expressed by neurons, that guide axons to their appropriate targets. What is less well understood is the molecular coupling between axon guidance receptors and the cytoskeletal regulatory apparati. Recent work has identified some prototypical regulatory molecules that feed directly into the cytoskeletal machinery for specific differentiation processes. For example, the Par3-Par6-aPKC complex is known to be important for establishing neuronal polarity 1, 2. The Rho family of GTPases, including Rho, Rac, and Cdc42, are known to regulate the elongation, growth, and guidance of neurites3. Also, the Ena/VASP family of actin regulators is known to be important for controlling the structure and motility of the growth cone – the motile end of a growing neurite4, 5. We have established a method that combines in vivo electroporation 6, 7 with Gal4-based transgenic zebrafish lines 8 that can be used to specifically target developing neurons of the olfactory bulb. Neurons targeted through this method show stereotypical axon projections of mitralcells 9, the major output cells of the olfactory bulb. In addition to spatial targeting of GFP expression, this technique also allows us to incorporate a loss-of-function reagent at a specific stage in neural development, providing excellent temporal control of the knockdown. Here we use this approach to determine the function of the Rac1 GTPase for the growth and guidance of the mitral cell axon projection. We target the function of Rac1 by co-electroporating an expression plasmid coding for a dominant negative form of Rac1 (T17N). We have characterized the phenotype of Rac loss-of-function using confocal microsopy and three-dimensional reconstruction of the morphology of the olfactory bulb projection. We show that embryos expressing DN Rac1 display shorter axon projections down the lateral olfactory tract, and havedrastically reduced crossing of axons to the contralateral side. Thus, Rac1 function appears to benecessary for proper formation of the axon projection of mitral cells from the olfactory bulb.

Program/Abstract # 359
The methyltransferase NSD3 is required for neural crest migration
Jacques-Fricke, Bridget; Gammill, Laura, University of Minnesota Genetics, Cell Biology and Development, Minneapolis, United States

Neural crest cells are avertebrate stem cell population that arise from the dorsal neural tube, migrate extensively to reach their final targets, and form a variety of structures. While methylation is known to impact neural crest specification, we have identified aneural crest-essential methyl transferase that shows methylation independently regulates neural crest migration as well. The lysinemethyl transferase nuclear receptor SET domain-containing 3 (NSD3) is expressedin premigratory and migratory neural crest cells. Disrupting NSD3 by preventing its production or blocking its function results in reduced premigratory Sox10 expression and decreased migration distance, but not alterations in cell death or proliferation. Temporally restricting NSD3 loss of function to migratory stages reveals that NSD3 directly regulates neural crest migration. NSD3 overexpression also impairs neural crest migration, suggesting that a precise level ofNSD3-mediated methylation is required for proper neural crest development. As NSD3 methylates histone H3K36 in vitro, we are currently evaluating NSD3-dependent changes in histone methylation status in chick cells. Surprisingly, we found NSD3 is expressed in both the nucleus and cytoplasm of migratory neural crest cells, suggesting that NSD3 maymethylate non-histone substrates as well. Altogether, our work reveals an indispensable role for NSD3 in neural crest cell migration.
Funded by NSF IOS-1052102 and NIH F32 DE021651.

Program/Abstract # 360
The interplay of actomyosin contraction and post-translationally modified microtubules regulates adhesion maturation and cell migration
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Although much is known about how individual cytoskeletal systems contribute to cellular locomotion, how these different systems coordinate their functions to achieve physiological migration is still poorly understood. Here we show that human fibroblasts and organ explants reciprocally coordinate levels of acetylated microtubules and activity of actomyosin contraction to modulate the surface density of integrin and the progression of adhesion maturation, which dictate the migration rates of fibroblasts. Experimentally reducing contraction increased the level of acetylated microtubules. Conversely, increasing microtubule acetylation decreased cellular contraction. This inverse, reciprocal interaction between acetylated microtubules and contraction was achieved by competitive myosin phosphatase interactions with either MLC or HDAC6, which affected the activation state of either protein. This balance of contractility and acetylated microtubules controlled the surface density of the alpha5beta1 integrin, which affected adhesion maturation into fibrillar adhesions. Hyper acetylation of microtubules decreased endocytosis of the alpha5beta1 integrin, and the decreased rate of migration due to hyperacetylation of microtubules was partially rescued by inhibiting the alpha5beta1 integrin. Thus, a homeostatic balance between contractility and acetylated microtubules is achieved through controlled activation and deactivation of myosin II and HDAC6, which regulates the surface density of alpha5beta1 integrin and maturation of adhesions, thereby governing the rate of cell migration.

Program/Abstract # 361
Golgi orientation directs early cerebellar Purkinje cells migration through axon specification
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Neuronal migration is a fundamental process for central nervous system development. Neuronal migration is accomplished together with cytoskeletal and secretory pathway polarization. Golgi apparatus is an early compartment in protein secretory pathway. Golgi reorientation has been found to occur during neocortical and cerebellar neuronal migration. During early cerebellar Purkinje cell (PC) migration, Golgi reorientates from the base of the leading process to the opposite pole. However, it is not clear whether Golgi polartiy leads to a directional neuronal migration. Moreover, how Golgi orientation governs neuronal migration remains unknown. In this current work, we show that Golgi relocates to the base of the leading process during early PC migration, which may specify the leading process into axon. Disruption of Golgi orientation suppresses axon specification in cultured PCs. In conditional inactivation of Smad1/5 in mouse cerebellum, a subpopulation of PCs failed to migrate and displayed random and dispersed Golgi positioning, which suggests a close relationship between Golgi orientation and PC migration. We further demonstrated that, in later developmental stages of Smad1/5 mutant mice, those PCs which failed to migrate were not only lack of polarized Golgi but also axon protrusions. Our results suggest a correlation between Golgi orientation and early PC migration. It implicates that Golgi orientation may specify axon during PC early polarization, which maybe important for PC further migration.

Program/Abstract # 362
Characterizing M9.17, a strong dominant enhancer of the trio and abl mutant phenotypes
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We explore signaling networks involving the trioguanine-nucleotide-exchange factor using second site modifier genetics. Trio is expressed in axonal growth cones, and so we hope to further define networks controlling targeted axon outgrowth. M9.17 is a mutation isolated as a strong dominant enhancer of the trio mutantphenotype. We have found M9.17 also functions as a strong dominant enhancer of the abl mutant phenotype, which is significant as abl is also highly enriched in growth cones. M9.17 was generated by gamma-ray mutagenesis and was mapped to 64 cM on the second chromosome. Fine mapping, complementation analysis and genomic DNA sequencing have shown that M9.17 is caused by a 23-base pair deletion within the sequoia gene. Sequoia is a zinc-finger transcription factor, originally isolated by its effects on axon and dendrite morphogenesis. This deletion cases a frame-shift resulting in 260 novel residues following sequoia's Q561. This novel sequoia allele is a neomorph as neither sequoia-null deficiencies nor seq[vr5-5] (Q641STOP) function as dominant enhancers of the trio mutant phenotype. Unexpectedly, M9.17's enhancement of the trio mutant phenotype is not due to disruptions of early CNS architecture. Rather, these animals survive normally to 3rd-instar larvae, but then fail to pupate. Classic behavioral assays have demonstrated they fail to switch from "forager" to"wanderer" 3rd-instar larvae. Other work on this behavioral switch has implicated the larval mushroom body. Therefore our future work will focus on trying to understand how M9.17's strong, dosage sensitive genetic interaction with both trio and abl may affect mushroom body neuronal circuitry involved in the forager-to-wanderer behavioral switch.

Program/Abstract # 363
TrkB, TRPC3, and Ca2+ regulation of primary afferent extension in the embryonic avian spinal cord
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During neural development, dorsal root ganglia (DRG) sensory axons extend into the spinal cord at the dorsal root entry zone (DREZ), then branch to grow longitudinally along the rostral-caudal axis and finally grow into the grey matter. Our laboratory is interested in the regulation of longitudinal growth of sensory axons in the developing spinal cord. Using an *in vitro* preparation, we have shown that axon extension in the longitudinal pathway is significantly reduced by inhibition of TrkB or BDNF in the St. 25 chicken embryo. While the data suggest a role for TrkB and BDNF in this growth pattern, the mechanism is unknown. BDNF is an activator of a cation current that has been shown to require components of the BDNF induced PLCγ intracellular signaling pathway including phospholipase C, IP3 receptors, release of Ca2+ stores and Ca2+ influx. In addition, transient receptor potential canonical subfamily (TRPC) channels, specifically TRPC3, have been shown to co-localize with TrkB and become activated in a PLCγ dependent manner. Here we show immunoreactivity for TRPC3 in the DRG, DREZ and longitudinal pathway at St. 25. Further, we show that removal of extracellular Ca2+ or application of U7322, an inhibitor of PLCγ, significantly decreases DRG axon extension along the longitudinal axis of the embryo. These data suggest that TRPC3 and the PLCγ signaling pathway may mediate the role of BDNF and TrkB in DRG axon extension in the developing longitudinal pathway.

Program/Abstract # 364
Cdc42ep1 facilitates the efficient migration of cranial neural crest cells
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CDC42 is a small GTPase of the Rho-subfamily, which regulates signaling pathways that control diverse cellular functions including cell morphology, migration, endocytosis and cell cycle progression. We have identified a cdc42 effector protein cdc42ep1 in *Xenopus* embryos that is predominantly expressed in premigratory and migrating neural crest cells. Depletion of Cdc42ep1 with antisense morpholino oligonucleotides causes cranialneural crest cells to migrate significantly shorter distances, preventing their segregation into distinct migratory streams. At later stages, this results in severe defects in cartilage formation. Analysis of cranial neural crest cells reveals that Cdc42ep1 is required for the formation of filopodial protrusions, and the polarized distribution of Cdc42. Taken together, these results suggest that Cdc42ep1 interacts with Cdc42 in controlling cell polarization and protrusive activities. Selective expression of Cdc42ep1 in neural crest cells thus facilitates efficient migration of this unique cell population.

Program/Abstract # 365
Proteolytic processing of cadherins in chick cranial neural crest cells
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Misregulation of molecular pathways controlling the epithelial-to-mesenchymal transition (EMT) underlying cranial neural crest emigration, including adherens junction disassembly, may adversely affect neural crest development and result in craniofacial defects. Cranial neural crest cells undergo EMT en masse to acquire motility, subsequently migrating throughout the head and differentiating into a diverse range of cell types. In chick midbrain premigratory neural crest cells, N-Cadherin (N-Cad) and Cadherin6B (Cad6B) proteins are down regulated prior toEMT, with their turnover coordinated independently of transcriptional repression. We hypothesize that proteolytic processing depletes cadherin levels to facilitate neural crest EMT. We have verified proteolytic cleavage of Cad6B and N-cad *in vivo* at stages immediately following EMT by immunoblotting of midbrain lysates. To identify the protease(s) responsible for this cleavage, we have co-expressed relevant candidate metalloproteinases observed in the chick midbrain with Cad6B or N-cad *in vitro* and have identified members of the A Disintegrin and Metalloproteinase (ADAM) family to be important for cadherinc leavage. We will now perform over expression and knock-down assays *in vivo* to confirm these *in vitro* results and to assess subsequent effects on cadherin localization and neural crest cell EMT and migration. Collectively, our work will facilitate a better understanding of the molecular mechanisms underscoring normal developmental and disease-related EMTs.

Program/Abstract # 366
Regulation of Slit-Robo signaling by Comm-family members in insects
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Slit-Robo signaling is a key mediator of axon guidance decisions in divergent organisms ranging from planaria to vertebrates. In *Drosophila melanogaster*, Commissureless (Comm) is a key post-translational regulator of the Robo receptor and functions to prevent cell surface accumulation of Robo. Two additional Comm-family members are found in *Drosophila* and they vary in their ability to regulate Robo receptors. Although all three Comm-family members can complex with Robo receptors, only Comm and Comm2 regulate Robo receptor distribution in a Drosophila S2 cell culture assay. *In vivo* loss-of-function and gain-of-function assays reveal prominent roles for Command minor roles for Comm2 during embryonic development. Structure-function studies suggest that the functional differences amongst Comm-family members map to the trans-membrane and juxta-membrane regions of these proteins. Results from these ongoing studies will be presented. We are also investigating the evolution of Comm-like genes and regulation of Slit-Robo signaling in insects. The presence of Comm-family members in some insect orders (Diptera and Hemiptera) and apparent absence in others (Lepidoptera and Hymenoptera) suggests a more ancient origin of Comm and the loss of Comm-like genes in some but not all insect lineages. We are addressing the functional properties of divergent Comm-family members from a variety of insects using several approaches, including the S2 cell culture assay. This work has been supported by a grant from the National Science Foundation.

Program/Abstract # 367
Behavioral phenotypes after selective abrogation of Arx from the developing dorsal telencephalon
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The aristaless-related homeobox gene (Arx) is mutated in many neurodevelopmental disorders all including neurocognitive phenotypes. During development Arx is expressed in progenitor cells throughout the forebrain. In the pallium Arx is expressed in the proliferative ventricular zone (VZ) where the excitatory neurons of the cortex are born. Arx-/y mice have decreased proliferation in the VZ of the cortex resulting in a thinner cortex, however, these mice die soon after birth precluding behavior analyses (Kitamura et al. 2002). Mice that have a point mutation or a poly-alanine track expansion in Arx survive to adulthood and both were found to have learning deficits with the poly-alanine track expansion mice also exhibiting anxiety and hyperactivity (Kitamura *et al*. 2009). To evaluate how Arx is affecting the development and function of the excitatory neurons of the cortex, we conditionally eliminated Arx selectively from the precursors to all cerebral cortical projection neurons without affecting the interneuron population. These mice lose upper layer neurons resulting in a thinner cortex. The corpus callosum and the anterior commissure are hypoplastic or absent suggesting cortical and possibly amygdala and hippocampal, connectivity are perturbed. Behaviorally these mice are less anxious, more active, less social, and have focused learning deficits when compared to their wild type littermates. Furthermore, compared to all other Arx mutant mice that have been studied, they do not have seizures. This mutant mouse provides an important tool in which to study the function of Arx in brain development and behavior without seizures confounding the analysis.

Program/Abstract # 368
A screen to identify interactors of the antiphosphatase Paladin in the neural crest
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Neural crest cells arise in the dorsal neural tube and migrate into the periphery, forming diverse structures including sensory neurons and much of the craniofacial skeleton. Research into the molecular mechanisms of neural crest migration has established a gene regulatory network (GRN) of transcription factors that produces cells capable of migration. However, expression of the transcription factors within the neural crest GRN does not guarantee eventual migration as a neural crest cell. As previous studies have shown that phosphoregulation is required for migration, regulators of phosphorylation status may control the acquisition of migratory ability. We have identified a novel phosphoregulator, Paladin, in a screen for genes upregulated as a late consequence of neural crest induction. Our work has demonstrated that Paladin acts as an antiphosphatase and is required for normal neural crest migration. To understand how Paladin exerts its effects, we aimed to define the proteins with which Paladin interacts. We have performed a yeast two-hybrid screen to identify the targets and binding partners of Paladin in a cDNA library that includes premigratory and migratory stages of neural crest development. Our screen has revealed novel Paladin protein interactions, including a phosphorylation-dependent interaction with myosin heavy chain 9, a non-muscle motor protein. Experiments to confirm these interactions in chick neural crest cells are currently underway. Our data identify new candidate regulators of neural crest development and support a role for Paladin-modulated differential protein activity in regulating neural crest migration. Funding: U of MN UROP; F32DE019973 and K22DE015309; Minnesota Medical Foundation

Program/Abstract # 369

TashT: A new model for Hirschsprung disease

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Hirschsprung disease (HSCR) or aganglionic megacolon is a severe congenital anomaly of the enteric nervous system (ENS) with an incidence of 1/5000 in newborns and a 4:1 male to female sex ratio. This lethal condition is characterized by a lack of intestinal motility due to the absence of neural ganglia in the terminal region of the gut. The lack of gut innervation results from improper colonization by enteric neural crest cells (eNCC). The genetics of HSCR is complex, involving mutations in multiple genes such as members of the RET and EDNRB signaling pathways. However, mutations in known genes account for only about half of the cases of megacolon observed. Moreover, although environmental factors are known to affect HSCR pathogenesis, the contribution of such non-genetic factors is poorly understood. TashT is a novel mouse model that has been generated via an insertional mutation screen for genes involved in NCC development. As for human HSCR, TashT homozygote pups display megacolon with variable penetrance and a male sex bias (~5:1). We have localized the transgene insertion site in a 3.3 Mb gene desert on chr.10B2, syntenic to human chr.6q16 and devoid of HSCR associated gene. TashT homozygote embryos exhibit defective gut colonization by eNCC. This defect is characterized by a migration delay as well as a reduced number of eNCC; the status of Ret and Ednrb signaling pathways is currently being evaluated using gut explants. Interestingly, although very few neurons are present in the resulting aganglionic zone, they are sufficient for colonic motility for most mutants. However, other data suggest that such marked reduced number of neurons sensitizeTashT homozygotes to exogenous insults affecting neuron survival.

Program/Abstract # 370

Parallel integrin-associated pathways regulate gonadal distal tip cell migration and turning in *Caenorhabditis elegans*

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The U-shaped morphology of the *C. elegans* hermaphroditic gonad arm relies on the migration of the distal tip cell (DTC). DTCs first migrate longitudinally away from the midbody along the ventral basement membrane, then turn to migrate longitudinally backtoward the midbody along the dorsal basement membrane. Our previous work has shown that the DTC's turn back toward the midbody relies on integrin-associated Nck-interacting kinase (MIG-15) and MIG-38, a novel protein. Here, we describe the requirement for a parallel integrin-related pathway identified by analyses of LET-607 and cbp-1, a transcription factor and coactivator, respectively. DTC-specific RNAi knockdown of either let-607 or cbp-1 resulted in failure to return to the midbody by more than two-thirds of DTCs, resulting in gonad arms that extended to the pharynx or thetail. Because both let-607 and cbp-1 exert transcriptional control, we took a candidate gene approach to identify their target genes that have a rolein DTC turning. Curiously, this screen uncovered target genes shared between LET-607 and CBP-1 that encode integrin adhesion receptors and integrin signaling proteins. In particular, expression of src-1 and talin, which encode a non-receptor tyrosine kinase and an integrin activator, respectively, depends on LET-607 and CBP-1. Double RNAi knockdown of src-1 and talinphenocopies cbp-1 knockdown, suggesting that these two genes are mainly responsible for CBP-1-dependent turning. Furthermore, the overexpression of SRC-1 in DTCs restores turning

back to the midbody in cbp-1 RNAi-treated animals. We propose that LET-607 and CBP-1 coregulate the expression of these integrin-associated target genes that function in parallel to the MIG-15/MIG-38 pathway.

Program/Abstract # 371
Nodal signaling regulates endodermal cell motility and actin dynamics via Rac1 and Prex1
Woo, Stephanie; Housley, Michael; Weiner, Orion; Stainier, Didier, UC SF, United States
During organ morphogenesis, fate specification and differentiation decisions are translated into dynamic cell behaviors in order to form the three-dimensional shape of the organ. In the zebrafish embryo, endodermal cells are specified prior to gastrulation and subsequently undergo a series of complex movements to bring initially dispersed cells into a coherent epithelium that will ultimately line gastrointestinal tract. In order to investigate the molecular mechanisms underlying these early morphogenetic movements, we generated a novel transgenic line that labels the endodermal actin cytoskeleton, which allowed us to track actin dynamics and cell motility at high resolution *in vivo*. We observed that during early gastrulation, endodermal cells first migrated in a random, non-persistent manner and exhibited a high degree of actin dynamics. However, at late gastrulation, actin protrusions were more persistent and migration became more oriented as endodermal cells converged on the dorsal side of the embryo. We show that the dynamic actin and random motility characteristic of early gastrulation are dependent on Nodal signaling, and that Nodal signaling regulates activity of the Rho GTPase Rac1. Furthermore, we identified the Rac exchange factor Prex1 asa Nodal target and found that Prex1 is also required for random motility. Reducing Rac1 activity in endodermal cells caused them to bypass the random migration phase and resulted in cells aberrantly contributing to mesodermal tissues. Together our results reveal a novel role for Nodal signaling in regulating actin dynamics and migration behavior, which are crucial for endodermal morphogenesis and cell fate decisions.

Program/Abstract # 372
Annexin A6 modulates cranial neural crest cell migration
Wu, Chyong-Yi; Taneyhill, Lisa, University of Maryland Animal Sciences, College Park, United States
The vertebrate neural crest is a transient population of migratory cells that originates in the dorsal aspect of the embryonic neural tube. These cells undergo an epithelial-to-mesencyhmal transition (EMT), delaminate from the neural tube and migrate extensively to generate an array of differentiated celltypes. Elucidating the gene regulatory networks involved in neural crest cell induction, migration and differentiation are thus crucial to understanding vertebrate development. To this end, we have identified Annexin A6 as an important regulator of neural crest cell EMT and migration. Annexin proteins comprise a family of calcium-dependent membrane-binding molecules that mediatea variety of cellular and physiological processes including cell adhesion, migration and invasion. Our data indicate that Annexin A6 is expressedin the proper spatio-temporal pattern in the chick midbrain to play a potential role in neural crest cell ontogeny. To investigate Annexin A6 function, we have depleted or overexpressed Annexin A6 in the developing midbrain neural crest cell population. Our results show that knockdown or over expression of Annexin A6 reduces or expands the migratory neural crest cell domain, respectively. Importantly, this phenotype is not due to any change in cell proliferation or cell death but most likely caused by changes in the premigratory neural crest cell population. Taken together, our data indicate that Annexin A6 plays a pivotal role in modulating the formation of migratory neural crest cells during vertebrate development.

Program/Abstract # 373
Pku190 controls the spacing and periodical deposition of neuromasts by regulating Cxcr7 and Fgf signals in zebrafish
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Collective cell migration is a fundamental and essential process for embryonic development and cancer metastasis, but the molecular mechanism of many critical steps in this process remains largely unknown. Zebrafish lateral line provides a robust model to investigate the interactions between different signaling pathways during collective cell migration. Here, we report the identification and functional analyses of a novel zebrafish gene pku190, which encodes a protein containing a fibronectin domain and is specifically expressed in the lateral line system during embryonic development. Pku190 was identified from mp190, a fish line obtained from a large scale Tol2 transposon-mediated enhancer trap screen, where the EGFP reporter gene faithfully recapitulates the expression pattern of pku190. Time lapse recording of mp190 showed that the EGFP periodically expresses in the trailing region of the primordium and especially strongly expresses in pro-neuromast just before deposition. Morpholino knocking down of pku190 led to reduced number of neuromast and absence of their correct deposition in the posterior lateral line, though the posterior lateral line primordium migrated normally. The expression pattern of cxcr7b in the path of migration was altered though cxcr4b was still detectable. Moreover, fgf3, fgfr1

and their target pea3 were down-regulated in pku190 morphants but not fgf10. All these data suggest that pku190 is important and necessary for the correct timing and spacing of pro-neuromast deposition during lateral line development and may function through modulating the expression, activation as well as the coordination of Fgf signal and chemokine receptor Cxcr7b.

Program/Abstract # 374
Pax3 splice form expression and isoform function in the trigeminal placode
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Pax3 encodes a transcription factor that is necessary for normal ophthalmic trigeminal (opV) placode development and sensory neuron differentiation. In opV placode cells, Pax3 is expressed from the time of cell specification through ganglion formation. We show through quantitative RT-PCR that alternative splice forms of Pax3 are present during opV development. We have named these splice forms, Pax3V1 and Pax3V2, and show that alternative splice forms are expressed at different transcriptional levels over time. To determine whether the Pax3 isoforms serve functionally distinct roles during developmental progression of opV sensory neurons, we performed *in ovo* electroporation of each isoform prior to significant cell specification. For all isoforms, targeted cells remained in the ectoderm as undifferentiated cells significantly more than in controls. Misexpression of the Pax3 isoforms also caused a decrease in the number of differentiated (Islet1+) cells in the opV ganglion. A more careful evaluation of cell count data revealed subtle but statistically significant differences between the differentiation potential of each isoform. To better understand the roles of Pax3 isoforms, we repeated the experiments while simultaneously enhancing neurogenesis by blocking Notch signaling with DAPT. Using this method we clearly observed significantly more differentiation in Pax3V2-targeted cells than in full length Pax3- or Pax3V1-targeted cells. These results show that the Pax3V2 isoform may contribute to the neuronal differentiation of opV placodal cells, while full length Pax3 and the isoform Pax3V1 may need to be down-regulated to allow for neuronal differentiation.

Program/Abstract # 375
Functional characterization of Rdh10 during pancreas development in the mouse
Arregi, Igor; Iliev, Dobromir; Ahmed, Emad; Steinkogler, Karina; Semb, Henrik (Lund University, Lund, Sweden); Liliana, Minichiello (University of Edinburgh, Edinburgh, United Kingdom); Pera, Edgar (Lund University, Lund, Sweden)

Maternal vitamin A-derived retinoic acid (RA) is an essential signalling molecule in embryonic development, and misregulation of this pathway leads to severe malformations. We and others have shown that retinol dehydrogenase-10(Rdh10) is a key enzyme in RA biosynthesis and is controlled by RA negative feedback regulation in vertebrate embryos (1-4). Here we present a new conditional knockout model to study Rdh10 function in the mouse. Rdh10 null mutants die around embryonic day E12.5 and show typical signs of RA deficiency including craniofacial, limb and internal organ defects. Immunohistochemical analysis shows expression of Rdh10 in the pancreas epithelium. In Rdh10 null mutants the dorsal pancreas does notform, and the ventral pancreas is reduced in size. Analysis with molecular markers suggests that the mutant ventral pancreas fails to form a tubular network and exhibits reduced endocrine and exocrine differentiation. Together, our results reveal novel functions of Rdh10 in pancreas specification, morphogenesis and cell differentiation. References: 1. Sandell LL, Sanderson BW, Moiseyev G,Johnson T, Mushegian A, Young K, Rey JP, Ma JX, Staehling-Hampton K, Trainor PA(2007). *Genes Dev* 21(9):1113-24. 2. Strate I, Min, TH, Iliev D, Pera EM (2009). *Development* 136, 461-472. 3. Rhinn M, Schuhbaur B, Niederreither K, DolléP. (2011) *Proc Natl Acad Sci U S A*. 108(40):16687-92. 4. Ashique AM, May SR, Kane MA, Folias AE, Phamluong K, Choe Y, Napoli JL, Peterson AS. (2011) *genesis* 10.1002/dvg.22002.

Program/Abstract # 376
Apical/basal polarity and differentiation within ESC-derived neural rosettes
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Embryonic stem cell (ESC) *in vitro* neurogenesis resembles the development of the central nervous system in the early mammalian embryo. Using a well-defined monolayer protocol that utilizes the BMP-antagonist Noggin, we observe formation of neural rosettes, in which differentiating neural stem cells (NSCs) are radially arranged around a lumen and exhibit interkinetic nuclear migration. Additionally, these neural rosettes display a focal localization of the adherens junction protein N-cadherin at the lumenal surface, as well as localized β1-integrin staining at the periphery, suggesting formation of an epithelium with apical/basal polarity reminiscent of the embryonic neural tube and developing neocortex. Notch signaling plays an important role both in the maintenance of this polarized epithelium and in regulating symmetric

versus asymmetric cell divisions critical to cell fate decisions. A polarity complex composed of the proteins aPKC-Par6-Par3 maintains active Notch signaling in the most apical domain of rosette neural stem cells. Notch signaling is inhibited at basolateral portions of the cell by Numb. We show here that inhibition of Notch signaling by treatment with the gamma-secretase inhibitor DAPT results in a reduction in symmetrical cell divisions and an increase in asymmetrical cell divisions, and therefore increased production of neurons relative to neural stem cells. We also demonstrate that treatment with aurothiomalate (ATM), a small molecule inhibitor that prevents formation of a functional aPKC-Par6-Par3 polarity complex, disrupts the subcellular localization of Par3 and Numb and reduces the number of asymmetric cell division events. Under these conditions, the extent of neuron production is reduced. Additionally, we show that rosettes require active maintenance of apical/basal polarity, as ATM treatment disrupts these structures.

Program/Abstract # 377

The Bmp antagonist Noggin paradoxically induces the chondrogenic program in post-migratory, neural crest-derived facial mesenchyme

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The bone morphogenetic protein (BMP) antagonist, Noggin, and Retinoic acid (RA) have previously been shown to induce a homeotic transformation within the face of chicken embryos. The maxillary prominence was converted into the frontonasal mass, thereby duplicating the facial midline. (Lee et al. 2001). The phenotype consists of the formation of an ectopic cartilage which acquires the identity of the interorbital septum. Here, we examined the genesis of the cartilage and determined that a Noggin bead alone was sufficient to induce the cartilage. This transformation from osteogenic to chondrogenic fate only occurred in stage 15 embryos. By stage 20, treated embryos lost various intramembranous bones in the palate but did not form cartilage. To test whether Noggin could directly activate the chondrogenic program we created micromass cultures from stage 15 and 20 maxillary prominence, transfected a Col2-Luciferase reporter gene, which is based on the binding site for SOX5, SOX6 and SOX9 in Col2a1. Noggin treatment of stage 15 mesenchyme significantly stimulated the reporter whereas Noggin treatment at stage 20 had no effect on Col2-LUC activity. The induction of SOX9 RNA by Noggin at stage 15 but not at stage 20 mirrored the stage-specific results from the luciferase reporter experiments. Our results suggest that Noggin at early stages of craniofacial development takes on a different role, unrelated to being a BMP antagonist. Instead, Noggin activates transcription factors that promote chondrogenesis. We conclude that the response of the mesenchyme is altered at stage 20 due to changes in the molecular landscape. This work was supported by GACR (grant 304/09/0725) to MB and CIHR grant toJMR.

Program/Abstract # 378

The Hippo pathway member Nf2 regulates trophectoderm/inner cell mass specification.

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The first lineages to be specified during mouse preimplantation development are the trophectoderm (TE) and the inner cell mass (ICM). Proper segregation of these two lineages requires that TE-specific transcription factors such as Cdx2 be restricted in their expression to the outer, future-TE cells of the embryo. This Cdx2 expression pattern is regulated by the transcriptional co-activator Yap, whose nuclear localization and resulting transcriptional activity is controlled by the kinases Lats 1 and 2 (Lats1/2). The factors acting upstream of Lats1/2, Yap and Cdx2 during TE/ICM specification have not yet been described. Here, we demonstrate a role for the Hippo scaffolding protein Nf2 in the preimplantation embryo. Injection of a dominant negative form of Nf2 (dnNf2) causes a cell autonomous accumulation of Yap in the nuclei of inside cells of the embryo, where it is normally cytoplasmic, and an increase in Cdx2 expression in these cells, where it is usually absent. Consistent with an role upstream of Lats1/2, the effects of dnNf2 injection can be rescued by over-expression of wild-type Lats2. In contrast to the strong and consistent effects of dnNf2 injection, we find that Nf2-/- embryos have mild defectsin Yap localization and Cdx2 expression, and that these defects become more severe as blastocyst development proceeds. Additionally, in wildtype embryos, Nf2 mRNA and protein can be detected before the onset of zygotic transcription, suggesting that maternally supplied Nf2 is masking a stronger phenotype in these mutants. Taken together, our results demonstrate that Nf2 acts upstream of Lats1/2 and Yap to regulate Cdx2, and that maternally supplied Nf2 may play an important role during TE/ICM specification.

Program/Abstract # 379

Identification of transcription factors involved in differentiation of late-born ventral spinal neurons

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The regulatory networks that control lineage specification in the developing central nervous system are not completely understood. We have recently identified a novel population of neurons that originate in the ventral neural tube at stages when spinal cord progenitors are committed to glial fates. They are a late-born subset of V2 cells and were identified as CerebroSpinal Fluid-contacting Neurons (CSF-cN). The aim of this work is to determine the mechanisms involved in the differentiation of CSF-cN. Genetic lineage tracing in vivo experiments and expression analysis in mice indicate that CSF-cN progenitors express both the proneural transcription factor Ascl1 and the zinc finger containing transcription factor Gata3. We have found that CSF-cN are missing in Ascl1 mutant mice, while other neuronal cell types generated earlier from the same domain remain unaffected. In order to assess when Ascl1 is needed, we took advantage of the temporally restricted expression of Ascl1 conditional mice, revealing that Ascl1 exerts its critical actions around the time of CSF-cN differentiation. Moreover, our results indicate that Gata3 also plays a key role in CSF-cN development, as this population is severely affected in conditional CNS Gata3 knockouts. We determined that Ascl1 precedes Gata3 expression and that Gata3 is absent in ventral progenitors from Ascl1 mutants. On the contrary, we did not find a change in Ascl1 expression in Gata3 cKOs. In summary, we identified two key and specific regulators of CSF-cN specification. Our results show that Ascl1 and Gata3 act sequentially in controlling late neurogenic events in the ventral neural tube.

Program/Abstract # 380
Transcription factor dynamics in single mouse ES cells during germ layer commitment
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Embryonic stem cells can differentiate into all the cell types in the body. It remains unclear though how intracellular signaling and gene expression dynamics control fate choices of individual cells. Our previous work showed that the dynamic expression ratio of two pluripotency factors governs the earliest fate choice an embryonic stem cell makes, between neuroectodermal and mesendodermal progenitors (Thomson, 2011). Here, our goal is to identify the key transcription factors and their dynamics that govern specification of single cells into progenitors of one of the three germ layers. We measured the expression dynamics of pluripotency factors (Oct4, Nanog) and germ layer-specific factors (Sox1, Brachyury, Gata6) in single mouse embryonic stem cells during early differentiation. We used these dynamics to identify and purify subpopulations of cells that appear en route to germ layer specification. Comparing gene profiles of these purified subpopulations, we identified other transcription factors whose expression dynamics correlated with differentiation. We found that expression of a subset of transcription factors (Dnmt3a, Eomes, Snail, Foxa2, Gata4) correlated with intermediate transitions between pluripotency and germ layer commitment. Future experiments to interfere with these transcription factors during differentiation will determine the importance of these dynamics on the fate choice single cells make between the three germ layer progenitors. Through these experiments, our aim is to develop a general approach to make quantitative predictions about the dynamics of key transcription factors that control developmental decisions.

Program/Abstract # 381
The role of voltage-gated calcium channels in neuronal phenotype specification
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In addition to their role in neurotransmission in the mature nervous system, voltage-gated calcium channels are implicated in a wide variety of developmental processes. Recent literature has demonstrated the role of calcium activity in neural induction, neurotransmitter phenotype specification, axon pathfinding, and dendrite outgrowth. Given that the voltage-gated calcium channels are expressed at an appropriate time and place during development, we hypothesize that the alpha1 subunits mediate the spontaneous calcium activity that is important for neurotransmitter phenotype specification. To address this hypothesis, a morpholino “knockdown” approach is used to prevent the expression of the calcium channels. Thus far we have employed morpholinos to knockdown Cav2.1. This particular calcium channel subunit was chosen because it expressed widely inthe neural tube of *Xenopus laevis* during the early stages of development.The morpholino-injected embryos are then raised to the swimming tadpole stage and observed for any morphological, behavioral, and gene expression differences. The morpholino-injected embryos have displayed less swimming movement and an underdeveloped nervous system. Additionally, we are using a bioinformatics approach to analyze the upstream regulatory elements of Cav2.1, by aligning this upstream sequence with the upstream regions of other calcium channels and among species. Using a transgenics approach, we are currently testing the hypothesis that the conserved sequences are important regulatory elements.

Program/Abstract # 382
A low level of Hedgehog signaling in the notochord is sufficient for normal ventral pattering in the embryonic

spinal cord.

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Current models of neural patterning emphasize a role for the length of time progenitor cells are exposed to the morphogen Sonic Hedgehog (Shh) as the key determinant in generating pattern in the ventral vertebrate spinal cord. Although a Shh concentration gradient is thought to pattern the neural tube, a temporal mechanism implies that small amounts of morphogen are required to initiate the diversification of ventral spinal cord cell types. We utilized a genetic approach to test this idea during mouse spinal cord development. Because Patched1 is both the receptor and negative regulator of Shh signaling, its mutation sensitizes the embryo to Hedgehog signalling. We employed a novel Patched1 mutation (called Wiggable) that removes its carboxyl-terminus, which is required to inhibit Hedgehog signal transduction. This mutant is less severe than the Patched1-null and shows expanded ventral character in the developing spinal cord, consistent with increased Shh signalling. We then tested whether Wiggable mutants can rescue ventral patterning in situations where Shh levels were either entirely absent or severely reduced. We accomplished this by using the Shh-null and Hedgehog acteyl transferase (Hhat)-null mouse mutants, respectively. Hhat encodes a palmitoylase that is required to produce secreted forms of Hedgehog proteins, and is thus crucial for the establishment of the Shh signaling gradient. In its absence, the spinal cord develops without the ventral-most cell types such as floorplate cells and V3 interneurons. Surprisingly, Hhat/Wiggable double mutants show completely normal spinal cord patterning, while Shh/Wiggable mutants lacked only floorplate cells. The principal difference between Shh and Hhat mutants being that Hhat mutants still have low amounts of Hedgehog signaling in the notochord. Thus, our findings show that remarkably little Hedgehog signalling from the notochord is all that is required to initiate the full range of neuronal identities in the vertebrate spinal cord.

Program/Abstract # 383

CCAR1 is required for Ngn3-mediated endocrine differentiation

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Neurogenin3 (Ngn3) is a basic helix-loop-helix transcription factor that specifies pancreatic endocrine cell fates during pancreas development. It can also initiate a transdifferentiation program when expressed in pancreatic exocrine and ductal cells. However, how Ngn3 initiates a transcriptional cascade to achieve endocrine differentiation is still poorly understood. Here, we show that cell cycle and apoptosis regulator 1 (CCAR1), which is a transcriptional coactivator for nuclear receptors, also interacts with Ngn3. The association between Ngn3 and CCAR1 was verified by pull-down assays and co-immunoprecipitation analyses. Using gene reporter assays, we found that CCAR1 is essential for Ngn3 to activate the expression of the reporter genes containing the NeuroD promoter. Moreover, down-regulation of endogenous CCAR1 in the PANC-1 pancreatic ductal cell line inhibits the transdifferentiation program initiated by Ngn3. CCAR1 is, therefore, a novel partner of Ngn3 in mediating endocrine differentiation.

Program/Abstract # 384

Cell cycle exit is required for cell invasive behavior

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Cell invasion through basement membranes is an essential cell biological process that occurs during development, immune surveillance, and is mis-regulated during cancer metastasis. While cell cycle exit is a hallmark of differentiated cell types (i.e., muscle and neurons), it is unknown whether cells must exit the cell cycle to acquire the ability to invade. To investigate cell cycle control of the invasive phenotype, we are using a simple *in vivo* model of cell invasion that occurs during *C. elegans* larval development, where a specialized gonadal cell, the anchor cell (AC), invades through the uterine and ventral epidermal basement membrane to contact the developing vulval epithelium. We performed a uterine-specific RNAi screen targeting 598 *C. elegans* transcription factors, identifying the tailless/TLX ortholog, NHR-67, as a novel regulator of the AC cell cycle. Loss of function of NHR-67 results in the presence of multiple ACs that fail to breach the basement membrane. Through the use of cell cycle reporters we show that depletion of NHR-67 results in the normally post-mitotic AC failing to exit the cell cycle and proliferating. To determine whether cell cycle defects were functionally correlated with defects in invasion we manipulated the AC cell cycle machinery. Loss of genes required for cell cycle exit phenocopies depletion of NHR-67, resulting in multiple ACs that fail to invade. Conversely, prevention of cell cycle entrance in NHR-67-depleted animals rescues the invasion defect. To better understand the link between cell cycle exit and invasion, we are quantifying the dynamics ofthe actin cytoskeleton during proliferative and invasive states. Additionally, we are examining AC-specific gene expression during these disparate states and have identified that proliferative ACs fail to express MMPs that are transcriptional targets of the AP-1 transcription factor FOS-1A. Together, our data demonstrates a functional link between cell cycle exit and invasion and raises the possibility that invasive cells should be classified as a

differentiated cell type, which may have broad implications in the treatment of disorders associated with cell invasive behavior.

Program/Abstract # 385
Investigating the role of lin-42, the *C. elegans* period homolog, in developmental timing
McCulloch, Katherine, University of Minnesota, MinneapolisUnited States; Wohlschlegel, James (Los Angeles, CA, United States); Rougvie, Ann (Minneapolis, United States)

Precise temporal control is a critical component of developmental regulation. In humans, for example, puberty, the juvenile to adult transition, is regulated by a highly complex combination of genetic and environmental factors. Aberrant timing of puberty is predictive of serious health problems later in life, so understanding these processes is critical. Significant insights into developmental timing mechanisms have been achieved through studies in *C. elegans*, which uncovered several conserved temporal regulators termed heterochronic genes. These include lin-28, which has since been implicated in timing the onset of puberty in mice and humans. In addition, study of developmental timing revealed the first miRNAs identified: lin-4 and let-7. Let-7 miRNA is of particular interest. Its sequence is identical between *C. elegans* and humans, and in both systems, let-7 activity promotes differentiation. Another highly conserved heterochronic gene is lin-42, the *C. elegans* period homolog. period is a key component of the fly and vertebrate circadian clock, which synchronizes behavior and gene expression with the 24 hour cycle. In *C. elegans*, however, lin-42 acts largely as a developmental timer. In lin-42 mutant animals, the larval-to-adult transition occurs too early. Although lin-42 has long been known as a key component of the heterochronic pathway, the placement of lin-42 in the pathway and its molecular mechanism is unclear. We have found that one role of lin-42 is to act in parallel with lin-28 to regulate the accumulation of let-7 miRNA. Studies are ongoing to determine at what step in miRNA biogenesis lin-42 acts and how precocious expression of let-7 affects its targets in the pathway. In addition, we are using proteomic techniques to identify LIN-42 binding partners that will further elucidate its role in timing.

Program/Abstract # 386
Plasticity of patterning information in the blastema during limb regeneration in *Ambystoma mexicanum*
McCusker, Catherine D.; Gardiner, David, University of California at Irvine, United States

The amphibian limb regenerate, called the limb blastema, has the fascinating capacity to replace exactly the missing limb structures regardless of its location along the proximal/distal limb axis. How is pattern established in the blastema? The popular hypothesis is that early blastema cells, which are dedifferentiated adult limb cells, retain information about their original position in the limb and independently establish the pattern of the regenerate. However, ourfindings using GFP-labeled blastema grafts suggest that the early blastema remains under the influence of the differentiated stump tissue for patterning information. To understand how changes in the blastema microenvironment correspond to the establishment of pattern we performed a detailed characterization of the organization of blastema cells, the surrounding extracellular matrix, and location of cell proliferation in the blastema as it develops. We found that the early blastema and the apical tip of the lateblastema share a similar microenvironment. By performing additional grafting experiments, we have found that patterning in the apical tip of the late blastema is plastic, while the basal regions closest to the stump are specified. Our hypothesis is that patterning information is gradually “hardwired” in the blastema starting with the blastema cells closest to the differentiated stump. Our current focus is on how the blastema microenvironment controls the level of pattern commitment in the blastema cells.

Program/Abstract # 387
The role of Notch signaling in neurotransmitter phenotype specification and secondary neurogenesis in *X. laevis*
McDonough, Molly; Tellis, Athena; Koshiya, Hitoshi; Saha, Margaret, College of William and Mary, Williamsburg, United States

The acquisition of a neurotransmitter phenotype by individual neurons is crucial for the formation of a functioning nervous system. During primary neurogenesis, the Notch signaling pathway regulates neural differentiation via lateral inhibition. We hypothesized that Notch signaling may also regulate the specification of excitatory and inhibitory neurotransmitter phenotypes. We perturbed Notch signaling by injecting sense mRNA for different components of the pathway into *Xenopus* embryos at the two-cell stage. Embryos were then analyzed for gene expression using wholemount ISH. Results indicate that perturbing components of Notch signaling atearly stages of development does not affect specification of GABAergic or glutamatergic fates but does alter neural versus non-neural fate differentiation in early stages of neurogenesis. However, gene expression at later stages of development appears to compensate for these earlier effects. We utilized qPCR to determine the extent to which that compensation is occurring. Possible mechanisms of compensatory regulation were analyzed by performing ISH for neural progenitor markers and immunohistochemistry with PCNA to

determine cell cycle activity in response to Notch perturbations. Additionally, TUNEL assays were performed to examine altered rates of apoptosis in embryos in which Notch signaling was perturbed. These experiments elucidate the possible role of Notch signaling in secondary neurogenesis. We also injected embryos with mRNAs coding for hormone-inducible fusion proteins to activate Notch perturbations at later stages of development. We subsequently assayed these embryos using ISH to determine the potential role of Notch in GABAergic versus glutamatergic specification.

Program/Abstract # 388

The Dkk1 receptor Kremen1 regulates progenitor cell identity during mechanosensory organ formation.

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Canonical Wnt signaling regulates many cellular behaviors in development and disease. Here, we investigate how Wnt signaling is modulated in the context of the posterior lateral line primordium (pLLp) migration. The pLLp is a cohort of ~100 cells that collectively migrate along the trunk of the developing zebrafish embryo. ThepLLp is comprised of proliferating progenitor cells and differentiated cells that will form the mechanosensory organs of the pLL. Canonical Wnt signaling isactive in the leading progenitor zone of the pLLp and restricted from the differentiated trailing zone by activity of the secreted Wnt inhibitor Dkk1. Abrogation of Wnt signaling by ectopic expression of Dkk1 leads to decreased cellular proliferation and a concomitant increase in cell death in pLLp progenitors. We have identified a zebrafish strain that carries a mutation in kremen1 gene, a Dkk1 receptor. Previous studies showed that Kremen1 negatively regulated Wnt signaling. Surprisingly, we found that kremen1 mutants exhibited phenotypes associated with the loss rather then overactivation of Wnt signaling, including loss of proliferation in presumptive progenitor cells and an increase in cell death. Expression of Wnt target genes were progressively and prematurely downregulated in kremen1mutants. Mosaic analysis revealed that the effects of loss of Kremen1 arenon-cell autonomous. Based on our results, we propose that Kremen1 is required to sequester Dkk1 in the trailing zone of the pLLp and loss of Kremen1 leads toan ectopic expansion of Dkk1, down regulating Wnt signaling in the leading progenitor cells. Understanding the precise regulation of Wnt signaling during development may shed light on how the pathway is misregulated in disease.

Program/Abstract # 389

Re-examination of the primordial germ cells of the mouse: a general stem cell pool for building the posterior region?

Mikedis, Maria; Downs, Karen, University of Wisconsin-Madison Cell and Regenerative Biology, Madison, United States

Over the past several years, results from our laboratory have revealed that the posterior region of the mouse conceptus is more architecturally complex than was previously known. In particular, we have reported the existence of a putative stem cell reservoir, called the Allantoic Core Domain (ACD), within the mouse allantois, or precursor umbilical cord. The ACD is required, at a minimum, for allantoic elongation to the chorion to create the chorio-allantoic placenta. The presence of the ACD in the posterior region spatiotemporally coincides with the presence of STELLA-positive putative primordial germ cells (PGCs), which also localize to the proximal region of the allantois. It is thought that these STELLA-positive cells translocate to the hindgut endoderm and, from there, migrate to and colonize the gonads. However, contrary to previous reports, we have discovered that STELLA is not confined to the putative PGC lineage. Rather, it is found in multiple cell types within the posterior conceptus, including the amnion, allantois, hindgut, and tailbud. Fate mapping the posterior region, including the STELLA-positive ACD and adjacent embryonic component of the primitive streak, has revealed that STELLA-positive cells within both regions contribute to the allantois, hindgut, and tailbud, and raise the question of whether the cells traditionally regarded as PGCs in mouse maybe part of a generalized stem cell population required to build the posterior end of the fetus. Future work will examine PGC formation and development in mutants for Brachyury (T), which is required for the formation of the ACD.

Program/Abstract # 391

The characterization of GABAA α and GABAB receptor subunits and the role of calcium activity in the developing nervous system of *Xenopus*

Rabe, Brian A.; Kaeser, Gwendolyn; Saha, Margaraet, The College of William and Mary, Williamsburg, United States

The predominating inhibitory neurotransmitter in the adult nervous system, gamma-aminobutyric acid (GABA), acts primarily in an excitatory manner during early neural development, causing the depolarization of cell membranes. During development, GABA has been implicated as a factor involved in multiple processes including cell migration, proliferation, synapse formation, and neurotransmitter phenotype specification. It has been hypothesized that GABA signaling affects these processes by altering calcium activity in developing neural cells. We predicted that GABA receptor subunits are expressed in embryos at the neurula stages of development which serves as a critical period for neurotransmitter specification. Using *in situ* hybridization and semi quantitative real time RT-PCR to show spatiotemporal expression of

five GABAA α subunits and the two GABAB¬ subunits, we have demonstrated that these subunits are expressed throughout the developing nervous system. In order to determine the function of the GABAB receptors during development, we have injected translation blocking morpholino oligonucleotides (MOs) against the GABAB subunits in *Xenopus* resulting in a reduction of the anterior nervous system including the brain and retina. In order to investigate the role of calcium activity on neural cell gene expression, cells from early neural plates were grown in primary culture in media with either 2mM or 10mM calcium. After verifying increased calcium activity in the cells grown in 10mM calcium using laser confocal microscopy and the calcium sensitive compound Fluo4-AM, total RNA was extracted from these cells and subject to microarray analysis to determine differential gene expression between the two groups.

Program/Abstract # 392
C. elegans as a model to investigate the molecular functions of CHD-7, the homolog of the CHARGE syndrome gene
Roiz Lafuente, Daniel; Rimann, Ivo; Hajnal, Alex, Intitute Molecular Life Science, University of Zurich, Zurich, Switzerland

Human CHARGE syndrome results in severe malformations of the heart, reduced neural stem cell proliferation in the olfactory epithelium and developmental defect in the inner ear among other deviations from the normal development. Many patients with CHARGE syndrome carry mutations in the chd-7 gene, which encodes a Chromo-domain helicase DNA binding protein.CHD-7 is a core component of a conserved multi-protein chromatin-remodelling complex that contains, among others, PBRM-1 and BAF-47. In a forward genetic screen for genes controlling vulval development in the Nematode *C. elegans*, we have isolated a loss-of-function mutation in chd-7. Our phenotypic analysis indicates that CHD-7 regulates together with BAF-47 and PBRM-1 the sensitivity of the vulval precursor cells (VPCs) to receiveor transduce a graded Wingless (Wnt) signal, which renders the VPCs competent to adopt vulval cell fates. In chd-7 mutants, anterior VPCs that are far away from the Wnt producing cells and hence receive a low dose of the Wnt signal stop proliferating and fuse with the surrounding epidermis, while the posterior VPCs receiving a high dose of Wnt are not affected. We will present the results of our genetic and biochemical analysis of chd-7complex to define its role in Wnt signal transduction.

Program/Abstract # 393
Roles revealed: Compound mutants define cooperative activities for BMP antagonist genes
Stafford, David A., UC Berkeley, United States

The induction and differentiation of many tissues and structures are known to be regulated by BMP antagonists. However, a comprehensive understanding of antagonist function in a given process, as well as the identification of additional antagonist-dependent events, is complicated by the functional redundancy exhibited by many of these molecules. To address these issues, we generated compound mutant embryos using conditional alleles for three antagonist genes, Noggin, Gremlin1, and Follistatin. We find that despite dynamic and non-overlapping expression, these antagonists cooperate in the patterning and development of multiple structures. We have shown that that Noggin and Gremlin1 cooperate to maintain a BMP signaling-free zone required for Hh-mediated sclerotome specification. We also observe that Noggin and Gremlin1 act together in differentiation and elaboration of the telencephelon and hindbrain. Preliminary analysis implicates BMP-mediated activation of Dkk1 in this phenotype. Similar to Gremlin, ablation of Follisatin exacerbates the skeletal phenotype exhibited by Noggin deficient embryos. We are analyzing the Follistatin promoter for sequence elements that mediate a repressive BMP signal. Based on our comparisons of double mutant embryos to single mutant siblings, we speculate that many additional developmental processes are modulated by the combined activity of multiple BMP antagonist genes.

Program/Abstract # 394
An Lmx1b-miR135a2 regulatory circuit modulates Wnt1/Wnt signaling and determines the boundaries of the midbrain dopaminergic progenitor pool
Awatramani, Raj B., Northwestern U – Neurology, Chicago, IL, United States

Borders are defined when territorial disputes are resolved. In early embryonic CNS development, neighboring progenitor zones compete for territory, ultimately settling the conflict by agreement on a sharp boundary. Transcription factor dosage is likely to play a key role in this process, yet mechanisms controlling their stoichiometry remain to be elucidated. Here we reveal an autoregulatory negative feedback loop between the transcription factor Lmx1b and microRNA135a2, the stoichiometric balance of which resolves the territorial dispute between the Lmx1a/b+ dopamine and adjacent Nkx6.1+ red nucleus progenitors in the ventral midbrain. First, forced maintenance of Lmx1b results in an expanded dopamine progenitor pool, whereas removal of Lmx1b results in a decreased dopamine progenitor pool. Next, we provide evidence that microRNAs are involved in restricting dopamine progenitor allocation. Conditional loss of Dicer1 in ES cells results

in expanded Lmx1a/b+ progenitors and reduced Nkx6.1+ progenitors. In contrast, elevated microRNA135a2 during an early window *in vivo* phenocopies the Lmx1b conditional knockout, in that the proportion of Lmx1a/b+ progenitors is selectively reduced. We demonstrate that these mutants display alterations in Wnt1/ canonical Wnt signaling, which at least in part may underpin these phenotypes. MicroRNA modulation of Lmx1b/Wnt1 dosage thus determines allocation of dopamine progenitors within the midbrain floor plate. Further, since canonical Wnt activity has recently been recognized as a key ingredient for programming ES cells towards a dopaminergic fate in vitro, these studies could impact the rational design of such protocols.

Program/Abstract # 395
Bacterial regulation of a developmental switch in choanoflagellates
King, Nicole, UC Berkeley, United States.

The evolution of animals from their single celled ancestors represents one of the major transitions in life's history. The origin of animals was shaped by extensive genomic and gene regulatory innovations, co-option of pre-existing genes to new functions in cell adhesion and signaling, rising atmospheric and oceanic oxygen concentrations, and the subsequent influence of new selective pressures. By studying choanoflagellates, the closest living relatives of animals, my lab aims to reconstruct the biology of the last common ancestor of animals. I will discuss our recent findings regarding the ancestry of animal gene families and the potential connection between genome evolution and animal origins. In addition, I will describe our development of a colony-forming choanoflagellate, *Salpingoeca rosetta*, as a new model for investigating the origin of animal multicellularity. Through our study of *S. rosetta*, we have discovered that a developmental switch in choanoflagellates is regulated by a secreted signal from environmental bacteria. Explaining how the intersection of genetic novelty, gene co-option, and environmental interactions contributed to the transition to multicellularity has important implications both for understanding early animal evolution and for identifying the foundations of animal cell biology.

Program/Abstract # 396
Multiscale mechanisms of neural crest migration: Theory and experiment
Kulesa, Paul, Stowers Institute for Medical Research, Kansas City, United States.

Long distance cell migration is an important dynamic feature of embryonic development in which cells are driven to precise peripheral targets. Although some cell guidance signals have been identified, the mechanisms that coordinate and direct subpopulations of cells to distinct targets are poorly understood. Here, we use the embryonic neural crest (NC) in tandem with theoretical studies to evaluate model mechanisms of long distance cell migration. We find that a simple chemotaxis model is insufficient to explain our experimental data. Instead, model simulations predict that NC cell migration requires leading cells to respond to long-range guidance signals and trailing cells to short-range cues in order to maintain a directed, multicellular stream. Experiments confirm differences in leading versus trailing NC cell subpopulations, manifested in unique cell orientation and gene expression patterns that respond to non-linear tissue growth of the migratory domain. Tissue transplantation experiments and model predictions that move trailing NC cells to the migratory front or vice-versa reveal that cells adopt a gene expression profile and cell behaviors corresponding to the new position within the migratory stream. These results offer a mechanistic model in which leading cells create and respond to a cell-induced chemotactic gradient and transmit guidance information to trailing cells that use short-range signals to move in a directional manner.

Program/Abstract # 397
The reactivation of the epithelial-mesenchymal transition in organ degeneration
Nieto, Angela, Inst Neuroc Alicante, Spain

The epithelial-mesenchymal transition (EMT) occurs during embryonic development for the formation of many tissues and organs, but also occurs in the adult as a physiological response to injury and during the progression of cancer and other pathologies (Nieto, *Ann Rev Cell Dev Biol*, 2011). Although the relevance of the EMT in human disease been debated until recently, it is now established as an important step in the metastatic cascade of epithelial tumors and it is emerging as fundamental in organ fibrosis. The EMT was recruited during evolution to define embryonic territories and to control epithelial plasticity. Therefore, the embryo holds the clues to the molecular and cellular mechanisms operating after its reactivation in the adult, despite the peculiarities associated with different pathological EMTs. Among the transcription factors that trigger EMTs, Snail genes are a good example to understand both physiological and pathological EMTs. I will discuss the pleiotropy of this gene family and its involvement in the reactivation of the EMT during the development of organ fibrosis.

Program/Abstract # 398
From signals to shapes in tissue morphogenesis
Shvartsman, Stas, Princeton University, United States

The epithelial sheet that envelops the *Drosophila* oocyte gives rise to the eggshell, a complex structure that controls several critical aspects of embryonic development. Eggshell morphogenesis can be viewed as a two-step process, in which localized chemical cues induce highly robust tissue deformations. In the first step, a small set of signals establish two-dimensional expression domains of dozens of genes, which collectively control mechanical forces and properties of cells across the epithelium. In the second step, these forces act on the epithelium, transforming it into a three-dimensional structure. The anatomical simplicity of the developing egg makes it an ideal system for establishing multiscale models of epithelial morphogenesis. I will present the results of our efforts to develop and experimentally test quantitative descriptions of eggshell morphogenesis, starting from network biology of gene expression and then moving to biomechanical models of tissue deformations.

Program/Abstract # 399
The enhancer disruption (ED) screen in zebrafish.
Villar-Cerviño, Verona; Molano-Mazón, Manuel (U Miguel Hernandez, Spain); Catchpole, Timothy (UTSW Med Ctr); Valdeolmillos, Miguel (U Miguel Hernandez, Spain); Henkemeyer, Mark (UTSW Med Ctr); Martínez, Luis M.; Borrell, Víctor; Marín, Oscar (U Miguel Hernandez, Spain).

Transposon-mediated enhancer trap screens in zebrafish have been reported as an effective way to screen for new genes involved in early developmental processes. Although this approach is well suited for the creation of gene reporter lines, it is relatively inefficient for the generation of mutations. Insulator sequences have been identified which can block transcription due to their interference with enhancer activity. We have exploited such insulating activity to build an innovative enhancer trap vector, based on the Tol2 transposon, whose mutagenic potential has been greatly enhanced by including an insulator sequence. As a classical enhancer trap, this tool is able to uncover genes with tissue-specific expression patterns and permit the isolation of their respective reporter lines. Moreover, due to its insulator activity, this transposon is also able to generate regulatory mutations through the disruption of tissue-specific enhancer activity. Additionally, we have incorporated the ability to discriminate between 5´and 3´enhancer activity relative to the transposon's insertion point. Here we will present results of a genetic screen performed with this tool which we refer to as an Enhancer Disruption (ED) vector and will discuss its efficient mutagenic activity and its impact in genomic regulatory landscapes.

Program/Abstract # 400
Cellular tiling in the cerebral cortex through contact repulsion
Bessa, José; Luengo, Mario; Rivero-Gil, Solangel; Ariza-Cosano, Ana; Naranjo, Silvia; Campaña, Francisco; Caballero, Pablo; Gómez-Skarmeta, José Luis, Centro Andaluz de Biología del Desarrollo, Spain

Cajal-Retzius (CR) cells play a fundamental role in the development of the mammalian cerebral cortex. They control the formation of cortical layers by regulating the migration of pyramidal cells through the release of Reelin. The function of CR cells critically depends on their disposition throughout the surface of the cortex, but little is known about the events controlling this phenomenon. We found that migrating CR cells repel each other upon contact, which leads to their random dispersion throughout the cortical surface and to the formation of a dynamically stable pattern of distribution that optimizes surface coverage. This process of cellular tiling is mediated by bi-directional Eph/ephrin interactions. Our observations reveal a novel mechanism that controls the even distribution of neurons in the developing brain.

Program/Abstract # 401
Capicua regulates proliferation and survival of RB-deficient cells in Drosophila.
Krivy, Kate; Bradley-Gill, Mary-Rose; Moon, Nam, McGill University, Montreal, Canada

Mutations of rbf1, the *Drosophila* homolog of the RB tumour suppressor gene, generate defects in cell cycle control, differentiation and cell death during development. Previous studies have established that EGFR/Ras activity is an important determinant of proliferation and survival of rbf1 deficient cells. Here, we report that Capicua (Cic), an HMG box transcription factor whose activity is regulated by the EGFR/Ras pathway, regulates both survival and proliferation of rbf1 mutant cells. We demonstrate that cic mutations allow rbf1 mutant cells to bypass developmentally controlled cell cycle arrest and apoptotic pressure. The cooperative effect between Cic and RBF1 in promoting G1 arrest is mediated, at least in part, by limiting Cyclin E expression. Surprisingly, we also found evidence to suggest that cic mutant cells have decreased levels of Reactive Oxygen Species (ROS), and that the survival of rbf1 mutant cells are affected by changes in

ROS levels. Collectively, our results elucidate the importance of the crosstalk between EGFR/Ras and RBF1 in coordinating cell cycle progression and survival.

Program/Abstract # 402
An investigation of Blastoderm specific gene 25D, a potential pole cell specifying gene.
Kowanda, Michelle A.; Yee, Stephanie; Liu, Niankun, McGill University, Montreal, Canada; Lécuyer, Eric (Institut de Recherches Cliniques de Montréal, Montreal, Canada); Lasko, Paul (McGill University, Montreal, Canada)
Specific localized mRNAsare essential for germline specification in *Drosophila melanogaster*. These mRNAs accumulate in the posterior pole plasm of the early embryo and are takenup by the pole cells, or primordial germ cells. While numerous mRNAs have beenfound to localize at the posterior of the early embryo, the functions of manyin germ cell development have not been determined. Additionally, a mechanism of active transport has been found to localize a subset of germ plasm mRNAs to thepole cells. Active transport along astral microtubules creates a feature called‘RNA islands’ in stage 3 embryos, ensuring that specific mRNAs are incorporated into the primordial germ cells. Interestingly, many well-characterized mRNAs that perform crucial roles in pole cell development are recruited to RNA islands, such as *germ cell-less* and *arrest*. *Blastoderm specific gene 25D* (*Bsg25D*) mRNA also localizes to RNA islands but its function has not been characterized. We have found that Bsg25D protein localizes to the posterior of region 2b of the germarium and posterior of the oocyte during stages 1-10 of oogenesis. Bsg25D translation is reduced in homozygous mutant $vasa^{1}/vasa^{1}$ovaries, although *Bsg25D* mRNA is localized to the oocyte throughout stages 2-10 of oogenesis. Finally, Vasa binds in vitro to the 3’UTR of *Bsg25D* containing a U-rich motif similar to the Vasa-binding site in *mei-P26* mRNA. Thus far, our analysis suggests that *Bsg25D* is a target of Vasa-mediated translationalactivation.

Program/Abstract # 403
Identification of a conserved motif in mRNAs that localize to RNA islands during *Drosophila* embryogenesis
Yee, Stephanie; Kowanda, Michelle, McGill University Biology, Montreal, Canada; Li, Xiao; Morris, Quaid; Lipshitz, Howard (University of Toronto, Toronto, Canada); Lecuyer, Eric (Institut de Recherches Cliniques de Montreal, Montreal, Canada); Lasko, Paul (McGill University Biology, Montreal, Canada)
During early *Drosophila* embryogenesis, post-transcriptional control of gene expression is essential for directing development. The posterior localization of maternal transcripts to the pole cells of the embryo establishes a molecular asymmetry, which is necessary for germ line specification. According to two ongoing large-scale *in situ* hybridization screens performed on *Drosophila melanogaster* embryos, a small fraction of maternally contributed mRNAs localize to the pole cells (Lecuyer et al., 2007; Tomancak et al., 2007). The posterior localization of those transcripts that later enrich in the pole cells can be divided into three distinct categories: 1) localization to the pole plasm during oogenesis, 2) enrichment to the pole plasm followed by formation of RNA islands around pole cell nuclei in the stage 3 embryo, and 3) accumulation in the pole cells by stage 4. Using fluorescent in situ hybridization, we found that most mRNAs that accumulate in *D. melanogaster* pole cells also do so in *D. simulans* and *D. virilis*. We have used bioinformatics tools to identify a consensus motif within the 3’UTR of *D. melanogaster* mRNAs that localize to RNA islands. The functional significance of the putative motif is currently being tested by expressing transgenes with the motif disrupted in the 3’UTRs of gcl, gwl and pgc , three mRNAs that contain the motif, and assessing for defects in RNA localization. Altogether, our studies will provide further mechanistic insight into germ cell development through the identification of conserved sequences and structural motifs.

Program/Abstract # 404
A structure-function study of Vasa in *Drosophila* early development
Dehghani, Mehrnoush; Lasko, Paul, McGill University, Montreal, Canada
Vasa is a DEAD-box RNA-binding protein essential for germ cell specification in a variety of organisms. DEAD-box helicases, including Vasa, were previously believed to be non-sequence specific, yet Vasa is known to regulate translation of specific mRNAs. Previous work in our lab (Liu et al. 2009) indicated that Vasa can distinguish a (U)-rich motif in the 3’UTR of its mRNA targets such as mei-P26, and that this specificity is conferred by the part of Vasa sequence residing outside of the motifs conserved among DEAD-box proteins. We hypothesize that the sequence-specific binding is associated with one or more RGG motifs in the N-terminus of Vasa, since these sequences act as auxiliary motifs that confer specificity to some other RNA binding proteins. To investigate this, we generated gfp-vasa constructs encoding proteins with progressive deletions in their N-termini to eliminate different numbers of RGG domains. These were expressed in vasa-null flies to study their localization pattern as well as their ability to rescue different aspects of Vasa function. Our initial data suggest that the RGG motifs in Vasa sequence act redundantly. Furthermore, proteins lacking more than six RGG motifs can still partially restore Vasa function; however, expressing such proteins at a high level

disrupts ovary development even more severely than vasa-null. We propose that this effect is associated with a decreased specificity of the protein, essentially blocking some other proteins from their mRNA targets. To confirm this, we will use deep sequencing analysis to compare RNAs bound to different Vasa truncates used in this study.

Program/Abstract # 405
Understanding the craniofacial defects produced by inhibition of folic acid metabolism
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It has been demonstrated that women who take folic acid prior to and during pregnancy reduce their chance of a number of structural birth defects, primarily neural tube defects but also facial defects including. The mechanisms by which folic acid reduces birth defects, and conversely, the mechanisms by which a reduction of folic acid and folate one-carbon metabolism contribute to birth defects, are not fully understood. The developmental consequences of reduced folate one-carbon metabolism have been studied for the neural tube and heart but less well understood for craniofacial regions. The zebrafish embryo can be studied in large numbers and have been demonstrated to have similar folate related one-carbon pathways as mammals and can provide insight into the mechanisms by which folate deficiency can impact human birth defects. We have determined that, by using the antifolate drug methotrexate (MTX) during early gastrulation through early neural crest migration stages, there is a dose-dependent effect on the size but not the patterning of cartilage elements when analyzed later in the developing larva. We seek to interrogate the neural crest to determine what developmental process is primarily disrupted in this model system.

Program/Abstract # 406
Hypertrophic chondrocytes contribute directly to the osteoblast and osteocyte lineage in endochondral bones *in vivo*
Cheah, Kathryn S.; Yang, Liu; Tang, Tiffany; Tsang, Kwok Yeung; Dung, Nelson WF; Chan, Danny, University of Hong Kong, Hong Kong

It is widely accepted that in vertebrates, bone formation occurs via one of two processes - membranous bone formation in which osteoblasts directly differentiate from mesenchymal cells or via endochondral ossification (EO), a multistep process wherein chondrocytes differentiate from mesenchymal condensations, to form a cartilaginous template, proliferate, exit the cell cycle to undergo hypertrophy and terminal differentiation. Vascular invasion occurs, and hypertrophic chondrocytes (HCs) are thought to undergo apoptosis, while bone is laid down by osteoblasts from the periosteum and bone collar surrounding the hypertrophic zone, replacing cartilage. However whether in vivo, all HCs undergo apoptosis in EO or can become osteoblasts has been a subject of considerable controversy. To address this controversy we have followed the fate of HCs using the Cre-loxP system in mice. We used homologous recombination to generate two cre lines, *Col10a1-cre* and *Col10a1-creER^{TM}*, which express Cre recombinase under the control of HC-specific *Col10a1* gene. We genetically tagged HCs by crossing *Col10a1-cre* mice to Cre-reporter mice (Rosa-26R-LacZ or Rosa-26R-YFP) and performed cell-lineage analyses to track the fate of HCs in fetal and postnatal stages. These experiments in combination with pulse-chase experiments using tamoxifen induction of Cre activity in *Col10a1-creER^{TM}*; Rosa-26R-LacZ mice, show that *in vivo*, HCs contribute directly to the osteoblast and osteocyte lineage of ALL endochondral bones. These osteoblasts of HC origin (HCObs) contribute to about a third of bone cells in long bones. In a bone-injury model we further show that HCObs contribute to new bone formation in the healing process. These discoveries impact on our current understanding of the origin of bone cells and have translational implications for regenerative medicine.

Program/Abstract # 407
Development of the autopod, but not of proximal skeletal elements, is impaired by misexpression of the BMP-binding molecule Chordin-like 1 in the chick limb
Allen, Justin, Boston Children's Hospital, Brookline, United States; McGlinn, Edwina; Tabin, Cliff; Warman, Matthew (Boston, United States)

The BMP-binding molecule Chordin-like 1 (Chrdl1) is expressed in mesenchymal lineages, including progenitors of skeletal tissues, but its role in skeletal development has not been examined in detail. We report that Chrdl1 is strongly expressed in limb buds and in somites of chick embryos. To further investigate the function of Chrdl1 in skeletal development, we utilized a gain-of-function approach using the RCAS viral expression system to misexpress Chrdl1 in developing limbs. Overexpression of Chrdl1 in the chick limb resulted in a severe oligodactyly phenotype, with a loss of anterior digits and of distal phalanges, although skeletal elements proximal to the autopod developed normally. Whole mount *in situ* hybridization analysis of genes important for limb development revealed that Shh and FGF signaling were upregulated, and FGF signaling from the apical ectodermal ridge persisted later in development in Chrdl1-overexpressing

limbs. Apoptosis was also perturbed, leading to abnormal overgrowth of the developing autopod as well as ectopic soft tissue outgrowths. SOX9 was not detected at sites of presumptive digit formation in the anterior limb, instead markers for the interdigital mesenchyme Msx1 and Msx2 were ectopically expressed. The expression of markers for the anterior limb however were not affected. These data suggest that the absence of anterior and distal skeletal elements in Chrdl1-overexpressing limbs is likely due to inhibition of chondrogenic differentiation rather than a defect in patterning the anterior limb.

Program/Abstract # 408
Hedgehog signaling acts upstream of Foxd1 to control the renal capsule
Martirosyan, John; Rosenblum, Norman (Toronto, Canada)

The renal capsule is a flattened layer of cells which invest the kidney and give rise to stromal cells in the kidney cortex. Differentiation of capsule cells is known to be dependent on the transcription factor Foxd1. While expression of Indian Hedgehog (IHH) and Gli1 in murine embryonic capsule cells suggest HH activity, HH functions in this domain are undefined. We hypothesize that HH activity controls capsule morphogenesis in the embryonic kidney. Mice with loss of HH signalling in capsule cells were generated by interbreeding Foxd1-Cre and Smoothened-loxP (Smo loxP/loxP) mice. Compound mutant mice were characterized by decreased expression of Gli1 and Ptch1, markers of HH activity, in capsule cells. Analysis of newborn mutant mouse kidney using histology and scanning electron microscopy demonstrated regions on the surface of the kidney where no capsule cells were present. Furthermore, the outer cortex underlying these regions was interrupted by tubules and mature glomeruli, which normally exist in the inner cortex. The discontinuous capsule phenotype was observed only after E13.5 by which stage capsule cells lost expression of the markers Foxd1 and Raldh2 and demonstrated decreased proliferation by 54%. Mice with a severe loss of renal capsule died immediately after birth while less severely affected mice survived to adulthood with normal kidney function. These results indicate that HH signalling acts upstream of Foxd1 to control capsule cell differentiation and proliferation.

Program/Abstract # 409
Stromally expressed ß-catenin regulates branching morphogenesis and nephrogenesis during kidney development
Boivin, Felix, McMaster University, Dundas, Canada; Bridgewater, Darren (Hamilton, Canada)

Formation of the mammalian kidney is dependent upon branching morphogenesis, defined as growth and branching of the ureteric epithelium, and nephrogenesis, the formation of nephrons from the kidney mesenchyme. Deletion of ß-catenin from the ureteric epithelial cells or kidney mesenchyme results in severe renal dysplasia, suggesting an essential role for ß-catenin in kidney development. We recently demonstrated ß-catenin localizes to the nuclear compartment of the renal stroma in embryonic and mature kidneys suggesting a functional role in kidney development. We hypothesize that stromally expressed ß-catenin plays an essential role in regulating branching morphogenesis and nephrogenesis. To support our hypothesis we generated a mouse model whereby ß-catenin is deleted in the renal stroma. Gross anatomical and histological analysis revealed renal hypodysplasia, pancake-like kidneys, a lack of renal capsule, ill-defined nephrogenic zone, and reduced condensed mesenchyme. The analysis of ureteric branch patterning revealed elongated and disorganized epithelial branches by E12.5. To provide support for a molecular mechanism we generated a mouse model that overexpresses ß-catenin exclusively in the renal stroma. Gross anatomical and histological analysis demonstrated bilateral renal aplasia or severe hypodysplasia, markedly increased condensed mesenchyme, absence of nephrogenic structures, and increased renal stroma. Analysis of branch pattern revealed disorganized and reduced epithelial branching. Taken together, these studies indicate that ß-catenin plays an essential role in the formation of the renal stroma and in the regulation of ureteric branching and nephrogenesis.

Program/Abstract # 410
Elucidation of the role of Rasip1 and Arhgap29 in blood vessel lumen formation
Koo, Yeon, , Dallas, United States; Xu, Ke; Fu, Stephen; Chong, Diana; Skaug, Brian; Chen, Zhijian (Dallas, United States); Davis, George (Columbia, United States); Cleaver, Ondine (Dallas, United States)

Cardiovascular function depends on patent blood vessel formation by endothelial cells (ECs). Blood vessel development initiates via the aggregation of ECs into tubes with a central lumen that allows blood flow. However the mechanisms underlying vascular ‘tubulogenesis’ are only beginning to be unraveled. A recent study by our lab has demonstrated the requirement for a novel GTPase-interacting protein called Rasip1, and its binding partner the RhoGAP, Arhgap29, for blood vessel lumen formation. Rasip1 null mice showed disrupted localization of polarity and junctional complexes, and loss of adhesion to extracellular matrix (ECM), resulting in failure of functional blood vessel formation. Depletion of either Rasip1 and Arhgap29 in cultured endothelial cells caused increased RhoA/Rock/Myosin II activity, suggesting that Rasip1 and Arhgap29 may function together to suppress RhoA mediated internal contractility. In vitro studies also

demonstrated that integrin dependent maturation of adhesion contacts require Rasip1 and Ahrgap29 function, indicating that blood vessel lumen failure may result in part from failed EC-ECM contacts. From these studies, we propopse that Rasip1 and Arhgap29 regulate multiple cellular processes required for functional vascular tubulogenesis. Current studies are aimed at elucidating the mechanisms by which Rasip1 and Arhgap29 regulate adhesion contacts.

Program/Abstract # 411
GTPase control of blood vessel development
Cleaver, Ondine, UT Southwestern Medical Center, Dallas, United States; Koo, Yeon; Barry, David (UT Southwestern Medical Center, Dallas, TX, United States); Xu, Ke (Harvard University, Cambridge, MA, United States)
Embryonic viability depends on proper development of its cardiovascular system, including blood vessel formation by endothelial cells (ECs). However, the mechanisms underlying development of functional vascular tubes are only beginning to be unraveled. We recently showed that endothelial tubulogenesis requires the GTPase interacting protein, Rasip1, and its binding partner the RhoGAP Arhgap29. Rasip1-/- embryos fail to form patent lumens in all blood vessels, including the early endocardial tube. Rasipl null angioblasts display adhesion defects both between ECs and to surrounding extracellular matrix (ECM). Similarly, depletion of either Rasip1 or Arhgap29 in cultured ECs blocks in vitro lumen formation. Here, we examine how Rasip1 and other GTPase effectors coordinate signaling decisions to ensure proper blood vessel formation.

Program/Abstract # 412
RhoA signaling controls development of Kupffer's vesicle and cardiac left-right asymmetry in zebrafish
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Rho GTPase signaling cascades have been implicated in establishing cardiac left-right (LR) asymmetry, but the underlying mechanisms remain unclear. We are using the zebrafish embryo to identify and characterize role(s) of RhoA signaling during LR asymmetric heart development. The small GTPase RhoA is a molecular switch that can activate downstream effectors including Rho kinases to modulate cytoskeletal dynamics and control several cell behaviors. Previously, we found that the Rho kinase Rock2b is involved in establishing an anteroposterior (AP) asymmetric distribution of motile cilia in Kupffer's vesicle (KV), which is necessary for these cilia to generate asymmetric fluid flow and direct normal cardiac LR development. Partial depletion of RhoA protein levels with previously characterized morpholinos reproduced these LR defects in the heart and AP asymmetry defects in KV, but also revealed defects in KV cilia formation. KV cilia in RhoA depleted embryos were significantly shorter than controls. While this phenotype was not observed in Rock2b deficient embryos, antisense depletion of another Rho kinase, Rock2a, also resulted in shorter KV cilia and cardiac LR defects. Importantly, co-injection of mRNA encoding a constitutively active Rock protein partially rescued LR defects in Rock2a or RhoA morpholino-depleted embryos. Videomicroscopy of beads injected into KV demonstrated that depletion of Rock2a or RhoA disrupted asymmetric fluid flow in KV. These results indicate RhoA signals through different downstream effectors (Rock2a and Rock2b) to control multiple steps of Kupffer's vesicle development.

Program/Abstract # 413
The dimple mutation uncovers a link between mouse gastrulation and mitochondrial function
Garcia-Garcia, Maria J.; Duran, Ivan, Cornell University, Ithaca, United States
During mouse gastrulation, a group of cells in the primitive streak, an area located at the posterior side of the embryo, delaminate from the embryonic epithelia and migrate to form mesodermal and endodermal lineages. To identify novel genes regulating these processes, we performed a forward mutagenesis screen and found dimple, a recessive mutation that causes early embryonic lethality and severe gastrulation defects. In dimple embryos, cells delaminate to form mesoderm, but are unable to migrate and accumulate close to the primitive streak area. Analysis of molecular markers revealed that several signaling pathways required for gastrulation are up-regulated in dimple mutants, including NODAL, WNT3 and FGF. Interestingly, Fgf8 is up-regulated in dimple embryos before morphological phenotypes are observed, suggesting that increased FGF signaling is primarily responsible for the developmental defects of dimple mutants. Positional cloning revealed that dimple disrupts SLC25A26, a transmembrane protein located in the inner mitochondrial membrane and responsible for the transport of S-Adenosylmethionine (SAM). Preliminary characterization of mitochondrial function in dimple embryos indicates absence of mitochondrial stress and apoptosis levels similar to those of wild type embryos. Together, these results indicate that the gastrulation defects of dimple mutants are not caused by an energetic imbalance, but rather to a role of mitochondria in modulating the signaling pathways that control mouse gastrulation.

Program/Abstract # 415
Spatiotemporal biomechanical variation in the avian embryo during primitive streak morphogenesis

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During embryonic development, the primitive streak (PS) is the organizing center of gastrulation, an essential process that results in the formation of the three germ layers and requires complex biochemical and mechanical spatiotemporal signaling. Current models for PS formation and gastrulation largely ignore the biomechanical environment. Here, for the first time, we present biomechanical properties of the early avian embryo, a classical model for studying gastrulation. To test our hypothesis that the spatiotemporaldifferences in stiffness were due to actomyosin contraction, we inhibited actomyosin contractility via the Rho kinase (ROCK) pathway using the small-molecule inhibitor Y-27632. Explants were taken from pre- and post-PS embryos and indented using an atomic force microscope. Electroporation and time-lapse microscopy combined with individual cell tracking provided cell convergence velocities. Treated samples were exposed to 100 mM Y-27632 for 1 h. ROCK-mediated actomyosin contraction appears to be essential for PS formation, the convergence of cells to the PS during gastrulation, and the increased stiffness of the PS relative to pre-PS stages and other regions of the post-PS embryo. Further, we found that regions of the embryo outside the PS soften relative to the pre-streak embryo. These data suggest that genetically-regulated tissue relaxation may be as essential as contraction during morphogenesis. Future models of the fundamental early morphogenetic movements should account for 1) the critical role of Rho kinase-mediated actomyosin contractility and 2) the significance of tissue softening as well as contraction to guide development.

Program/Abstract # 416
PCP pathway controls polarized actomyosin localization through septin 7 during collective cell movements
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The Planar Cell Polarity (PCP) pathway is a critical regulator of cell behaviors during development. Convergent Extension (CE) is an essential collective cell movement regulated by the PCP pathway. Although PCP signaling is necessary for establishing cell polarity during CE, little is known about how it controls the cytoskeletal machinery, such as actomyosin, that execute cell behaviors. We have previously shown that septins have a role in controlling cortical stability downstream of PCP signaling. In the present study, we show that septin 7 and F-actin accumulate at the medio-lateral cell cortices. While, activated actomyosin, as assessed by phosphorylation of myosin regulatory light chain (pMYL), localizes at the antero-posterior cell cortex. Septin 7 KD disrupts these polarized F-actin and pMYL localizations, as we observed ectopic accumulation at the cortices. Additionally, pMYL protein level is increased in septin 7 KD cells. Finally, we find that septin 7 is required to maintain polarized cortical forces, which enable cells to intercalate between each other along the medio-lateral axis. From these results, we conclude that polarized actomyosin distribution is regulated by the PCP pathway through septin 7 to generate the directional force driving collective cell movement.

Program/Abstract # 417
Uncovering the function of TMED2 during trophoblast differentiation
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Transmembrane emp24 domain trafficking protein 2, (TMED2) is a member of the p24 family of proteins involved in vesicle transport between the ER and Golgi. During vesicular transport between the ER and Golgi p24 proteins function as receptors for both cargos and coat proteins. Our group showed that Tmed2 is required for normal embryo and placental development in mouse and that syncytiotrophoblast cells of the mouse labyrinth placenta failed to differentiate in homozygous mutant embryos. In human placenta, we showed expression of TMED2 between 5.5 and 40 weeks of gestation in all trophoblast cell types. We noted that early in gestation TMED2 was more highly expressed in cytotrophoblast cells versus syncytiotrophoblast. The choriocarcinoma cell lines BeWo and JEG-3 are widely used for the study of trophoblast differentiation. These cells share many properties with villous trophoblast in terms of their morphology, biochemical markers, and hormone secretion. We found that TMED2 was more highly expressed in a choriocarcinoma cell line, BeWo, which can be induced to differentiate and form syncytiotrophoblast when compared to the JEG-3 cell line, which does not fuse to form syncytiotrophoblast. We hypothesized that TMED2 is required for fusion of trophoblast cells during syncytiotrophoblast differentiation. To test this hypothesis we are examining the function of TMED2 during trophoblast differentiation of BeWo and Jeg-3 cell lines. We will show our plans to ectopically express TMED2 in Jeg-3 cells and to knockdown TMED2 expression in BeWo choriocarcinoma cells with shRNA. Our work suggests that TMED2 is required for trafficking cargoes that are essential for placental development.

Program/Abstract # 418
Epithelial intercalation drives elongation of the mouse neural plate
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Mark (National Cancer Institute, Frederick, United States); Sutherland, Ann (University of Virginia, Charlottesville, United States)

The early embryo forms an elongated body axis through the mass movements of convergence and extension (CE) of the tissue. While the mechanisms driving CE are well described in the frog and fish, our understanding of CE and the resulting axial elongation forces in mammalian embryos is still rudimentary. We have shown by direct observation that the paraxial mesoderm of murine embryos undergoes CE by mediolaterally polarized cell intercalation driven by bipolar protrusive activity, but the cellular behaviors underlying epithelial cell rearrangement in neural tissue have not been identified. To determine what cell behaviors lead to neural CE in the mouse, we examined the neural plate of live, fluorescently labeled embryos using time-lapse confocal microscopy. We found that mouse neural cells undergo mediolateral cell intercalation and exhibit both apical junctional rearrangement and basolateral protrusive activity. Neural cells form rosettes, similar to those identified as driving Drosophila germ-band extension, but this pattern of cell rearrangement is only one of a number of cell behaviors that lead to neural cell intercalation in the mouse. Neural cell intercalation is disrupted in embryos with mutations in the genes encoding PTK7 and Vangl2, leading to failure of neural tube closure. By creating a conditional knockout of PTK7 in the mesoderm only, we found that while normal neural cell intercalation is sufficient to drive closure of the anterior neural tube, CE of mesoderm is required for closure of the posterior neural tube and full elongation of the A-P axis. This is the first demonstration of a cellular mechanism for CE in mouse neural plate, and for its role in overall axial elongation.

Program/Abstract # 419

Prolonged FGF signaling is necessary for lung and liver induction in *Xenopus*

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FGF signaling plays numerous roles during organogenesis of the embryonic gut tube. Mouse explant studies suggest that different thresholds of FGF from the cardiogenic mesoderm induce the lung, liver and pancreas lineages from ventral foregut progenitors. The mechanism that regulates the doseof FGF in vivo is unknown. Here we use *Xenopus* embryos to examine the hypothesis that a prolonged duration of FGF signaling is required to induce foregut organs. We show that the cardiac mesoderm and FGF signaling are required for liver and lung development in *Xenopus*; formally demonstrating that this important step in organ induction is conserved with other vertebrates. Moreover we show that prolonged contact with the mesoderm and persistent FGF signaling over an extended period of time is required for complete organ induction. Inhibiting FGF signaling for a short time during the induction process or blocking just the MEK or PI3K branches of FGF intracellular transduction results in intermediate phenotypes. Cell autonomously blocking FGF activity in the foregut progenitors using dominant negative FGF receptors results in decreased contribution of the cells to the lung and liver buds, but nochange in contribution to the intestine, pancreas, or stomach. Additionally, FGF inhibition arrests foregut cells in a progenitor state. These results suggest that the high dose of FGF needed to induce lung and liver is achieved *in vivo* through prolonged FGF signals which has important implications for efforts to direct the differentiation of stem cells into foregut lineages. In addition this study forms the foundation for further mechanistic studies on foregut organogenesis using the experimental advantages of the *Xenopus* system.

Program/Abstract # 420

A mouse model for juvenile hydrocephalus

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Juvenile hydrocephalus, the accumulation of cerebrospinalfluid (CSF) in the ventricles of the brain, causes significant morbidity among human children affecting roughly 1 in 500 newborns. The disease manifests due to overproduction, decreased absorption, or restricted flow of CSF. Few genetic causes of this disease are known, and therefore animal models can prove beneficial in identifying candidate genes and studying relevant pathways. The Juvenile hydrocephalus (Jh) mouse line contains a lacZ transgene integration on mousechromosome 9. Homozygous Jh mice exhibit hydrocephalus by two weeks of age and few survive beyond eight weeks. Histological analysis showed a patent aqueduct with no overt brain malformation, indicating communicating hydrocephalus. Scanning electron microscopy found reduced density and loss of orientation of ventricular ependymal cilia, which may be involved in the development of the hydrocephalus. This phenotypere presents a novel cause of the disease since no known hydrocephalus mutations map to this genomic region. Analysis of the integration site showed disruption of an uncharacterized gene, 4931429I11Rik, here called I11. The lacZ transgene is expressed in pineal gland, hypothalamus, neuroepithelium lining the aqueduct of Sylvius and choroid plexus, suggesting the expression pattern of I11. The predicted protein product of I11 has no recognizable functional domains,

however, and its role in CSF maintenance is unknown. Future research will focus on definitively establishing the role of I11 in hydrocephalus and dissecting its normal function.

Program/Abstract # 421

The Bardet-Biedl syndrome modifier CCDC28B participates in ciliogenesis and modulates mTORC2 function

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Bardet-Biedlsyndrome is a rare genetic disorder characterized by retinal degeneration, obesity, learning difficulties, polydactyly and malformations of the gonads and kidneys. To date, sixteen BBS genes have been identified (BBS1-12, MKS1, CEP290, FRITZ/C2ORF86, SDCCAG8) and their encoded proteins have been shown to participate in different aspects of the biology of ciliaincluding cilia formation/maintenance and modulating cilia-dependent signaltransduction, findings that have prompted the classification of BBS as aciliopathy. Although historically considered an example of Mendelian trait, the identification and mutational screening of different BBS genes has demonstrated that in some families at least, mutations at more than one *bona fide* BBS locus or second site modifiers segregate with the disease and modulate its penetrance and expressivity. CCDC28B (MGC1203) is a second site modifier of BBS encoding a protein of unknown function. Here we report the first functional characterization of this protein and show that it affects ciliogenesis in zebrafish and consequently causes a number of phenotypes that are characteristic of cilia dysfunction including hydrocephalus, left-right axis determination defects and renal impairment. Furthermore, we show that CCDC28B interacts with SIN1, a structural member of the mTOR complex 2 (mTORC2), and modulates its activity both *in vitro* and *in vivo*. Importantly, the mTOR pathwayhas been associated recently to the cilium whereby both cilia are required for the correct regulation of the pathway and mTOR participates in ciliogenesis.Therefore, our results shed light into the biological function of the BBSsecond site modifier CCDC28B, providing novel insight to understand the pathogenesis of this human condition, and potentially implicates mTORC2 signaling in the organization of cilia.

Program/Abstract # 422

Dosage effect of Six3 in the pathogenesis of holoprosencephaly

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Holoprosencephaly (HPE) is the most common forebrain malformation. It is defined as the incomplete separation of the two cerebral hemispheres. Based on the severity of the defect, HPE is divided into three forms, alobar, semi-lobar and lobar. The pathology of HPE is variable, even within families carrying the same mutation. The cause(s) of this variability remains unknown. Here we demonstrate that variability in Six3 dosages will lead to different forms of HPE. The semi-lobar HPE phenotype is the result of a severe down-regulation of Shh expression in the midline of the ventral forebrain. Consistent with this conclusion, elevation of the Shh signaling activity by deleting one allele of Ptch1 fully rescued the semi-lobar HPE phenotype. This study is the first study to show that different forms of HPE can originate from variations in the dosage of a transcription factor and provides a possible explanation for the variable pathology of HPE.

Program/Abstract # 423

Identification of predominant pattern of co-regulation among kinetochore genes

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The NCI-60 cell line panelis the most extensively characterized set of cells in existence. It provides multiple forms of data on different cancertypes, and has been used extensively as a screening tool for drug discovery. Previously, the potential of this panel has not been applied to the fundamental cellular processes of chromosome segregation. In the current study, we used data from multiple microarray platforms accumulated for the NCI-60 to characterize an expression pattern of genes involved in kinetochore assembly. This analysis revealed that 17 genes encoding the constitutive centromere associated network of the kinetochore core (the CCAN complex) plus four additional genes with established importance in kinetochore maintenance (CENPE, CENPF, INCENP, and MIS12) exhibit similar patterns of expression in the NCI-60, suggesting a mechanism for co-regulated transcription of these genes which is maintained despite the multiple genetic and epigenetic rearrangements accumulated in these cells. Multiple potential regulatory influences are identified for these genes, including transcription factors, DNA copy number, and microRNAs. In addition, alterations of expression levels of these genes are associated with karyotypic complexity. Thus, our results provide a prerequisite for experimental studies on

regulation of genes encoding kinetochore proteins, the process that, when aberrant, leads to the aneuploidy that is a hallmark of many cancers. We suppose that the comparison of expression profiles in the NCI-60 cell line panel could be a tool for the identification of other genes groups whose products are involved in the assembly of organelle protein complexes.

Program/Abstract # 424
Dietary cholesterol triggers Hedgehog-dependent follicle stem cell proliferation in the *Drosophila* ovary
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The long term maintenance and function of stem cells depend on signals generated by the local stem cell microenvironment, or niche. While significant progress has been made in identifying the niche-generated factors necessary for stem cell regulation, little is known about the mechanisms that control stem cell responses to systemic changes such as nutritional status. In the fly ovary, germline stem cells (GSCs) and follicle stem cells (FSCs) proliferate actively in the presence of abundant food, but rapidly arrest proliferation when nutrients are limited. Here, we molecularly define the mechanism that translates changes in nutrient status to FSC proliferation control. Specifically, we find that dietary cholesterol triggers release of Hedgehog (Hh) molecules sequestered on the surface of Hh-producing cells within the ovary. The resulting accumulation of Hh ligand in the FSC niche drives FSC proliferation. This mechanism enables a rapid, tissue-specific response to global changes in nutrient availability, thus tailoring ovarian stem cell divisions and egg production to environmental conditions that are sufficient for progeny survival.

Program/Abstract # 425
Notum/Wnt antagonism controls planarian brain patterning and size in regeneration.
Hill, Eric M.; Petersen, Christian P., Northwestern University, Evanston, United States
Adult regeneration perfectly restores the form of an injured animal. Therefore, regeneration must involve size-scaling mechanisms to ensure that new tissues are present in correct proportions. Planarians are bilaterally symmetric flatworms capable of regenerating any damaged tissue, including their central nervous system, so they must possess robust scaling mechanisms to appropriately regulate the extent of regeneration. We report a novel role for notum in regulating brain size in the planarian *Schmidtea mediterranea*. Notum proteins are conserved secreted hydrolases that have been proposed to modulate cell signaling by cleaving glycophosphatidylinositol (GPI) anchors and cause shedding of a subset of cell-surface proteins. Inhibition of notum by RNAi resulted in a net shrinking of the brain that involves formation of ectopic anterior neural structures (photoreceptors and brain tissue) and loss of posterior brain regions during regeneration of decapitated head fragments. notum mRNA is expressed at the anterior pole of the planarian brain, suggesting NOTUM may act as a positional cue to control organ size. In planarians and *Drosophila*, Notum proteins have been shown to inhibit Wnt signaling. A previously described planarian Wnt gene, wntA, is expressed in the posterior of the planarian brain, and wntA RNAi has been shown to cause a posterior enlargement of the brain. Simultaneous RNAi of notum and wntA in decapitated head fragments suppressed the highly penetrant notum RNAi brain phenotype, suggesting that notum antagonizes wntA function to control brain size. notum expression depends on wntA activity, suggesting that feedback inhibition along the AP axis of the brain provides robust control of organ size during regeneration.

Program/Abstract # 426
Head to heart: transformation of skeletal muscle stem cells to cardiac muscle
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The population of cells known as the secondary heart field, which forms the anterior parts of the heart in the vertebrate embryo, also contributes substantially to the mesoderm of the head and the skeletal muscles formed from it. We have found that the stem cells (satellite cells) of adult head muscles still retain the competence to form cardiomyocytes, whereas this is not the case for satellite cells from muscles in other parts of the body. We have isolated satellite cells from the jaw muscles of adult mice and treated with signaling molecules similar to those experienced by cells of the secondary heart field during cardiac development. Initially the cells upregulate transcription factor genes characteristic of cardiac development (e.g. *Nkx2.5, Isl1, Tbx1, Tbx5*), and subsequently they express markers of differentiated cardiomyocytes (e.g. *MLC2v, Cx43, Cav1.2, cTnT*). Some of the cells undergo spontaneous contraction. Action potentials are 100-300 msec in duration, similar to embryonic cardiomyocytes but much longer than skeletal muscle. They are sensitive to nifedipine, which blocks calcium channels in cardiomyocytes but not skeletal muscle. The cells will also participate in the formation of beating structures when co-cultured with embryonic heart from an early mouse embryo. When muscle satellite cells from leg muscles are similarly treated, there is no cardiac development. Instead, myogenic transcription factors characteristic of skeletal myogenesis are expressed as normal. This work has significance both for basic developmental biology and for translational medicine. For developmental biology it demonstrates that an adult stem cell population can retain competence from its

embryonic precursors to form an alternative cell type. For translational medicine it introduces a new possible source of cardiomyocytes, derived from an adult tissue, which might be used for drug testing or for therapeutic transplantation.

Program/Abstract # 427
Specification of the ascidian larval PNS
Zeller, Robert W.; Chen, Jerry; Tang, Joyce (San Diego State University, United States)
The ascidian larval peripheral nervous system (PNS) consists of a series of paired sensory neurons, called epidermal sensory neurons (ESNs) that run along the midline of the larva. Other laboratories have shown that both BMP and FGF signaling are required for the early specification of these cells and that the pattern of ESNs is further refined by Notch-Delta signaling. Here we show that the entire larval epidermis is neurogenic and can be converted into ESNs by the expression of the single ascidian Pou4 class transcription factor. In the context of a larger gene regulatory network (GRN) for the larval PNS, we have identified at least four microRNAs and at least four transcription factors that operate downstream of Notch-Delta signaling to pattern the larval ESNs. In *Ciona intestinalis*, the microRNA miR-124 is expressed in both the PNS and central nervous systems. We computationally identified miR-124 targets and show that key miR-124 targets include the Notch receptor, the three HES family genes that mediate Notch signaling and neutralized, which regulates Delta levels. We show that miR-124 feeds back on Notch-Delta signaling and plays a role in ESN specification. In addition to Pou4, we have identified at least three additional transcription factors that pattern the ESNs. By analyzing a matrix of gene expression patterns in transgenic embryos ectopically expressing these transcription factors we have established a temporal and spatial cascade of regulatory gene expression in the PNS. Interestingly, the microRNAs and transcription factors we have identified play roles in the specification in the hair cells of the vertebrate inner ear.

Program/Abstract # 428
Requirements for posterior growth in sequentially segmenting arthropods
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A key feature of the arthropod body plan is the presence of segments along the body axis. Evolution of segments has permitted broad diversification of arthropods. Most arthropods develop their segments in the same fashion: they add them one by one from the posterior in a region commonly called the “growth zone”. Although this is the most common mode of arthropod segmentation, the growth zone is mostly unstudied. We are studying the growth zone at the cellular level in two groups within the Pancrustacea, crustaceans: the branchiopod *Artemia* and *Thamnocephalus* and an insect: the beetle, *Tribolium*. Both exhibit features likely ancestral for their respective lineages: larval addition of segments in the branchiopods and sequential addition of segments in the beetle embryo. Recently, a common set of genes - caudal, even-skipped and Wnts – have been hypothesized to control the growth zone in diverse arthropods. We have characterized the dimensions of the growth zone and analyzed the behavior of cells relative to the dynamic patterns of gene expression in the posterior of the developing larvae and germ band. We are also completing a fate map of the blastoderm by tracing the fates of small clusters of cells subsequent to activation of a photo activatable dye. Our results provide evidence for divergent modes of sequential segmentation in branchiopods and beetles: in the branchiopod posterior growth contributes continuously to the segmentation process, whereas posterior growth makes a discontinuous contribution to the segmentation of the *Tribolium* germ band.

Program/Abstract # 429
A genetic circuit conferring robustness to dorsal patterning in *Drosophila*
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The robust and invariant development of the wild-type organism is widely thought to be adaptive and essential. Using quantitative analyses of dorsal patterning in the *Drosophila* embryo we have assessed the robustness of this event and determined the genetic mechanism required for invariance of wild type patterning. The DV axis of both arthropods and vertebrates is patterned by the activity of Bone Morphogenetic Protein (BMP) signaling. In *D. melanogaster* the BMP ligand Decapentaplegic (Dpp) is essential for the specification of dorsal fates in the pre-gastrular embryo. During cellularization, Dpp signaling is initially localized in a low intensity, broad dorsal domain due to the activity of extracellular ligand binding proteins. At the onset of gastrulation, the Dpp signaling domain is intensified and refined into a sharp stripe on the dorsal midline. Previous work indicated that positive feedback was required for the formation of this final signaling domain. Our current work characterizes and defines a component of the feedback circuit, and its interaction with a BMP signaling antagonist. We find that the combined activity of positive and negative factors leads to the

invariance of the BMP signaling domain in the D. melanogaster embryo. Additionally, we have found two Drosophila species which lack the early activity of these components and have consequently variable patterning. We hypothesize that this circuitry buffers against the inherent noise of the early embryonic patterning mechanism and genetic perturbations. Furthermore, the existence of species lacking such circuitry suggests evolutionary trajectories which can allow for relaxation of this buffering activity.

Program/Abstract # 430

Gastrulation in *Drosophila melanogaster* and *Drosophila pseudoobscura*: a comparison of folded gastrulation and T48 expression profiles.

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During gastrulation prospective mesodermal cells must be brought onto the inside of the embryo. In *Drosophila melanogaster* this requires precise spatial and temporal regulation of folded gastrulation (fog) and T48 gene expression. The fog and T48 gene products then activate Rho mediated cell signaling pathways. This in turn leads to constriction of the apical side of the cells, thereby initiating the internalization of the prospective mesoderm. This process of apical constriction and many of the molecular components involved are conserved in other morphogenetic events and in other species (including neural tube formation in vertebrates). However, direct homologs of fog and T48 in vertebrates have not been identified. We are interested in understanding the evolution of this morphogenesis pathway and have begun by identifying fog and T48 homologs in other dipterans. Sequence analysis of the identified fog homologs shows fog to be a rapidly evolving gene while T48 is evolving less rapidly (55 and 89% sequence similarity between *D. melanogaster* and *D. pseudoobscura* respectively). We have also analyzed the expression of fog and T48 in *D. pseudoobscura* embryos. Initial results show T48 expression to be conserved in the prospective mesoderm of both species at the onset of gastrulation. The temporal and spatial expression of fog is also conserved. However, the level of fog expression appears to be lower in *D. pseudoobscura* than in *D. melanogaster*. We are currently confirming these observations using Q-PCR and examining possible morphological consequences of lower fog expression. Ultimately we hope these studies will provide insight into the evolutionary processes that shape the developmental pathways of morphogenesis.

Program/Abstract # 431

Dissecting physiologically and developmentally relevant genetic regulation of mammalian chromosome biology with murine interspecific backcrosses, Y chromosomes, unstable inverted repeat (IR) Sry loci, sex reversal phenotypes, viral Oris and HJ-replication restart complexes: A synopsis

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Regulation of chromosome biology is conserved, chromosome/locus specific, with 875 genes modulating genomic stability in yeast (*S. cerevisiae*). Interspecific combinations of genomes and Y chromosomes result in rearrangements and uncoupling of Y linkage including in the sex determining ~200 kb Sxr-region spanning the 34 kb IR Sry locus/gene. Although Sry is required for testis determination between d10-d12.5, chimeric adult males (>90% XX somatic/testicular tissues) confirm its dispensability at this stage. Using this and 3 methods we have shown that high frequency instability of Sry, in all tissues, germlines, fertile males of all strains, is genetically modulated. As predicted by IR replication, a spectrum of deletion products/amplicons from the HMG cds/flanks is detected. A putative retrotransposon fusion ORF of SRY-HMG with (Cpa6) LINE-1 ORF1 (transposase-22) domains at a cryptic splice site joint and a partial ORF-2 (RT) has also been identified. Spectra of rearranged products at Sry and yeast IR are similar with those in *S.c.* being sites of meiotic/mitotic DSBs rarely processed into double Holliday Junctions (dHJ) recombination intermediates and their products. Dissolution or resolution of mitotic dHJ in *S.c.* is determined by the interactions of Sgs1 helicase/Top3 topoisomerase/Rmi1 heteromer with Rad51 - orthologous to mammalian complexes. EMSAs and 'Pull-Down' assays showed that Rmi1 and Top3/Rmi1 preferentially bind HJs over 10 structures representing recombinational or replicational intermediates with the dimer also stimulating Sgs-1 N domain/HJs complexes. Our data suggest a distinction between helicase and dHJ dissolution/resolution activities of Sgs1 imposed by DNA structure at stalled forks with the unloading of Sgs1 from dHJ followed by its dimer promoted reloading. Sxr instability is also consistent with its structural similarities

with fork retarding Replication Slow Zones RSZs/Fragile sites and cis suppressors of viral Oris. Systematization of chromosome biology by scoring lesions/microarray analysis in combinations of Collaborative Cross Strain genomes and Y chromosomes represents an unbiased integrative approach taking advantage of the breadth of evolutionarily selected dys-regulation.

Program/Abstract # 432
Mechanisms of ROS mediated longevity in *C. elegans*
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Aging is a natural process that occurs in most species and despite years of research, is a process which is still poorly understood. Previous research using model organisms has identified three pathways which can affect aging: caloric restriction, insulin/IGF-1 signalling, and mitochondrial signalling. In *C. elegans*, two mutations that affect mitochondrial electron transport chain subunits result in increased production of reactive oxygen species (ROS). Animals carrying these mutations have a significantly enhanced lifespan relative to the wild type. It has also been shown that treatment with pro-oxidants such as paraquat (PQ) can significantly increase wild type lifespan but has no effect on the lifespan of these mitochondrial mutants. Furthermore, treatment with anti-oxidants such as N-acetyl-cysteine (NAC) and Vitamin C can decrease the longevity of these mutants to the wild-type level. These and other results suggest that increased levels of ROS act as a signal to extend lifespan in C. elegans. In order to determine the mechanisms involved in ROS signalling, microarrays were performed on long-lived mitochondrial mutants and wild type animals treated with PQ. Between the two mitochondrial mutants tested, an 80% overlap of the genes that were upregulated was detected. Most significantly, 50% of the genes found in this overlap were also upregulated in the wild type animals treated with PQ. This pattern was also observed for downregulated genes. This suggests that the genes that were upregulated by both the mitochondrial mutations and by PQ (~500) are likely to be involved in ROS mediated longevity. An RNAi screen of the genes found to be commonly upregulated is currently being performed in the mutant backgrounds.

Program/Abstract # 433
Characterising the role of a regulator of G protein signalling in cranial sensory ganglia formation
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The cranial sensory ganglia, which are responsible for relaying a variety of sensations in the head, form in part by migration of neuroblasts delaminating from specialised thickenings of surface ectoderm called placodes. Here we investigate the molecular mechanisms governing neuroblast generation and migration from the placode in the developing chick embryo by characterising the role of a regulator of G protein signalling, RGS3. We initially identified RGS3 in a microarray screening for molecules upregulated during neuroblast migration. We show it to be specifically expressed in cranial sensory neuroblasts during ganglia formation. In addition, knockdown of RGS3 expression via short-hairpin RNA results unexpectedly in precocious extension of axon projections and neuroblast migration. Future work elucidating this function will include differential analysis of naturally-occurring isoforms of RGS3, as well as the broader role RGS3 may play in G protein signalling.

Program/Abstract # 434
The role of NFAT/calcium pathway during kidney development and polycystic kidney disease
Saban, Jeremy; Miller, Michelle; Corsini, Rachel; Iglesias, Diana; Goodyer, Paul (MCHRI, Westmount, Canada)
In 2005, Simons *et al*. proposed that during kidney development there is a switch from canonical to non-canonical Wnt signalling. Failure to do so could result in a cystic kidney phenotype. Our lab described that canonical Wnt signalling is high during normal kidney development and is down regulated once nephrogenesis is completed. This is also the case in two mouse models of polycystic kidney disease (PKD). We hypothesize that non-canonical Wnt signalling could be abnormally regulated in PKD. We studied the role of the NFAT/calcium non canonical pathway during kidney development and explored a potential role in PKD. We employed an NFAT-luciferase reporter mouse, where NFAT is the primary downstream signaling molecule of the Wnt-calcium pathway. We found that Wnt/calcium signaling peaks from embryonic day E13-E16 in wild type embryonic mouse kidneys. This reporter was responsive to non-canonical Wnts such as Wnt5a and Wnt11, and the effect of the Wnts could be abrogated by cyclosporin-A, a specific inhibitor of the pathway. To study the functional role of this pathway in the developing kidney, we examined Wnt-calcium signaling in different kidney cell lines, finding that turning on the Wnt-calcium pathway restricts cell motility, without affecting cell proliferation or apoptosis. In addition, *in situ* hybridization showed that Wnt-calcium signaling is located in the nephrogenic zone; however, preliminary data shows that activity seems to be located in the ureteric bud (UB) cells. This may indicate Wnt-calcium signaling is active at the UB tips and could be involved in branching morphogenesis. Preliminary data indicates that the pathway may be down regulated in a polycystic kidney disease mouse model.

Program/Abstract # 435
TNF regulates dual death pathways in mice at E10.5
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Caspase-8, the initiator caspase of the death receptor pathway of apoptosis, and its catalytically inactive homologue FLIP are both essential for embryonic development as these knockout animals die at E10.5 due to a failure of yolk sac vascularization. Recent studies show that ablation of RIPK3, a kinase that promotes a form of programmed, necrotic cell death, rescues development in caspase-8-deficient mice. In contrast, mice lacking both FLIP and RIPK3 still die at E10.5, but via apoptosis rather than necrosis, suggesting that inhibition of both death pathways is necessary for successful development. We investigated whether TNF triggered these dual death pathways at E10.5 via ablation of the death receptor Tnfr1 in both Casp8-/- and FLIP-/-RIPK3-/- mice. Both Casp8-/-Tnfr1-/- and FLIP-/-RIPK3-/-Tnfr1-/- mice survived past E10.5 until E15.5, implicating TNF as the regulator of E10.5 lethality and suggesting that others signals regulate both apoptosis and RIPK3-mediated necrosis later in development.

Program/Abstract # 436
Gata3 antagonizes prostate cancer progression through modulation of PI3K-Akt pathway
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Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer, which leads to activation of the PI3K/AKT pathway. Using a prostate-specific Pten-inactivation model, we show that the transcription factor Gata3 is lost primarily at the protein level during tumor progression. Using conditional loss- and gain of function approaches, we show that acute inactivation of Gata3 in these tumors accelerates tumor invasiveness, whereas enforced expression of GATA3 impedes tumor progression by preventing polarity loss, EMT and the loss of differentiation markers when Gata3 was present in the nucleus. In contrast, prostatic ducts expressing cytoplasmic or no Gata3 developed carcinoma. We show that in this model GATA3 acts by preventing PI3K/Akt pathway upregulation. In turn, the PI3K/Akt pathway plays a role in Gata3 cytoplasmic localization, thereby generating a mutual repression system between Akt signalling and Gata3 that provides a molecular rationale for Gata3 inactivation during prostate cancer progression. Remarkably, a similar mislocalization of GATA3 was observed in human prostate tumors, where GATA3 is lost or sequestered to the cytoplasm in the majority of castrate-resistant tumors. In addition, GATA3 expression level in prostate tumors prior to treatment holds predictive value for tumor recurrence. Together, these results conclusively identify GATA3 as a critical player in prostate homeostasis and cancer progression.

Program/Abstract # 437
Exploring the roles of two ciliary genes, *Cluap1* and *Ccdc42*, in mammalian development and reproduction
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Cilia and flagella are highly conserved microtubule structures found on most eukaryotic organisms. These organelles are essential to human development, and the study of cilia genes has advanced our understanding of this organelle's fundamental roles in development. Two genes, *Cluap1* and *Ccdc42*, have been identified as potential mammalian cilia genes. However, their functions have not been explored. Here we demonstrate that *Cluap1* is widely expressed in both the embryonic and adult mouse. Furthermore, *Cluap1* is necessary for both ciliary assembly and embryonic development. Interestingly, *Ccdc42* null mice, do not display obvious developmental defects, but rather show profound defects in flagella assembly of the developing spermatogonia, rendering males infertile. Studies of cilia and flagella in mammals will continue to shed light on how they serve as signaling hubs in development as well as demonstrate their more specialized roles.

Program/Abstract # 438
Prdm13, a direct target of Ptf1a, executes neuronal specification in dorsal spinal cord
Chang, Joshua C.H., UT Southwestern Medical Center Neuroscience, Dallas, United States

Generating a balanced network of inhibitory and excitatory neurons during development requires precise transcriptional control. Ptf1a, a basic helix-loop-helix (bHLH) transcription activator, maintains this delicate balance by inducing

homeodomain (HD) transcription factors like Pax2 that specify the inhibitory GABAergic lineage while suppressing HD factors like Tlx1/3 that specify the excitatory glutamatergic lineage in the dorsal spinal cord. Utilizing deep sequencing of chromatin immunoprecipitation (ChIP-seq) and gene expression profiling (RNA-seq) in Ptf1a mutant versus wildtype neural tubes, we identified Prdm13, a putative chromatin-remodeling zinc finger transcription factor, as a direct downstream target of Ptf1a for explaining how Ptf1a can suppress gene expression. Prdm13 is lost in Ptf1a domains in the Ptf1a null mouse. In both gain and loss of function experiments in chick neural tube, Prdm13 phenocopies Ptf1a by inducing $Pax2^{+}$ /GABAergic neurons and suppressing the $Tlx1/3^{+}$ /glutamatergic neurons. This neuronal cell-fate specification function by Prdm13 requires the zinc finger domains and transcriptional repressor activity. Epistasis experiments confirm Prdm13 functions downstream of Ptf1a, and Ptf1a requires Prdm13 for its function in neuronal specification. Furthermore, Prdm13 regulates this neuronal specification through directly repressing transcription of dI5/dILB lineage genes through DNA binding activity. This activity of Prdm13 acts antagonistically to the bHLH factor Ascl1, which at this stage directs progenitors to the Tlx1/3 glutamatergic lineage. Our findings demonstrate that Prdm13 is a novel component of a highly coordinated transcriptional network necessary to mediate the balance of inhibitory versus excitatory neurons generated in the dorsal neural tube.

Program/Abstract # 439

Sulfatase 1, an extracellular regulator of the motoneuron to oligodendrocyte cell fate choice in the ventral spinal cord

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In the developing vertebrate spinal cord, oligodendrocyte precursor cells (OPCs) mainly originate from ventral neural progenitors of the pMN domain, marked by Olig2 expression. These progenitors first generate motoneurons (MNs) and switch to an OPC fate after completion of MN generation. We previously evidenced that Sulfatase 1 (Sulf1), a secreted endosulfatase, is upregulated in ventral neural progenitors immediately prior to OPC specification. Sulf1 is known to regulate the sulfation state of heparan sulfate proteoglycans (HSPGs), extracellular matrix molecules involved in regulating various signaling pathways. To assess Sulf1 function in the MN/OPC switch, we recently analyzed OPC development in mice lacking Sulf1 function. Our results clearly showed that specification of ventral OPCs is severely affected in Sulf1-/- mutant mice. Indeed, the efficiency of OPC induction is reduced, only few pMN progenitors switch to an OPC fate while they continue to generate MNs passed the normal timing of the MN/OPC switch. Moreover, using chick spinal cord explants, we showed that the deficiency in OPC production in Sulf1 loss of function is not a consequence of an early phenotype but results from the timely regulated expression of Sulf1. Finally, we bring arguments supporting that Sulf1 controls the MN/OPC switch by regulating Shh activity. Our work then establish that Sulf1 is a major component of the mechanisms that cause neural progenitors of the pMN domain to stop producing neurons and switch to an OPC fate.

Program/Abstract # 440

Removal of Polycomb Repressive Complex 2 makes *C. elegans* germ cells susceptible to direct conversion into specific somatic cell types

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How specific cell types can be directly converted into other distinct cell types is a matter of intense investigation with wide-ranging basic and biomedical implications. We have recently shown that the removal of the histone chaperone LIN-53 (called Rbbp4 and Rbbp7 in vertebrates) permits ectopically expressed, neuron-type-specific transcription factors (terminal selectors) to convert C. elegans germ cells directly into specific neuron types. How the LIN-53 protein protects the germ cell genome from being converted was unclear since histone chaperones like LIN-53 function in a number of distinct, chromatin-related processes, including nucleosome assembly, remodeling, and various types of gene activation and repression events. We show here that the function of LIN-53 in the germ cell to neuron conversion process can be phenocopied by loss of members of the histone 3 lysine 27 (H3K27) methyltransferase complex PRC2. Terminal selector-induced germ cell to neuron conversion can not only be observed upon genome-wide loss of H3K27 in PRC2(-) animals, but also upon genome-wide redistribution of H3K27 in animals which lack the H3K36 methyltransferase MES-4. Manipulation of the H3K27 status not only permits neuronal terminal selector-dependent conversion of germ cells into neurons, but also permits hlh-1/MyoD -dependent conversion of germ cells into muscle cells, indicating the PRC2 protects the germline from the aberrant execution of multiple distinct somatic differentiation programs. Taken together, our findings demonstrate that the normally multi-step process of development from a germ cell via a zygote to a terminally differentiated somatic cell type can be shortcut by providing an appropriate terminal selector transcription factor and manipulating histone methylation patterns.

Program/Abstract # 441

Regulatory logic of pan-neuronal gene expression in *C. elegans*

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The adult *C. elegans* nervous system consists of 302 neurons grouped in 118 anatomical classes. The morphological and functional diversity of mature differentiated neurons of each class is reflected in their different molecular composition. However, all neurons in a nervous system have common characteristics namely cellular projections and synapses. The molecular correlates to those common features are encoded by pan-neuronal genes. The regulatory programs that govern pan-neuronal gene expression are poorly understood. In this study we have defined a group of genes consisting of synaptic proteins and neuronal-specialized cytoskeleton components that are broadly expressed in the nervous system and we are using promoter bashing analysis and fosmid recombineering technology to address how these genes are transcriptionally regulated. We have found that most of the genes under study are indeed expressed throughout the nervous system of the adult hermaphrodite worm but are also expressed in other tissues. Preliminary analysis on the temporal expression pattern shows that some of these genes are only expressed once postmitotic neurons have been born. In addition, promoter bashing analysis suggests a piecemeal model for the transcriptional regulation of the genes in combination with redundant elements for spatial expression. While there is probably not a common regulatory logic among all genes we investigate the possibility that functionally related genes might be regulated in a common manner. Progress on this analysis will be presented.

Program/Abstract # 442

Intercellular calcium signaling in a gap junction-coupled cell network establishes asymmetric neuronal fates in *C. elegans*

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The nervous system has an immense variety of neuronal cell types and subtypes that derive from a limited number of progenitors. One general way to diversify neuronal subtypes is to specify different identities and functions of individual cell types through stochastic neuronal fate choices. However, the mechanisms that generate stochastic cellular diversity in the nervous system are only partly understood. The *C. elegans* left and right AWC olfactory neurons specify asymmetric subtypes, one default AWC^{OFF} and one induced AWC^{ON}, through a stochastic, coordinated cell signaling event. Intercellular communication between AWCs and non-AWC neurons via a NSY-5 gap junction network coordinates AWC asymmetry. However, the nature of intercellular signaling across the network and how individual non-AWC cells in the network influence AWC asymmetry is not known. Here, we demonstrate that intercellular calcium signaling through the NSY-5 gap junction neural network coordinates precise $1AWC^{ON}$ /$1AWC^{OFF}$ decision. We show that NSY-5 gap junctions in *C. elegans* cells mediate small molecule passage. We expressed vertebrate calcium buffer proteins in groups of cells in the network to reduce intracellular calcium levels, thereby disrupting intercellular communication. We find that calcium in non-AWC cells of the network promotes AWC^{ON} fate, in contrast to the autonomous role of calcium in AWC to promote AWC^{OFF} fate. In addition, calcium in specific non-AWC promotes AWC^{ON} side biases through NSY-5 gap junctions. Our results suggest a novel model in which calcium has dual roles within the NSY-5 network, autonomously promoting AWC^{OFF}, and non-autonomously promoting AWC^{ON}.

Program/Abstract # 443

Voltage- and calcium-activated BK potassium channels establish left-right neuronal asymmetry in *C. elegans*

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Many highly specialized cells must derive from a limited number of progenitors during nervous system development. An example of neuronal diversification is establishment of asymmetric gene expression across the left-right (L-R) axis, which occurs in the *C. elegans* AWC olfactory neuron pair. Expression of the odorant receptor str-2 in AWC is random: either the right or left AWC expresses str-2 to become AWC ON, and the other cell becomes AWC OFF. The default AWC OFF fate is executed by a Ca 2+ -regulated kinase cascade that is activated by influx of Ca 2+ through the voltage-gated Ca 2+ channel UNC-2/UNC-36. Intercellular communication between the AWCs and other neurons through the NSY-5/innexin gap junction network induces AWC ON fate. nsy-5 may antagonize the Ca 2+ kinase cascade by inhibiting unc-2/unc-36 activity, but how signals from gap junctions are transmitted to Ca 2+ channels is unknown. Changes in membrane potential can be propagated by gap junctions, and voltage affects L-R asymmetry in *Xenopus* embryos. To determine if voltage plays a role in establishing AWC asymmetry, we investigated the role of the voltage-and Ca 2+ -activated BK potassium channels in AWC asymmetry. BK channels are outward-rectifying ion channels that play major roles in neurotransmission, but little is known about their role in cell fate determination. We find that two BK channels redundantly promote AWC ON

fate, potentially acting downstream of nsy-5 to antagonize Ca 2+ channel activity by decreasing voltage across the membrane. These results establish a novel role for BK channels in neuronal fate diversification.

Program/Abstract # 444
Sox genes in *C. elegans*: sox-2 role in postembryonic development
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Sox proteins are a highly conserved family of transcription factors involved in several developmental processes. Expression of SoxB genes correlates with the commitment of cells to a neural fate; however, the relevance of SoxB proteins in early vertebrate neurogenesis has been difficult to prove genetically due to embryonic lethality and presumed redundant functions. The nematode *C. elegans* has only 5 sox genes: sox-2 and sox-3 form the SoxB group while sem-2, sox-4 and egl-13 belong to other Sox groups. Our results show that sox-2 and sem-2 are the sox genes expressed earliest and in a broader manner during embryogenesis, being expressed in several neuronal progenitors. sox-3, sox-4 and egl-13 are expressed in few cells during late embryogenesis, when most neurons are already born. Both sox-2 and sem-2 null mutants are early larval lethal but do not show neuronal specification defects during embryonic development as indicated by quantification of a panneuronal reporter. Potential redundancy or compensatory mechanisms between different sox genes have been ruled out, strongly suggesting that sox genes are not required for specification of embryonically-derived neurons. However, at the first larval stage there are still several blast cells that will give rise to different postembryonic lineages, which generate several neurons amongst other cell types. Interestingly, sox-2 is expressed in many of these progenitor cells. Using mosaic analysis we have so far identified neurons derived from two different postembryonic lineages which fail to be generated in C. elegans sox-2 mutants. These results support the idea that postembryonic progenitor competence is compromised in the absence of sox-2.

Program/Abstract # 445
The induction of pluripotent mesoderm from axolotl animal caps by Brachyury and BMP-4
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We developed axolotl embryos as a model system to investigate the development of primordial germ cells (PGCs) from pluripotent cells. Axolotls are a model for the tetrapod ancestor and so accurately represent the amphibian ancestor to mammals. As such, they share the gene regulatory networks (GRN) that control mammalian development, including the GRN for pluripotency. In normal development axolotl PGCs develop in a posterior mesodermal compartment that acts as a germ cell niche. Importantly, this tissue is not conserved in *Xenopus* or zebrafish embryos, and so little is known about how it is specified. We showed that forced expression of Brachyury and BMP-4 induces pluripotent mesodermal tissue that will give rise to neural tissue, including a neural tube, in addition to PGCs, and other mesodermal cell types. In normal development this domain is induced by FGF and BMP signalling, in the absence of Nodal signals. We refer to it as pluripotent mesoderm, which is distinct from the typical somatic mesoderm that is induced by Nodal, and we postulate that it is homologous to the posterior mesoderm compartment that is responsible for axial elongation in mice. Discrimination between pluripotent mesoderm and somatic mesoderm is governed by Nanog activity, and it represents one of the earliest cell fate decisions in vertebrate embryogenesis. We are using differential transcriptomics to identify the GRN that governs development of this novel mesodermal tissue, and we are working to understand how FGF signalling interacts with Nanog to control its development.

Program/Abstract # 446
FGF signaling is required for lineage restriction but not onset of primitive endoderm program in the mouse blastocyst
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Emergence of pluripotent epiblast (EPI) and primitive endoderm (PrE) lineages within the inner cell mass (ICM) of the mouse blastocyst occurs in several steps involving initial co-expression of lineage-specific markers, subsequent mutually-exclusive marker expression and salt-and-pepper distribution of lineage-biased cells, and sorting of lineage-committed cells into respective layers. Precisely how EPI and PrE lineage commitment occurs is not entirely clear, however FGF/ERK signaling appears to be required. To gain insight into the role of FGF signaling we investigated the phenotype resulting from zygotic and maternal/zygotic Fgf4. Fgf4 heterozygous blastocysts exhibited increased numbers of EPI cells and reduced numbers of PrE cells suggesting that FGF signaling must be tightly regulated to ensure appropriate cell numbers for each lineage. Fgf4 mutants lacked PrE entirely; their ICM comprised exclusively NANOG-expressing cells. Notably, co-expression of EPI and PrE marker was initially established, even in the absence of FGF4. Thus, Fgf4 mutant

embryos showed normal onset of the PrE program but exhibited a defect in its restriction phase, where lineage bias is acquired. Sustained exogenous FGF4 failed to rescue the mutant phenotype. Instead, depending on concentration, we noted no effect, or conversion of all ICM cells to GATA6-positive PrE. This suggested that paracrine signaling and local availability of FGF results in a salt-and-pepper distribution of lineage-biased cells. Our data also revealed that XEN (eXtraembryonic Endoderm) cells could be derived from Fgf4 mutant embryos in which PrE had been restored, showing that FGF signaling is essential for PrE lineage choice but dispensable for XEN cell maintenance.

Program/Abstract # 447
A putative role for Yap1 phosphorylation during trophoblast differentiation in the laboratory opossum, *Monodelphis domestica*
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A pluriblast-trophoblast differentiation process in the unilaminar blastocyst of the laboratory opossum, Monodelphis domestica, commences on the fifth day of development, shortly after the blastomeres have adhered to the zona pellucida. This process is associated with the mutually exclusive expression of Oct4 and Cdx2 proteins in the pluriblast and trophoblast, respectively. Yap1 expression mirrors this pattern, being confined to the cytoplasm in the pluriblast cells but only in the nuclei of trophoblast cells. This suggests that, as in the mouse embryo, Yap1 is a transcription co-activator of the trophoblast-specifying protein Cdx2. Nuclear entry by Yap1 can occur when the protein is in its unphosphorylated state, permitting it to activate Cdx2. Conversely, phosphorylated Yap1 (pYap1) is unable to enter the nucleus, preventing it from carrying out its function. We tested the hypotheses that pYap1 expression in opossum blastocysts a) is confined to the cytoplasm of opossum pluriblast, and b) is not be observed in the trophoblast cells. Our results indicate that, as expected, pYap1 is undetectable in the pluriblast nuclei. However, pYap1 is reliably detected in trophoblast cells, specifically in a small restricted domain within the trophoblast nuclei. This suggests the complexity of Yap1 protein interactions, namely that Yap1 may gain nuclear entry irrespective of its phosphorylation status.

Program/Abstract # 448
O-fucosylation regulates zebrafish dorsal-ventral patterning by inhibiting BMP signaling
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Glycosylation, the modification of proteins with sugars, plays very important roles in all biological processes. O-fucosylation, which adds fucose to the serines or threonines of a wide variety of cell surface and secreted proteins, impacts nearly every stage of vertebrate development. However, early embryonic lethal phenotypes of homozygous mutants disrupting O-fucosylation prevent access to its contribution to later developmental processes. Therefore, our understanding of O-fucosylation function during early development is still sketchy. Many critical questions, including which signaling pathways and how O-fucosylation modulates their activity remain unclear. To address these questions we have developed a hyper-O-fucosylation model by overexpressing the endoplasmic reticulum GDP-Fucose transporter, slc35c2. Embryos treated with slc35c2 showed clear dorsalization phenotypes. When combined with excess GDP-fucose, treated embryos showed significant increases in the severity and penetrance of the dorsalization phenotypes, while GDP-fucose alone caused no phenotypes. The balance between BMP signaling and its antagonists are known to pattern the dorsoventral axis of zebrafish embryos. We observed that inhibition of BMP signaling underlies dorsalization caused by excess O-fucosylation. Diminished BMP signaling was evident in lessened Phospho-Smad1/5 level and reduced expression of ventral fates regulators, including transcription factors and BMP ligands from the earliest stages examined. Reciprocally, BMP antagonist expression was expanded. Furthermore, excess O-fucosylation induced dorsalization could be suppressed by overexpression of BMP7a and BMP2b ligands. Together these data indicate O-fucosylation regulates dorsal-ventral patterning in zebrafish by modulating BMP signaling.

Program/Abstract # 449
Role of acyl Co-A synthases in *Drosophila* embryonic development
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Gastrulation is one of the earliest observable morphological events which transform a blastula into a multilayered embryo with three germ layers. A key morphogenetic event in *Drosophila* gastrulation is ventral furrow formation and it leads to the internalization of mesodermal precursors. In order for the ventral furrow to form, cells destined to form the mesoderm constrict, shorten, flatten and then shift towards the interior of the embryo. Ventral furrow formation marks the beginning of gastrulation, and the cellular movements as well as the molecular players involved in this process are conserved across species. While genetic analysis has revealed the genes involved in the specification of mesodermal cells, many of the effector molecules that mediate cytoskeletal rearrangements during ventral furrow formation remain unknown. A unique opportunity to understand this patterning/effector gene interface is offered by bubblegum (bgm), an acyl Co-A synthase

involved in *Drosophila* ventral furrow formation. We have shown that bgm is transcriptionally regulated by products of the patterning genes dorsal, twist and snail, each critical to formation of the mesoderm. In turn, bgm likely contributes to the maintenance of optimal levels of cellular very long chain fatty acids and thus to cellular cytoarchitecture. We have shown using in situ hybridization studies that bgm transcripts are expressed in the ventral furrow forming cells. Importantly, in embryos lacking both maternal and zygotic contributions of bgm, cells undergoing ventral furrow formation move in an uncoordinated fashion and the embryos fail to gastrulate. Thus, our studies have identified a prominent molecular player in gastrulation.

Program/Abstract # 450
Timing of southpaw initiation in lateral plate mesoderm is altered in ccdc40 and pkd2 morphants
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Pkd2 is a geneimplicated in human Autosomal Dominant Polycystic Kidney Disease. In addition to kidney defects, mutations and knockdowns of pkd2 in mouse and zebrafish disrupt normal left-sided nodal expression in the lateral plate mesoderm (LPM). Although pkd2 has been shown to be important in creation and initial restriction of nodal expression domains to the left LPM, the link between pkd2 and the initiation of nodal expression is unknown. To probe the functions of PKD2 in embryonic left-right patterning we examined the effects morpholino knockdown of the gene has on the timing of the zebrafish nodal-related gene southpaw expression in the LPM. PKD2 protein appears to be important for correct timing of propagation of southpaw expression as well as unilateral restriction. Interestingly, knockdown ofCCDC40, a protein involved in correct cilia assembly and rotation, results in randomized expression of nodal in the LPM and a similar delay in southpaw expression. We use this approach of southpaw expression timing to elucidate the functions of different proteins in their roles establishment of left-right asymmetry.

Program/Abstract # 451
The Integrator Complex Subunit 6 is a negative regulator of the vertebrate organizer
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Dorsal-ventral patterning relies upon the mutual antagonism of competing signaling pathways to establish a balance between ventralizing BMP signaling and dorsal fate specification mediated by the organizer. In zebrafish, the initial embryo-wide domain of BMP signaling is refined into a morphogenetic gradient following activation of a maternal Wntpathway leading to nuclear accumulation of β-cateninon the dorsal side of the embryo, repression of BMP signaling dorsally, and theinduction of dorsal fates mediated by the Nodal and FGF signaling pathways. Incontrast, the Wnt8 pathway operating zygotically limits dorsal fatespecification and maintains the specification of ventrolateral non-axialmesoderm. We have isolated a recessive dorsalizing maternal-effect mutation affectingthe Integrator Complex Subunit 6 (Ints6), a member of a complex whose only reportedrole is to mediate 3' end processing of spliceosomal snRNAs. Due to widespread de-repression of dorsalizing genes, embryos born to mutant mothers fail to maintain expression of BMPligands, fail to maintain operation of the Wnt8 pathway, display delayed cell movements during gastrulation, and become severely dorsalized. Limitation of Nodalsignaling or the restoration of BMP signaling completely rescues the patterningof affected embryos. Preliminary data indicate that Ints6 is required on thedorsal side of the embryo to restrict organizer gene expression. We are engaged in experiments to determine if snRNA processing is affected in mutant embryos. Furthermore, we hope to determine whether Ints6 participates in embryonic patterning independently of the Integrator Complex or whether our data indicatea new role for this RNA processing machine.

Program/Abstract # 452
PIAS-like protein Zimp7 is required for Zebrafish organizer formation and dorsal mesoderm development
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Human ZIMP7 protein and its homolog ZIMP10 were initially identified as androgen receptor co-activators. Analysis of their sequence revealed the presence of an SP-RING/Miz domain, which is highly conserved in members of the PIAS family and confers SUMO-conjugating activity. The ZIMP proteins also interact with transcription factors such as p53 or Smad3/Smad4 and with BRG1, the catalytic subunit of the SWI-SNF remodeling complex. Accordingly tonalli, the drosophila orthologue of the Zimp genes, was shown to interact with subunits of the Brahma complex. Mutations in tonalli produce flies with homeotic phenotypes. In zebrafish zimp7 is ubiquitously expressed in embryos from one-cell up to bud stage. In this study we set out to analyze the role of zygotic Zimp7 in the early stages of zebrafish development. We found evidence indicating that Zimp7 is required for the formation of the dorsal organizer and for dorsal mesoderm development. The dorsal organizer is a structure that acts as an inducer of dorsal cell fates and as a regulator of dorsoventral axis

patterning. Signaling pathways involved in these processes include Wnt, Nodal and Bmp. We found that loss of zygotic Zimp7 with a morpholino results in dorsalization of embryos and upregulation of markers of the organizer and mesoderm (including boz and squint). In contrast overexpression of zimp7 by mRNA injection, leads to repression of these same genes and causes axial mesoderm defects resembling previously described mutants that affect the organizer such as ichabod and boz. Loss of dorsal expression of the no tail gene and the presence of cyclopia in the gain-of-function situation suggest that Zimp7 could interact with the Nodal-related pathway.

Program/Abstract # 453
Direct visualization of retinoic acid gradients in zebrafish embryos
Sosnik, Julian; Gratton, Enrico; Schilling, Thomas, University of California Irvine, United States;

Retinoic acid (RA) is a derivative of vitamin A that plays important signaling roles in development, regeneration and disease. Because it is not synthesized *de novo*, tracking and determining its endogenous localization has been elusive. The only data available for the distribution of RA are indirect, based on the localization of proteins involved in its synthesis, transport, signaling and degradation, or based on reporters that indicate RA responses. To address this we have taken a novel approach using Fluorescent Lifetime Image Microscopy (FLIM) in combination with a phasor approach to data analysis. Due to RA's unique lifetime of fluorescence, this methodology allows us to make direct observations in vivo of its endogenous distribution in developing embryos. FLIM analysis of zebrafish embryos during gastrulation and somitogenesis revealed higher levels of RA in somites where it is synthesized by Aldh1a2, and levels were reduced in Aldh1a2 mutants. In addition, we observed graded reductions both anteriorly, across the presumptive hindbrain, as well as posteriorly in the presomitic mesoderm. These observations are consistent with previous models in which RA acts as a graded morphogen both in hindbrain and somite segmentation. These measurements strongly suggest that the graded responses previously observed using transgenic reporters form at the level of the RA ligand itself. Taken together, these data constitute some of the first direct observations of the endogenous distribution of RA in vivo in the developing embryo. Present work includes the use of FLIM in the analysis of the crosstalk between RA and other signaling pathways and their effects in modulating the spatial and temporal distribution of RA.

Program/Abstract # 454
The role for proteoglycans in regulating early embryonic patterning and morphogenesis
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Growth factors and morphogens regulate embryonic patterning, cell fate specification, cell migration, and morphogenesis. The activity and behaviour of these signalling molecules are regulated in the extracellular space through interactions with proteoglycans. Proteoglycans are high molecular-weight proteins consisting of a core protein with covalently linked glycosaminoglycan (GAG) sidechains, which are thought to mediate the proteoglycan-ligand interaction. UDP-glucose dehydrogenase is an enzyme required for the synthesis of GAGs. Through germline replacement, I have generated a maternal zygotic Ugdh mutant (MZ jek) to determine the requirement for proteoglycans in the developing embryo. In accordance with mouse and Drosophila ugdh mutants, Fgf signalling is reduced in MZ jek. Intriguingly, MZ jek possess left-right patterning abnormalities, characterized by bilateral lefty2 expression in the lateral plate mesoderm. The midline appears to be intact in the mutant embryos, when examined by insitu hybridization for shh , no tail , and lefty1 gene expression. Kupffer's Vesicle formation and ciliogenesis proceed normally in MZ jek , generating the necessary stereotypical counterclockwise flow required for symmetry breaking. Examination of calcium signalling in the wildtype embryos reveals unilateral phosphorylation of CamKII on the left side of the KV, a kinase that becomes activated in response to increases in intracellular calcium levels. Interestingly, in mutant embryos P-CamKII is found on both sides of the KV, possibly representing bilateral calcium influxes and subsequent improper Nodal pathway activation.

Program/Abstract # 455
The role of HSPG in anterior-posterior axis formation.
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Nodal and Bmp signaling play a central role in establishing the anterior-posterior axis of embryo. These growth factors act spatially and temporally in embryo. Heparan sulfate proteoglycans modulate activity of growth factors such as TGF-β, Nodal, Bmp, Fgf, Wnt and Shh during various developmental stage by controlling extracellular stabilization, movement and retention both on the cell surface and on the extracellular matrix. Here, we investigated the candidate of heparin sulfate proteoglycans regulating the Nodal and Bmp signal in anterior-posterior axis formation. We found two new candidate genes in regulating of anterior-posterior axis.

Program/Abstract # 456
Thoracic primary afferents bundle in segmentally distinct patterns during longitudinal extension in the embryonic avian spinal cord
Ezerman, Elizabeth B.; Forehand, Cynthia, University of Vermont, Burlington, United States
As primary afferents grow into the spinal cord, they extend rostrally and caudally across multiple levels before entering the grey matter to make segmental connections. We have asked whether the primary afferent projections from different thoracic segmental levels of the chicken embryo intermingle as they extend longitudinally or remain in separate bundles. Specifically, do the rostral projections from separate levels bundle together or do all the rostral projections from a single segment bundle together and remain separate from those of other segments? How do the caudally projecting axons from a rostral segment relate to the rostrally projecting axons of a caudal segment? What happens at the dorsal root entry zone (DREZ) where entering fibers encounter those extending longitudinally from other segments? To investigate these questions, we have used several colors of fluorescent dextran amines to label sequential spinal nerves (SPN) or ganglia (DRGs). Distinct segmental (color) domains are seen some distance from the DREZ when adjacent segments are labeled. Closer to the DREZ, there is apparent intermingling of fibers. When projections from two ganglia are labeled and the fiber associations examined rostral to both ganglia, the rostral projections from the more rostral ganglion reside in a more ventrolateral position. Conversely, examination of the caudal projections caudal to both ganglia shows that the caudal projections of the more rostral ganglion are dorsomedial within the bundle. There is intermingling of fibers entering at a DREZ with both rostrally and caudally projecting axons running longitudinally through the DREZ.

Program/Abstract # 457
Role of zebrafish Vangl2, a Wnt/Planar Cell Polarity pathway component, in cell behaviors underlying convergence and extension gastrulation movements
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Initially discovered in *Drosophila*, where it is required for the organization of planar epithelial polarity, the PCP pathway is evolutionarily conserved in vertebrates. In contrast to its well-described function in the relatively static *Drosophila* epithelia, during vertebrate gastrulation, Wnt/PCP pathway is required for the regulation of the highly dynamic mesenchymal cells behavior. However, how Wnt/PCP pathway functions in this dynamic context remains a challenging open question. Zebrafish embryos carrying mutations in core PCP genes present characteristic phenotypes with shorter and wider body axes, a consequence of perturbed convergence and extension movements. During zebrafish gastrulation cell behaviors change in a stage and domain specific manner. From mid- to late gastrulation stages, mesodermal cell behavior changes from slow dorsal convergence of individual cells to fast convergence of tightly packed and highly polarized cells. In the zebrafish Wnt/PCP mutant trilobite, carrying a mutation in the vangl2 gene, the slow dorsal migration of mesodermal cells is normal. At late gastrulation, mutant cells fail to elongate and their movement is slow and less effective. We show that Vangl2 protein is recruited at the cell membrane at a specific time during gastrulation, correlating with modification of cell behavior. Interestingly, our cell behavioral analysis shows that the transition in cell behavior is a complex process with two independent steps: the cell shape modification and the cell body alignment along the embryonic axes.

Program/Abstract # 458
The function of Sox11 in neurogenesis
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Sox proteins comprise a sub-family of transcription factors that contain an HMG (high mobility goup) DNA-binding domain. They have various roles during development, including in chondrogenesis, sex determination and neural development. Based on the amino acid sequence similarity of the HMG domains, Sox proteins are divided into groups A-H. Sox11 belongs to the SoxC group of HMG-box transcription factors and recent studies have indicated that the SoxC group factors function downstream of the proneural bHLH proteins and are necessary for neuronal maturation in the chick spinal cord. To investigate the role of Sox11 in primary neurogenesis, we analyzed the result of loss-of -Sox11 function in the frog *Xenopus laevis*. These studies reveal that loss of Sox11 function decreases the expression of proneural genes and markers of mature neurons and slightly increases that of neural progenitor markers. Thus Sox11 is required for neuronal maturation but may function upstream of the proneural proteins. With this information, we will investigate how the level of Sox11 is controlled such that a population of neural progenitors is maintained for later development and only a subset of cells differentiate during primary neurogenesis.

Program/Abstract # 459

Identifying the critical amino acids of SOBP that mediate interaction with the transcriptional regulator *Sine oculis*

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In *Drosophila*, *Sine oculis* binding partner (SOBP) is a novel protein that has been shown to interact with *Sine oculis* (SO) both *in vivo* and *in vitro* (Giot et al., 2003; Kenyon et al., 2005). While the exact function of SOBP remains unknown, both SO and SOBP are co-expressed in the eye-antennal imaginal disc, posterior to the morphogenetic furrow. Taken together, these data suggest that the interaction between the two proteins may have functional consequences for the development of the fly visual system. In this study, we identified a specific subset of amino acids within SOBP that appear to facilitate its interaction with the Six type protein interaction domain of Sine oculis. Based on this determination, the known expression patterns of SOBP, and inherent characteristics of the SOBP protein, we propose several potential functions for the SO/SOBP complex within the developing *Drosophila* eye. In addition, we tested *Xenopus* homologues of each factor in the yeast system; initial results indicated that these interactions are conserved in frogs. This research may have relevant implications for mammalian development given the conserved nature of SOBP across phyla.

Program/Abstract # 460

A role for Casz1, a homolog of the *Drosophila* fate determination gene Castor, in murine retinal development.

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Background: During neural development, different types of neurons and glia are often generated in stereotyped sequences. For example, in the embryonic murine retina, retinal ganglion, horizontal, and amacrine neurons, as well as cone photoreceptors are generated first. Postnatally, progenitors then generate rod photoreceptors, bipolar neurons, and Müller glia. The temporal control of progenitor competence is best understood in *Drosophila*, where a cascade of transcription factors progressively controls daughter cell identity. This cascade includes the zinc-finger transcription factor Castor. A single orthologous gene, Casz1, is conserved in vertebrate genomes. Here, we elucidate the function of Casz1 in murine retinal development. Results: Casz1 mRNA and protein begins to be expressed in retinal progenitors prior to birth. Casz1 becomes strongly expressed in photoreceptors and some bipolar cells postmitotically. Progenitors transduced with Casz1-expressing retroviruses generate clones that exhibit similar size versus controls, suggesting that Casz1 does not significantly alter progenitor cell cycle parameters or cell death. However, Casz1 misexpression skews the cell-type composition of the resultant clones. When Casz1 is misexpressed in early-stage retinal progenitors, late-born photoreceptors and bipolar cells are disproportionately generated at the expense of early cell types and Müller glia. In accordance with gain-of-function experiments, RNAi-mediated knockdown favors Müller glia at the expense of photoreceptors and bipolar cells. Conclusions: Casz1 may control the competence of retinal progenitors to generate late-born neuronal types in a manner analogous to the temporal fate determinant Castor in *Drosophila*.

Program/Abstract # 461

Distinct lineage-specific roles for GLI3R mediated control of ureteric induction, branching morphogenesis, and urinary tract patterning.

Blake, Josh, University of Toronto, Toronto, Canada; Rosenblum, Norman (SickKids, Toronto, ON, Canada)

The transcription factor GLI3 is proteolytically cleaved to a transcriptional repressor (GLI3R) in the absence of Hedgehog signaling (HH). Loss of Sonic Hedgehog ligand in the developing mouse embryo causes renal agenesis or a single ectopic hypodysplastic kidney and implicates GLI3R as a negative regulator of urinary tract formation. Yet specific events perturbed by GLI3R remain undefined. $Gli3^{\Delta 699/\Delta 699}$ obligately express GLI3R and demonstrate marked renal hypoplasia (100%) and a double collecting system (~47%) at E15.5 (n=12) with hydronephrosis at E18.5 (100%, n=16). Renal hypoplasia is preceded by ureteric bud (UB) hypoplasia at E10.5 & E11.5 and reduced UB branching at E12.5 (51%, n=16). Double collecting systems arise from two primary UBs (67%, n=6) in E11.5 Hoxb7-cre; $Rosa^{lacZ/+}$; $Gli3^{\Delta 699/\Delta 699}$ mice. Cranial Wolffian duct to bud-site lengths indicated normal positioning of one UB with cranial ectopic positioning of the second (p=0.0026). All UBs failed to maintain position caudally with the CND at E11.5 (p=0.004) and were associated with blind-ended ureters at E16.5 (100%, n=6). UB specific expression of GLI3R in E15.5 *Hoxb7-cre;*$Gli3^{TFlag}$ embryos caused renal hypoplasia (100%, n=14) without duplex collecting system. Moreover, mesenchyme specific expression of GLI3R in E15.5 *Pax3-cre;*$Gli3^{TFlag}$ embryos caused renal agenesis. Our data demonstrate that GLI3R acts in the ureteric lineage to control bud size and branching morphogenesis and in non-ureteric lineages to control ectopic budding and

ureteric insertion into the bladder. Furthermore, mesenchyme-specific expression of GLI3R controls UB induction. Our findings identify multiple and distinct lineage-specific roles for GLI3R in early urinary tract development.

Program/Abstract # 462
Stromal signals regulate differentiation of the kidney progenitor population.
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Kidney development in mammals requires reciprocal interactions between the ureteric bud and the adjacent metanephric mesenchyme. Signals from the mesenchyme promote branching morphogenesis of the bud epithelium, while reciprocal signals from this bud epithelium are necessary for the survival and renewal of a progenitor population within the mesenchyme. A signal(s) from the bud also induces a sub-population of the progenitors to undergo a mesenchymal-to-epithelial transition (MET) and differentiate into epithelia that will give rise to the kidney nephrons. We identified Wnt9b as signal that is necessary for both progenitor renewal and differentiation. Here, we address the mechanism whereby the same molecule induces two seemingly contradictory processes. We show that signals from overlying stromal fibroblasts modify the progenitor cells' response to Wnt9b, determining whether they will proliferate or differentiate. This stromal signal is mediated, at least in part, by the atypical cadherin Fat4. Utilizing these two opposing signals, differentiation and growth of nephron progenitors is balanced, assuring proper organ size and function.

Program/Abstract # 463
ß-catenin controls branching morphogenesis via the Gdnf Ret Signaling axis during kidney development
Sarin, Sanjay, Hamilton, Canada; Li, Aihua; Bridgewater, Darren (Hamilton, Canada)
During kidney development, branching morphogenesis gives rise to the collecting duct system through growth, elongation and branching of the ureteric epithelium. The ureteric epithelial induces the metanephric mesenchyme (MM) to undergo nephrogenesis, the formation of nephrons. A failure in these two embryonic processes leads to renal dysplasia, the major cause of childhood renal failure. β-catenin is normally expressed in the MM and over-expressed in renal dysplasia suggesting an important role in kidney development. We generated a mouse model with β-catenin deficiency exclusively in the metanephric mesenchyme. Mutants demonstrated renal hypoplasia by E13.5 and reduced ureteric branching. In-situ hybridization of E11.5 kidneys revealed a marked reduction in Gdnf expression, but no changes in Ret or Wnt11, molecules required for ureteric branching. To support β-catenin controls branching morphogenesis via regulating Gdnf expression we generated a model of β-catenin over-expression exclusively in the metanephric mesenchyme. Mutants displayed severe renal dysplasia and the formation of 4-6 ectopic kidneys. Analysis revealed marked abnormalities in ureteric budding and branching. *In situ* hybridization of E11.5 kidneys demonstrated increased Gdnf, Ret and Wnt11 expression. A ChIP assay revealed that β-catenin directly bound to a TCF consensus binding site in the Gdnf 5' untranslated region. Molecular cloning of this 4.9kb fragment upstream of a luciferase gene revealed β-catenin regulates gene transcription from this site. Taken together, these data establish that β-catenin has an essential role in the metanephric messenchyme to guide ureteric budding and branching through the regulation of the Gdnf signaling axis.

Program/Abstract # 464
Integrin-linked kinase (ILK) controls ureteric bud (UB) gene expression via p38MAPK-dependent and -independent mechanisms
Smeeton, Joanna M.; Rosenblum, Norman, SickKids Toronto, Canada
In mammals, the renal collecting system is derived via growth, branching, and remodeling of the UB, a process termed renal branching morphogenesis (RBM). The intracellular signaling pathways that control RBM are largely undefined. Previously, we demonstrated that ILK controls UB branching via p38MAPK in vitro and is required for RBM in vivo (Leung-Hagestejn et al, Mol Cell Biol, 2005; Smeeton et al, Development, 2010). To identify genetic targets of Ilk that regulate RBM, we used whole transcriptome analysis of E12.5 kidneys from mutant mice with Ilk-deficiency in the UB and from controls. Microarray analysis identified 227 differentially expressed mRNA transcripts ($p<0.003$). Correlation with the Genito Urinary Development Molecular Anatomy Project database (gudmap.org) identified 14 UB-enriched genes whose expression was downregulated in mutant kidneys. Quantitative RT-PCR confirmed decreased expression of Ilk, critical gene targets in the Ret/GDNF pathway (Wnt11, Sox8, Myb, CXCR4) as well as 3 less well-characterized UB-specific genes (SCF, Krt23, Slco4c1). In embryonic mutant kidneys, phosphorylation of p38MAPK was decreased by 70% ($p=0.004$). The functional requirement for p38MAPK activation in Ilk-mediated gene expression was investigated via p38MAPK inhibition in kidney explant culture. p38MAPK inhibition did not alter Sox8, Myb or CXCR4 mRNA expression but significantly decreased expression of Wnt11 (50%), SCF (45%), Krt23 (85%), and Slco4c1 (58%) ($p<0.05$). We conclude that ILK regulates downstream gene targets through both p38MAPK-dependent and -independent

pathways to control UB branching and that ILK regulates of key components of the Ret/GDNF signaling pathway in the developing UB.

Program/Abstract # 465

Hox6 genes are important niche factors that play critical roles in the proper formation and maintenance of the pancreas

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Despite significant advances in our understanding of endocrine pancreatic development, the function of the pancreatic mesodermal niche in this process is less well understood. However, niche factors are necessary for proper endocrine development and are likely to be of critical importance in designing successful regenerative therapies aimed at replacing lost islet cells in diabetic patients. Preliminary data generated in our laboratory demonstrates a critical role for Hox6 genes in pancreatic organogenesis. Hox6 genes are expressed exclusively in the pancreatic mesenchyme (not epithelium) and suggest a primary role for Hox6 genes in proper development of the pancreatic niche. The phenotypic abnormalities observed in our Hox6 triple mutants confirm this, as total pancreatic volume in mutants is reduced compared to littermate controls and there is a drastic reduction of hormone-producing cells. Early pancreatic specification appears to be unaffected, however, as the total volume of epithelial progenitor cell population is unchanged in Hox6 mutants at early budding stages. Hox6 triple mutant pancreata also exhibit abnormal epithelial branching and reduced epithelial cell proliferation. Finally, while triple mutants die shortly after birth, surviving compound mutants exhibit hyperglycemia and impaired responses in glucose tolerance tests. Moreover, these defects are exacerbated with age and, as Hox6 genes remain expressed in the pancreas through post-natal and adult stages, suggest that Hox6 genes may contribute to post-natal endocrine maintenance as well. Overall, these data suggest that Hox6 genes are critical pancreatic niche factors, necessary for the proper development and maintenance of pancreatic organogenesis.

Program/Abstract # 466

An unexpected role of the vagal enteric neural crest cells on digestive smooth muscle differentiation

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The gastrointestinal (GI) tract is a vital organ, highly conserved across species, which motility is ensured by the correct coordination of the visceral smooth muscle cells and the autonomous enteric nervous (ENS) system. Understanding the molecular processes that govern the differentiation of these cell types and their interrelationships during development could offer insight into the mechanisms altered in human GI motility disorders. The GI musculature is initially composed of splanchnic mesoderm. As development proceeds, the splanchnic mesoderm differentiates into 4 distinct concentric layers among which the smooth muscle. How the radial differentiation of the gut mesenchyme is established during development remains to be clarified. Concomitant with the patterning of the mesenchyme across the radial axis, the GI tract is colonized by the vagal enteric neural crest cells (vENCC) to establish its innervation. Previous reports suggested that the coalescence of neurons and glia cells into ganglion plexuses coïncides with the differentiation of smooth muscle. However, whether vENCC has an impact on smooth muscle cells differentiation remains to be studied. Here, we first examined and compared the spatiotemporal expression pattern of smooth muscle and ENS genes during avian GI tract development. We found that in the stomach, smooth muscle differentiation is initiated after vENCC migration. In contrast, in the colon, smooth muscle differentiation is initiated well before vENCC migration. These results suggest that visceral smooth muscle does not follow a rostrocaudal gradient of differentiation along the AP axis. Finally, we show that a reduction in vENCC number by neural tube ablation at level 3-6 somites impairs smooth muscle differentiation in the stomach, suggesting a positive role of the vENCC in this process.

Program/Abstract # 467

Multiple roles of Polycomb Ezh2 in regulating cerebellum development

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Polycomb group (PcG) proteins control cellular fate and developmental processes by repressing gene expression during embryonic development. This repression is accomplished by modifying the chromatin structure around developmental genes. The PcG protein Enhancer of Zeste Homolog 2 (Ezh2) tri-methylates lysine 27 of histone H3 (H3K27me3). Here we report that loss of Ezh2 in dorsal neural tube leads to a series of developmental defects in the developing mouse cerebellum, including premature differentiation of inter-neurons at the expense of Purkinje cells. These developmental defects ultimately result in a smaller cerebellum with a less intricate foliation pattern and behavioral abnormalities on the adult. The growth defect of mutant cerebellum is mainly attributed to a proliferative defect of granule precursor cells that give rise to the largest number of neurons-granule neurons. The proliferation deficiency is likely due to the up-regulation

of p16, a known cyclin kinase inhibitor normally suppressed by H3K27me3 at embryonic stages. The less number of Purkinje cells also means the less production of mitogen Shh postnatally, which affects the sustaining proliferation of granule precursor cells and ultimately leads to the hypoplastic growth of mutant cerebellum. RNA-Seq analysis of embryonic cerebellums revealed that the pan neuronal marker Neurogenin1 (Ngn1) is consistently down regulated at early embryonic stages in the mutant cerebellum, which accounts for the defective neurogensis of interneurons and Purkinje cells. Along with the downregulation of Ngn1, several micro-RNAs that are predicated to target Ngn1 are up-regulated in the mutant cerebellum. We are currently investigating how regulatory networks governed by Polycomb group proteins control the spatial and temporal neurogenesis in the cerebellar ventricular zone. Taken together, our data indicate that Ezh2 not only controls the proliferation of granule precursor cells but also ensures proper sequential generation of Purkinje cells and interneurons.

Program/Abstract # 468

Numb mediated trafficking of Cyclic Nucleotide-Gated Channel to rod photoreceptor sensory

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The photoreceptor cell has aspecialized primary cilium, the outer segments (OS), which is a stack of disk membranes surrounded by a plasma membrane that serves as an exclusive housing unit for a specific pool of proteins involved in phototransduction. OS are critical for photoreceptor cell function and mutations in genes that affect the development and integrity of OS often lead to photoreceptor degenerative diseases in humans. The mechanisms regulating protein sorting to the OS remain unclear. Recent studies have shown that Numb, an endocytic adapter protein, plays a part in regulating directional protein trafficking in migrating cellsand neurons, raising the possibility that it might be involved in OS protein sorting. Consistent with this hypothesis, we found that Numb is expressed in a highly polarized manner exclusively in the calycal process of the photoreceptor, where protein sorting takes place. To study the role of Numb in photoreceptors, we have conditionally inactivated Numb and Numblike (Nbl; a homolog of Numb) in photoreceptors using a specific Cre mouse line (Opsin-Cre). Analysis of the conditional double knockouts (cDKO) revealed that the spatial arrangement of the OS was severely disrupted, which ultimately leads to retinal degeneration. Interestingly, the cDKO show abnormal accumulation of an OS plasma membrane protein, the cyclic nucleotide gated channel A1 (CNGC-A1), in cell bodies and inner segments of photoreceptors before retinal degeneration. Importantly, OS proteins localized to the disk membranes were not affected, suggesting a specific function for Numb in the localization of OS plasma membrane proteins. Our results indicate that Numb function is required for polarized protein sorting to the plasma membrane of OS in mouse photoreceptors, and identifies the Numb pathway as a potential player in photoreceptor function and degeneration in humans.

Program/Abstract # 469

Tissue specific Porcupine deletion reveals a novel role for ectodermal Wnts in musculotendon development

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The Wnt family of secreted proteins consists of 19 family members (in the mouse) and are known to signal through multiple pathways that regulate crucial processes in the development of almost all tissues. Dissecting the roles of individual Wnts has been hampered due to functional redundancy that exist between family members. We made use of a conditional allele of the O-acyltransferase, Porcupine (Porcn), that is required for the secretion of all Wnt ligands and the Msx2Cre deleter to eliminate the secretion of all Wnt ligands from the ventral limb ectoderm, ventral abdominal ectoderm, and urogenital ectoderm. Interestingly, newborn mice lack ventral digital tendons and the most superficial musculature in the regions of strongest and earliest deletion. Molecular analysis indicates that the initial defect is a loss of TCF4 positive muscle connective tissue. This leads to the secondary defect of a loss of musculature in the most heavily affected regions. Tendons are patterned normally initially and then are lost once they reach a developmental stage where they must interface with musculature. Thus we show a requirement for ectodermal Wnts in the induction of muscle connective tissue.

Program/Abstract # 470

The microRNA pathway and its central role in the hypoxia response in *Drosophila melanogaster*

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The hypoxia-inducible factors (HIFs) are a highly conserved family of transcription factors that constitute the major regulators of cellular oxygen homeostasis throughout the animal kingdom. We have previously defined a hypoxia-

responsive system in *Drosophila* that is homologous to mammalian HIF. Even though the core HIF pathway and a few canonical regulators have been broadly characterized latterly, a systematic loss of function screen has not been performed so far. Hence, we have conducted an unbiased genome-wide RNAi screen in *Drosophila* cells aimed to the identification of genes required for HIF dependent transcription. One remarkable hit was the *argonaute 1* gene, a central element of the microRNA (miRNA) machinery. Further analysis confirmed an absolute requirement of the miRNA machinery for the hypoxic response suggesting a physiological role of miRNAs in HIF activity. Our next goal is to identify the miRNAs and its mRNA targets responsible for regulating the transcriptional response to hypoxia. We are currently carrying out a reporter gene-based screen using transgenic *Drosophila* lines that over-express all the miRNA genes known in the Drosophila genome. We have tested 98 microRNAs so far and we have already identified three miRNAs whose over-expression enhances the expression of the reporter gene. The characterization of the miRNAs involved in regulating the hypoxic response and the identification of its target mRNAs will allow us to further understand the complex regulation of dHIF activity *in vivo*.

Program/Abstract # 471
Dicer1 knock down in the Sim1 domains affects mouse survivability
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Although micro-RNAs (miRNA) play an important role during the development of the brain, little is known about the function of miRNAs in the developing paraventricular nucleus (PVN) of the hypothalamus, a major regulator of homeostatic processes. Previous studies have shown that miRNA processing protein DICER is produced in the PVN. In order to determine whether miRNA processing is required for PVN development, we generated mice that were homozygous for a dicer conditional mutation and hemizygous for a Sim1-cre transgene, which is expressed in the PVN. We found that dicer mutant expressing cre die shortly after birth. We performed detailed pathological studies of the tissues expressing Sim1, including the brain, heart, kidney, pancreas and skeletal muscle. We found that the kidneys contained numerous cysts whereas the other tissues were histologically normal. We next analyzed the expression of several markers of the paraventricular nucleus (Ot, Avp, Trh, Crh) as well as the expression of a Sim1tau-lacZ knock-in allele in various domains of the developing brain. This marker analysis did not show any abnormalities. These studies suggest that micro-RNA processing is not required for the development of the PVN. The dicer mutant mice expressing cre appear to die because of cystic kidneys.

Program/Abstract # 472
WT1 is critical for the normal development of the peripheral taste system
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Despite the importance of taste in determining nutrient intake, genetic analyses of the processes that control taste cell development are lacking. Several early markers of taste cell development have been identified including Sonic-Hedgehog (Shh), Bone Morphogenetic Protein 4 (BMP4) and members of the Wnt signaling pathway. However, the regulation of these factors is not well understood. The developing tongue is useful to study development paradigms such as cell-fate determination and epithelial-mesenchymal interactions. Recent work in our laboratory has identified a critical role for the Wilms' tumor 1 protein (WT1) in taste cells. WT1 is a transcriptional regulator that is required for the normal development of multiple systems, including olfactory epithelia and retinal ganglia. In addition, several genes that are essential for normal taste cell development are known WT1 target genes. Since WT1 is important in epithelial-mesenchymal transitions, it may be important in the formation of the peripheral taste system. The focus of this study is to elucidate the role of WT1 in the development of the peripheral taste system. In mice lacking WT1, the peripheral taste system fails to develop normally. Chromatin immunoprecipitation (ChIP) and gene expression analyses were used to identify the target genes of WT1 that are important for taste cell development. When WT1 expression levels are reduced, expression of WT1 target genes, including Lef1 and Ptch1, that have key roles in peripheral taste development are dysregulated, supporting an important role for WT1 in the taste system. Our data identify WT1 as a new transcription factor that has an important role in regulating the development of the peripheral taste system.

Program/Abstract # 473
Identifying key early activators of Rax expression using transient BAC transgenesis in *Xenopus tropicalis*
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The retinal anterior homeobox (rax) gene product is a key transcription factor for specifying retinal tissue and is necessary for proper eye development. Null mutations in this gene in vertebrates result in the loss of eye formation and are lethal. A

rax mutant was recently found in *Xenopus tropicalis* via a target-selected TILLING screen, and is the first mutation of its kind in *Xenopus*. Similar to other vertebrates (human, mouse, and zebrafish), homozygous mutant animals from this line fail to form eyes, most fail to complete metamorphosis and all die before reaching sexual maturity. We have developed a fast, efficient method of transgenesis for use in *Xenopus* using Bacterial Artificial Chromosomes (BACs) to transiently drive specific and accurate transgene expression, and use this method to drive a rax-GFP fusion transgene capable of rescuing the mutant phenotype. One goal of this work is to identify the transcriptional activators leading to the initial gene expression of master eye genes like rax. We, along with others, have identified a highly conserved region upstream of the rax proximal promoter that appears to be necessary for initial rax activation. This region contains a number of conserved putative transcription factor binding sites, but it remains unclear which sites are necessary for rax activation, as this has not been tested systematically using an in vivo, functional assay. In these experiments we use our BAC transgenesis rax rescue assay to identify key transcription factor binding sites necessary for early rax activation by individually mutating highly conserved sites found in the proximal upstream enhancer and testing each contruct's ability to rescue the mutant phenotype.

Program/Abstract # 474
Imprinting centre acts simultaneously as promoter for lncRNA-mediated epigenetic silencing and insulator function in vivo
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Several imprinted genes are silenced on the paternal allele by the large non-coding RNA (lncRNA) Kcnq1ot1 on mouse Chr7. We described a GFP insertional allele in this domain, termed Tel7KI, behaving as a maternally expressed genes. The silencing of Tel7KI occurs in post-implantation embryos. Imprinting of the paternal is recapitulated in differentiating mESC where a role for the lncRNA Kcnq1ot1 was suggested via the introduction of a targeted deletion of the lncRNA promoter (IC2) in cis of the GFP allele in +/Tel7KI mESC. The +/Tel7KI-IC2KO mESC undergo an epigenetic switch and fail to silence the GFP reported in embryoid bodies. We have now obtained a meiotic recombinant between the Tel7KI and IC2KO alleles, bringing both alleles in a cis configuration. Paternal transmission of the Tel7KI-IC2KO haplotype provided *in vivo* confirmation that IC2 is required for the silencing of the paternal Tel7KI since we observed an epigenotype switch on the paternal Tel7KI-IC2KO haplotype. Consequently embryos of two different genotypes can express the GFP: Tel7KI/+ and +/Tel7KI-IC2KO. Surprisingly, these two genotypes exhibit different tissue-specific expression patterns in post-implantation embryos. We propose a model in which IC2 simultaneously plays a dual role: (i) as the imprinted Kcnq1ot1 promoter, and (ii) as a paternal allele-specific insulator element, regulating the expression patterns of linked loci. Whether the IC2 boundary element is also implicated in the epigenetic silencing of Kcnq1ot1 targets remains to be tested. Our work has important implications for the structure and evolution of imprinted centres and established Tel7KI as a sensitive reporter for postulated chromosomal looping during murine development.

Program/Abstract # 475
Proteolytic carving of the mammalian head by the Taspase1-TFIIA-CDNKN2A Axe
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The build of a mammalian head represents one of the most elegant creations by the Mother Nature and demands unimaginably complex yet precise signaling events, explaining our current limited understanding of this developmental process. Here our genetic and biochemical data indicate that Taspase1 a protease functions as a key orchestrator which cleaves TFIIA a so-called general transcription factor to assure the needed rapid expansion of forebrain during embryogenesis. Specifically, we discovered that both p16/Ink4a and p19/Arf are aberrantly upregulated in mice that are either knocked out of Taspase1 or knocked in of non-cleavable TFIIA. Most remarkably, the exhibited severe craniofacial defects can be largely rescued by genetic deficiency of the CDKN2A allele that encodes both p16/Ink4a and p19/Arf. Interestingly, the genetic non-cleavage of both MLL1 and MLL2, two known Taspase1 substrates, did not incur conspicuous craniofacial defects. Altogether, our study demonstrates a critical craniofacial program, which emanates from Taspase1 through TFIIA to CDKN2A that involves a highly conserved protease, a general transcription regulator, and a key cell cycle regulatory genetic locus. In conclusion, this sophisticated proteolysis-transcription-division circuit apparently constitutes a previously unknown critical genetic framework buried in the contemporary genetic construction blueprint for a mammalian head.

Program/Abstract # 476
Ectoderm-mesoderm separation is controlled through selective repulsion generated by specific pairs of ephrins and

Eph receptors.

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The physical segregation of embryonic tissues is a fundamental process in the development of multicellular organisms. Its perturbation leads to severe developmental defects, especially during phases of extensive cell movements, such as gastrulation. In vertebrate embryos, this process often depends on ephrins and Eph receptors. We have previously shown that ephrin/Eph-mediated repulsive signals generate cycles of adhesion and de-adhesion at the ectoderm/ mesoderm boundary. This mechanism explains how the two tissues can maintain intimate contacts with each other without mixing. The system is astonishingly complex, with both ectoderm and mesoderm expressing a variety of ephrins and Ephs, which could in principle signal and induce repulsion between cells within each tissue. Co-expression of ephrins and Ephs within the same cells has been also observed in the neural tissues, but how signaling is integrated under such conditions has remained largely unresolved. We present here a simple explanation for the strict confinement of repulsion to the ectoderm-mesoderm interface. We identify selective functional interactions between pairs of ephrins and Ephs, and we show that the asymmetric expression of these pairs is sufficient to account for selective cell detachments across the boundary. Ephrin-Eph signals also occur within the tissues, but their intensity is below the threshold required to disrupt adhesive bonds. These results provide a paradigm for how developmental systems may integrate multiple cues to control precisely morphogenetic processes, which may be applicable to other complex situations such as brain development.

Program/Abstract # 477

The role of Wnt9b-signaling in kidney development

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Acute kidney injury is a frequent clinical complication with high mortality. Recently it has been shown that the adult kidney retains some of the original progenitor cells. To understand how these cells may participate in the repair of nephron damage, it is crucial to understand how progenitor cells develop into nephrons during embryonic life. As the ureteric bud penetrates the column of mesenchymal progenitor cells, each branch tip secretes a soluble protein, WNT9b, a canonical WNT signaling molecule, which induces nearby mesenchymal progenitor cells to cluster into a tight “cap” of cells that differentiate into a single nephron. Only the immediate layer of renal progenitor cells responds to the WNT9b signal. This implies that the ureteric bud may control responsiveness to its WNT9b signal. We hypothesize that this is accomplished by release of microvesicles (MV) from the ureteric bud, carrying both, receptor and ligand. Frizzled (FRZ) proteins comprise a family of G protein-coupled receptors that transduce WNT signals. We discovered that renal progenitor cells from embryonic mice are responsive to WNT9b signals from co-cultured embryonic mouse ureteric bud cells, but not to co-cultured mouse fibroblasts that express WNT9b in the absence of FRZ4 and FRZ8, two main putative candidates. By RT-PCR we found that HEK293 cells did neither express FRZ4 nor FRZ8. Co-transfection of these cells with canonical WNT-reporter and co-culture with LWNT9b cells, that have been co-transfected with FRZ4 or FRZ8, lead to an increase in reporter activity. By immunoblotting we could demonstrate FRZs in the MV fraction of the transfected cells. This suggests that co-transfer of Wnt9b and FRZ receptors is required for canonical WNT signaling.