International Society of Developmental Biologists

17th International Congress of Developmental Biology

Cancun, Mexico
June 16 - 20, 2013

72nd Annual Meeting of the Society for Developmental Biology
VII Latin American Society of Developmental Biology Meeting
XI Congreso de la Sociedad Mexicana de Biologia del Desarrollo

ABSTRACTS

Program/Abstract # 1

Mechanical force controls chemical signals in creating plant pattern

Elliot Meyerowitz (Caltech, USA)

One pattern generated by the stem cells at the tip of each plant shoot – the shoot apical meristem – of flowering plants has held a fascination for generations of biologists and mathematicians. This is the phyllotactic pattern, the pattern of leaves and flowers around the stem. The most common such pattern is the spiral phyllotactic pattern, which creates the highly recognizable organization of compound fruits such as pineapples, of flowers like roses, and of inflorescences such as sunflowers. The model plant *Arabidopsis thaliana* also has a spiral phyllotaxis, and we have used genetic, genomic, and cell biological approaches to learn in detail how the cells of the meristem collaborate to generate this pattern. The major chemical signal is auxin, which has a specific transport system, in which a family of plasma membrane proteins directs the efflux of the hormone from cells. The efflux proteins are not uniformly distributed, thereby causing efflux directionally, leading to a net flow of auxin in complex patterns across the surface of the meristem. Auxin not only induces new primordia of leaves and flowers, but also changes the physical properties of the cell wall. These physical changes alter the stress pattern in the meristem surface, which in turn regulates the position of the auxin efflux carrier in anisotropically stressed cells. The feedback between auxin concentration and physical stress creates the dynamic auxin patterns that cause successive auxin peaks at positions approximately 130-140 degrees around the stem, creating the spiral (and other patterns of) phyllotaxis. The stress pattern in the meristem also regulates the microtubule cytoskeleton of meristematic cells, and consequently it may also dictate the plane of cell division. The stress pattern may also determine directions of cellulose synthesis in the cell wall, and thus the subsequent direction of cellular growth, and the anisotropy of cells – leading to changes in auxin flow, and, consequently, feedbacks on the stress pattern.

Program/Abstract # 2

Unusual patterns of Hox cluster evolution

Peter Holland, Ferdinand Marletaz, Laura Ferguson, Jordi Papas, (Oxford, UK), Fei Xu (Chinese Academy of Science, China), Willie Taylor (NIMR, UK), Pete Olson (Nat Hist Museum London, UK)

In the 1980s, the discovery of Hox gene clusters in very different animals paved the way for an integrated science in which principles and processes of embryonic development could be compared between widely divergent evolutionary lineages. A picture has emerged of a conserved Hox gene cluster pattering the ancient head-to-tail axis across all bilaterian animals. Yet there are modifications, and these differences between species may be very helpful in our attempts to link genotype evolution to phenotype evolution. Using examples from on-going research, I will discuss intriguing examples of Hox cluster breakage, Hox cluster expansion and Hox cluster shrinkage.

Program/Abstract # 3

Regulation of gene expression by RNA polymerase II promoter pausing during mouse embryonic development

Megan Jane Wilson (Univ. of Otago, New Zealand)

Proximal-promoter pausing by RNA polymerase II (RNA-polII) is a key rate-limiting step in transcription initiation. Recent genome-wide studies using chromatin-immunoprecipitation to detect stalled RNA-polII have shown that promoter-pausing occurs for a number of genes, particularly developmental control genes. This stalling is believed to be a mechanism of gene regulation, causing RNA-polII to be paused near a promoter region, ready to respond to environmental or developmental cues. Two transcription elongation factors DSIF and NELF control promoter stalling by RNA-polII. Our laboratory studies sex-specific differentiation of developing mouse embryo tissues and we have utilized this system to study RNA polII stalling and its effect on gene expression over key developmental stages. Using ChIP-seq with antibodies against RNApolII, we identified many promoters that have RNA-polII stalled in a sex-specific manor in both gonad and head tissue at 13.5 dpc. This corresponded to differences in gene expression between the sexes for the associated gene transcript (as assayed by qPCR). For some transcripts, RNA polII stalling marked them for future activation at a later time point in development. In other cases, paused RNA polII was associated with genes that were becoming down regulated. This data also reveals that promoter pausing can occur differently between sexes during development. We also have preliminary evidence that indicates that components of NELF and DSIF complexes are expressed differently between the sexes during embryogenesis. Together our data suggests that some genes are being poised to respond to a signal and then strongly upregulated, whereas transcription at other genes is stalled but not activated, perhaps in the absence of an appropriate signal.

Program/Abstract # 4

Withdrawn

Program/Abstract # 5

Characterization of human developmental enhancers: from whole genomes to single SNPs

Alvaro Rada Iglesias (Univ. of Cologne, Germany)

Distal regulatory elements, such as enhancers, play a preponderant role in the establishment of cell-type and developmental-stage specific gene expression profiles. However, these elements are difficult to identify, since they lack strong defining features and show limited sequence conservation. In the first part of my talk, I will summarize my postdoctoral work, in which epigenomic approaches were used to characterize the enhancer repertoire of pluripotent (i.e. human embryonic stem cells (hESC)) and multipotent (i.e. human

neural crest cells (hNCC)) human embryonic cell populations. In hESC, we uncovered a unique chromatin signature that identifies a novel class of enhancers, which are inactive but poised in hESC and that become active upon differentiation in a lineage-specific manner. Similarly, our epigenomic approach allowed us to characterize enhancers in hNCC, a hitherto largely inaccessible and biochemically intractable vertebrate-specific embryonic cell population that contributes to the formation of multiple tissues and organs, such as the peripheral nervous system and most of the facial bones and cartilages. Using the sequence information contained within hNCC enhancers, we uncovered NR2F1 and NR2F2, two orphan nuclear receptors, as novel neural crest and craniofacial regulators. Finally, I will briefly describe how the genomic characterization of human enhancers in relevant cell types might streamline the identification of functional non-coding genetic variants, which can have far-reaching implications in our understanding of the genetic basis of human complex diseases and human morphological evolution.

Program/Abstract # 6

Super-resolution imaging of regulatory chromatin dynamics in developing embryos

Alistair Boettiger, Xiaowei Zhuang (Harvard, USA)

The differentiation of embryonic cells into their appropriate developmental fates is mediated in part by fine scale structural changes to chromatin. Developmental specific transcription factors may shape this chromatin structure through a variety of mechanisms, such as re-positioning of histones (e.g. to restrict or increase access to DNA) or the generation of higher-order looped chromatin structures (e.g. to facilitate looping of distal regulatory sequences to target sites). These fine scale structural changes are mostly too small (10s of nanometers) to be observed with conventional microscopy techniques (limited to several hundred nanometer resolution), and have so far evaded in vivo observation in intact embryonic tissue. We present super-resolution imaging techniques which allow for the detection of changes in chromatin on the scale of tens of nanometers in developing embryos at gene locations of interest. We can detect locus-specific clusters of modified histones and regulatory chromatin proteins, and resolve sub-diffraction structural details of the chromatin region of interest. Because chromatin states are studied within the intact embryo, cell identity and the spatial relation of the cell to its neighbors and embryonic signals are still maintained. This allows us to follow how these structures and their protein composition change as a cell progresses through different stages of development, or as its daughter cells diverge into different fates. This approach allows a detailed view of regulatory modifications at the single cell level. Single cell analysis facilitates inference of causal relations between expression states and modification. It also allows for variation between identical populations to be measured and the frequency of each state within the population to be determined.

Program/Abstract # 7

HoxA genes regulation in developing limbs: from long-range control to cross-regulation

Marie Kmita (IRCM, Canada)

In most developing embryos, genes of each Hox cluster are activated sequentially in time and their expression patterns are differentially distributed along the main body plan. A similar “collinearity rule” applies in evolutionary novel structures such as limbs. Thorough analysis of HoxD genes regulation has revealed numerous remote enhancers triggering HoxD expression in limbs. In contrast to HoxD genes, HoxA expression does not follow the “collinearity rule” in limbs as Hoxa13 and Hoxa10 are robustly expressed distally while Hoxa11 is completely excluded from this domain. Such discrepancy between HoxA and HoxD expression raises the possibility that the regulation of HoxA genes in distal limb is distinct from that of HoxD genes. Mapping of genomic loci bound by proteins enriched at active enhancers allowed us to locate several putative enhancers, the transcriptional activity of which was confirmed using transgenic assays. These enhancers were tested for potential interaction with Hoxa13 using Chromosome Conformation Capture Carbon Copy (5C), which identified 10 remote enhancers “contacting” Hoxa13 . In addition, we identified one enhancer located in Hoxa11 intron. Interestingly, previous work uncovered the existence of Hoxa11 antisense RNA in distal limbs raising the possibility that this antisense transcription prevents Hoxa11 expression distally. By deleting Hoxa11 intron, we found that disruption of the antisense transcription results in ectopic Hoxa11 sense transcription in distal buds. We also found that Hoxa13 and Hoxd13 proteins trigger this antisense transcription revealing a cross-regulation mechanism in the control of Hoxa11 expression. Our data will be discussed in the context of limb development and evolution.

Program/Abstract # 8

Cell shape and morphogenesis: sub cellular and supra-cellular mechanisms

Matteo Rauzi, Uros Krzic, Timothy Saunders, Lars Hufnagel, Maria Leptin (EMBO, Germany)

The invagination of the ventral furrow during gastrulation in Drosophila is probably the best-studied example of epithelial folding. The genes that control the process are known, as are the mechanisms by which their products mediate the cell shape changes that bring about the formation of the furrow. While the ventral cells act autonomously to create the initial furrow, the other parts of the embryonic epithelium must participate to accommodate these changes, and perhaps to contribute to the evens that lead to the complete internalisation of the mesoderm. To be able to correlate the behaviours of all cells in the embryo with those of the invaginating mesoderm, and compare their shape changes and movements, it is necessary to follow them in the living embryo. In view of the speed of the process, the analysis requires very high temporal and spatial resolution. We have recorded embryos expressing plasma membrane-associated fluorescent markers and have made full 3D reconstructions at 20 second resolution. We find that the initial formation of the furrow occurs without participation of non-mesodermal cells. Rather, the reduction in cell surface in the invaginating cells is accommodated by an increase in the cell surface in the more lateral mesodermal cells. Once the mesodermal cells have

completed their constriction and have begun to translocate towards the interior of the embryo, the lateral and dorsal cells begin to move and change their shapes. We will present the dynamics of their behaviour relative to the mesoderm in wildtype, mutant and experimentally manipulated embryos.

Program/Abstract # 9

Functionally co-operating T-box transcription factors define neuro-mesodermal bipotency during vertebrate axial elongation

George Gentsch, Nick Owens, Stephen Martin, Michael Gilchrist, James Smith (NIMR, UK)

The development of effective cell replacement therapies requires detailed knowledge of how embryonic stem cells form primary tissues such as mesoderm or neurectoderm *in vivo*. Recent work indicates that the caudal region of the vertebrate embryo contains stem cells that can give rise to both mesodermal and neural derivatives. By combining genome-wide chromatin profiling and gain- and loss-of-function experiments *in vivo* as well as quantification of DNA binding dynamics *in vitro*, we provide here unprecedented mechanistic insights into T-box transcription factor-mediated cell fate switches to form correctly proportioned neural and mesodermal tissues along the rostro-caudal axis of the frog embryo. The T-box transcription factors Eomesodermin, VegT and Brachyury recruit RNA polymerase II and signalling mediators to run the transcriptional machinery for mesodermal cell fate acquisition. Consequently, their combined loss eliminates the mesodermal potential inherent to neuro-mesodermal stem cells and causes the embryo to develop a single oversized neural tube without any mesodermal derivatives including skeletal muscle, heart and pronephros.

Program/Abstract # 10

Transcriptional coding of muscle identity in Drosophila

Alain Vincent, Hadi Boukhatmi, Laurence Dubois, Mathilde De Taffin, Jean-Louis Frendo, Laetitia Bataillé, Michèle Crozatier (CNRS - Univ. de Toulouse, France)

The Drosophila larval musculature is composed of a stereotyped array of morphologically distinct muscles. Each skeletal muscle is seeded by a founder myoblast (FC) issued from the asymmetric division of a progenitor cell (PC) specified at a fixed position within the somatic mesoderm. It has been long proposed that each muscle identity - orientation, size, shape, skeletal attachment sites - could reflect unique combinations of "identity" transcription factors (iTFs) expressed by each FC. However, our understanding of this process is far from complete. By studying the Collier/EBF and Tup/Islet1 iTFs, we have recently shown that a key step is the segment-specific activation/cross regulation of iTFs, which occurs in each PC and integrates both positional and temporal cues. We have now undertaken genome-wide genetic and molecular screens in order to obtain a global view of muscle identity specification, focusing on dorsal and dorso-lateral muscles, including the alary muscles which "support" the heart. The results indicate a complex network of iTF regulation and establish new parallels between specification of pharyngeal muscles in chordates and dorsal muscles in Drosophila.

Program/Abstract # 11

Beta-catenin expression in Wolffian ducts is essential for Müllerian duct development during female sexual differentiation

Dibyendu Dutta, Thomas Marose, Calli Merkel, Thomas Carroll (UT Southwestern Med. Ctr., USA)

During female sexual differentiation in mammals, the Wolffian duct (WD) (that gives rise to male reproductive tracts) is degraded while the Müllerian duct (MD) differentiates into the female reproductive tract (oviduct, uterus and vagina). Although a great deal is known about the genetic regulation of sex determination, relatively little is known about the molecules that regulate the differentiation of the sex organs. We deleted beta-catenin from the mouse WD using the HoxB7Cre deleter strain and a floxed allele of beta-catenin. Mutant females showed a partial sex reversal where the WD was maintained while the MD did not form. Since HoxB7 is also expressed in the neural crest, we used alternative deleter strains including Pax2Cre (active in the WD) and Wnt1Cre (active in the neural crest) to determine in which cell type beta-catenin was required. Deletion of beta-catenin with Pax2Cre (but not Wnt1Cre) recapitulated the partial sex reversal phenotype suggesting beta-catenin was required in the WDs. Somewhat surprisingly, we found that the partial sex reversal was not due to inappropriate androgen production or masculinization of ovaries. Instead, it appears to be caused by premature differentiation of the WD rendering it impervious to the normal apoptosis program induced in females. Further, the differentiated ducts fail to express factors (including Wnt9b) necessary for MD migration. Thus, we have found that beta-catenin regulates reproductive tract differentiation in an androgen independent manner.

Program/Abstract # 12

Fat-PCP regulation of neuronal migration

Philippa H. Francis-West, Sana Zakaria (King's College London, UK); Yaopan Mao (Rutgers, USA); Anna Kuta, Robert Hindges, Sarah Guthrie (King's College London, UK); Kenneth Irvine (Rutgers, USA)

Planar cell polarity (PCP) is classically defined as the polarisation of tissue structures perpendicular to the apical-basal axis. In *Drosophila* and vertebrates, PCP is controlled by the Frizzled-PCP and Fat-PCP pathways. However, evidence for conservation of the Fat-PCP pathway in vertebrates is limited. During development, Facial-branchiomotor neurons (FBN) undergo tangential caudal and medio-lateral migrations within the plane of the neuroepithelium, and are a model system for the study of PCP. Previous studies have shown a critical role for Fz-PCP during caudal migration. We have examined the role of Fat-PCP signalling during FBN migration by analysing mouse mutant for *Fat4* and *Dchs1*, the vertebrate homologues of *Drosophila* Fat and Ds respectively. We show that in *Fat4* and *Dchs1* mouse mutants, FBN fail to undergo the medio-lateral migration and are not polarised appropriately. We also show that *Dchs1* and *Fat4* are expressed in opposing gradients along the medio-lateral axis suggesting that as in *Drosophila*, gradients of Fat

activity may determine the orientation of tissue structures. Indeed, chimeric analysis, which would disrupt these gradients, affects medio-lateral migration. This clearly demonstrates that Fat-PCP signalling is conserved in vertebrates and also implicates for the first time gradients of Fat4/Dchs1 activity in the establishment of PCP. This data also define Fat-PCP as novel neuronal guidance cues and show a novel system where Fz-PCP and Fat-PCP determine the polarisation of tissue structures along orthogonal axes.

Program/Abstract # 13

Systematic discovery of novel cilia and ciliopathy genes through functional genomics in the zebrafish

Semil P. Choksi, Deepak Babu (IMCB, Singapore); Malgorzata Szczepaniak, Rim Hjeij (Univ. Hosp. Muenster, Germany); Doreen Lau, Xianwen Yu (IMCB, Singapore); Petra Pennekamp, Claudius Werner, Niki T. Loges (Univ. Hosp. Muenster, Germany); Karsten Häffner (Univ. Hosp. Freiburg, Germany); Shunzhen Chen, Kangli Noel Wong (IMCB, Singapore); Gerard Dougherty (Univ. Hosp. Muenster, Germany); Ronald Roepman (Radboud Univ., Netherlands); Heymut Omran (Univ. Hosp. Muenster, Germany); Sudipto Roy (IMCB, Singapore)

Cilia are microtubule-based hair-like organelles that play many important roles in animal development and physiology. Ciliary dysfunction is implicated in a rapidly expanding spectrum of diseases, the ciliopathies. Primary ciliary dyskinesia (PCD), one of the most prevalent ciliopathies, is a genetically heterogeneous disease that arises from faulty motile cilia. As causative mutations in relatively few genes have been described, we sought to systematically identify new ciliopathy genes using functional genomics coupled with rapid phenotype screening in the zebrafish embryo. With this approach, we identified hundreds of cilia genes, a majority of which have never been associated with cilia before. We selected 50 genes at random and, remarkably, found that more than 60% are required for ciliary differentiation or function. In addition, we find that proteins encoded by 14 of the genes localize to motile cilia. By sequencing a cohort of PCD patients for mutations in these genes, we show that several of the novel candidates, including *ZBBX* and *MNS1*, are mutated in patients suffering from PCD. We find that these genes affect ciliary motility by disrupting the nexin-dynein regulatory complex and the outer dynein arm assembly, respectively. This large collection of functionally-validated motile cilia genes will be invaluable for the study of ciliary biology, and in the identification of new mutations causing ciliopathies like PCD.

Program/Abstract # 14

Sox10 regulates enteric neural crest cell migration in the developing gut

Mai Har Sham, Carly Leung, Mei Zhang, Hon Man Sit (U Hong Kong, China)

Sox10 is a HMG-domain containing transcription factor which plays important roles in neural crest cell survival and differentiation. Mutations of *Sox10* have been identified in patients with Waardenburg-Hirschsprung syndrome, who suffer from deafness, pigmentation defects and intestinal aganglionosis. Enteric neural crest cells (ENCCs) with *Sox10* mutation undergo premature differentiation and fail to colonize the distal hindgut. It is unclear, however, whether *Sox10* plays a role in the migration of ENCCs. To visualize the migration behaviour of mutant ENCCs, we generated a $Sox10^{NGFP}$ mouse model where EGFP is fused to the N-terminal domain of *Sox10*. Using time-lapse imaging, we found that ENCCs in $Sox10^{NGFP/+}$ mutants displays lower migration speed and altered trajectories compared to normal controls. This behaviour was cell-autonomous, as shown by organotypic grafting of $Sox10^{NGFP/+}$ gut segments onto control guts and vice versa. ENCCs encounter different extracellular matrix (ECM) molecules along the developing gut. We performed gut explant culture on various ECM and found that $Sox10^{NGFP/+}$ ENCCs tend to form aggregates, particularly on fibronectin. Time-lapse imaging of single cells in gut explant culture indicated that the tightly-packed *Sox10* mutant cells failed to exhibit contact inhibition of locomotion. We determined the expression of adhesion molecule families by qPCR analysis, and found integrin expression unaffected while L1-cam and selected cadherins were altered, suggesting that *Sox10* mutation affects cell adhesion properties of ENCCs. Our findings identify a de novo role of *Sox10* in regulating the migration behaviour of ENCCs, which has important implications for the treatment of Hirschsprung disease.

Program/Abstract # 15

Epigenetic Regulation of Intestinal Inflammation in Zebrafish

Lindsay Marjoram, Michel Bagnat (Duke, USA)

Our lab recently performed a forward genetic screen in zebrafish and identified mutants with perturbed epithelial integrity in the intestine. One of these mutants, *aa51.3*, shows excess epithelial cell shedding and apoptosis in the intestine. This phenotype is accompanied by loss of epithelial folds and nonpolarized distribution of cadherin. The observed defects are consistent with an inflammatory bowel disease-like phenotype. Tumor necrosis factor, a pro-inflammatory cytokine, plays a prominent role in inflammation and its overexpression is correlated with inflammatory bowel disease and defects in intestinal epithelial integrity. To monitor inflammation, we generated an inflammation-responsive tumor necrosis factor transgenic line, *TgBAC(tnfa:GFP)*. In *aa51.3* mutants, *TgBAC(tnfa:GFP)* is dramatically upregulated along the entire length of the intestine. Exome sequencing followed by positional cloning identified a causative splice-site mutation in the gene encoding Ubiquitin-like, Containing PHD and Ring Finger Domains 1 (Uhrf1), which plays a fundamental role in epigenetic regulation of gene expression by promoting DNA maintenance methylation. Expression of *uhrf1* is spatially restricted to the eye, liver and gut in five day post-fertilization larvae, a stage when we observe elevated intestinal *tnfa* expression. Our data suggest that loss of maintenance methylation in the *tnfa* promoter leads to elevated expression of *tnfa* in the intestine, which subsequently promotes the observed intestinal defects in *aa51.3* mutants. This research will provide insights into how changes in methylation may promote the development of inflammatory diseases and may uncover new therapeutic targets.

Program/Abstract # 16

"Paracrine endodermal signaling promotes tracheal cartilage development"

Debora Sinner, John Snowball, Richard Lang, Jeff Whitsett (Cincinnati Children's Med. Ctr., USA)

Tracheo-bronchomalacia is a condition in which the walls of the upper conducting airways are soft because of the lack of cartilage. While the prevalence of the disease is high (1:6000/live births), the etiology of tracheomalacia is unknown. Our **goal** is to understand how the trachea is patterned during normal development and in congenital airway malacia. We generated a mouse model where *Wls*, which mediates Wnt ligand secretion, was deleted from endoderm of the developing respiratory tract using ShhCre mice. In these embryos, the tracheal mesenchyme was mispatterned: Sox9 expression was not detected while the αSMA expression pattern was expanded into the ventral region of the trachea. Cartilage was near absent however no tracheoesophageal fistula was present. In contrast, expression pattern of conducting airway markers was unaltered in tracheal epithelium. We **hypothesize** that Wls modulates *Sox9* expression in tracheal mesenchyme. We crossed the Sox9 conditional KO mice to the mesenchymal driver Dermo1Cre and obtained mutants in which the cartilage and tracheal length was reduced, recapitulating in part endodermal deletion of *Wls* as well as *Wnt5a* null phenotype. Deletion of *Wls* in tracheal mesenchyme via Dermo1Cre or in chondroblast using Col2a1Cre, had not effect in cartilage formation, suggesting that Wls via a paracrine mechanism acts upstream of Col2a1 to induce cartilage. *In vivo* activation of canonical Wnt signaling did not rescue the lack of cartilage in embryos were Wls was deleted from pulmonary endoderm. We **conclude** that paracrine signaling from the respiratory endoderm modulated by Wls is required for Sox9 expression and patterning of the tracheal mesenchyme. *Partially supported by NIH-NHLBI 1K01HL115447-01 to DS.*

Program/Abstract # 17

TALE-mediated modulation of transcriptional enhancers

Justin Crocker, David Stern (HHMI Janelia, USA)

In eukaryotes, the pattern and level of gene expression is modulated by regions of DNA, called transcriptional enhancers, which encode binding sites for transcription factors. The majority of animal genomes may encode transcriptional enhancers, but we currently understand far less about the structure and function of enhancer regions. Our understanding of enhancer structure and function is derived mainly from reporter gene assays. However, these assays often fail to correctly recapitulate native expression patterns. Here we show that Transcription Activator-Like Effectors (TALEs) allow targeted repression and activation of enhancers in the *Drosophila* genome. TALE-repressors (TALERs) targeted against the promoter of the *even-skipped* gene (*eve*) caused repression of native *eve* expression in multiple cell types. Ubiquitously expressed TALERs with DNA binding specificity for each of the five "stripe" transcriptional enhancers of *eve* generated repression specifically of the focal enhancer, without disrupting the activity of neighboring enhancers. Ubiquitously expressed TALE-activators (TALEAs) targeted against individual enhancers caused increased expression specifically in cells normally activated by the focal enhancer. Phenotypic effects of these manipulations in embryos and adults are consistent with the modulations of *eve* expression caused by TALERs and TALEAs. TALEs thus provide a novel tool for detection and functional modulation of transcriptional enhancers in their native genomic context. Our results strongly support a model for combinatorial activation of independent, modular enhancers, and the view that repression acts in a dominant fashion to make silent enhancers inaccessible to transcriptional activators.

Program/Abstract # 18

Reunion after more than 700 million years of separation: fish transcription factors meet sea urchin DNA

Jongmin Nam (Rutgers, USA)

Commonality in gene regulatory systems is a key aspect of eukaryotic development, as it has been manifested by many successful trans-specific complementation of transcription factors. As transcription factors require specific DNA sequences to function properly, it stands to reason that their target sequences are also conserved. This assumption has been supported by numerous comparative studies between closely related species and has also served as a milestone for comparative genomics. However, there exists a knowledge gap in the deep evolution of transcription factors and cis-regulatory sequences: numerous transcription factors between different phyla share common sequence features and function, but the same cannot be said for cis-regulatory DNA sequences. This raises an interesting question about the prevalence of inter-phyletic cis-regulatory conservation. As a first step towards addressing this, we asked how many cis-regulatory modules (CRMs) that are active during early embryogenesis of the purple sea urchin are also active in the zebrafish embryo. Our results show that a high fraction of sea urchin CRMs, 58/109, promoted reporter expression in early zebrafish embryogenesis. When the same set of CRMs was tested in a more closely related species, the pencil sea urchin, a higher fraction of CRMs, 74/109, was active, reflecting a closer evolutionary relationship between the two urchins than to zebrafish. Our experimental and statistical analyses suggest that the majority of these inter-phyletic or inter-generic regulatory interactions are due to conserved regulatory interactions rather than opportunistic interactions. Thus, inter-phyletic functional conservation beyond "detectable" sequence conservation may be abundant.

Program/Abstract # 19

The weak shall lead the strong: The role of transcription factor binding affinity in morphogen gradient responses and enhancer evolution

Scott Barolo (U Michigan, USA)

Developmental cell signaling pathways relay patterning information to transcription factors (TFs), which in turn control cell fate by regulating gene expression. Because these pathways are highly pleiotropic, we are particularly interested in how *cis*-regulatory DNA sequences interpret such "generic" signals in a tissue-specific manner, and how they accurately read spatial information from morphogen gradients. 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 the *Drosophila* genes *wingless*, *dpp*, *stripe*, *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 "sign" of the response from activation to direct repression. This has important and complex implications for both the tissue specificity of responses to signaling and the patterning of morphogen gradient responses. I will address the implications of our recent findings for genetic, genomic, and computational approaches to the study of developmental transcriptional networks.

Program/Abstract # 20

Transcriptional and protein-protein networks in gynoecium and fruit development in Arabidopsis

Stefan de Folter, Víctor M. Zúñiga-Mayo, J. Irepan Reyes-Olalde, Paulina Lozano-Sotomayor, Daniela Ramos-Cruz, Jeanneth Pablo-Villa, Humberto Herrera-Umbaldo, Mariana Sotelo-Silveira, Ricardo Chavez-Montes, Rocio Escobar-Guzmán, Karla González-Aguilera (LANGEBIO, CINVESTAV-IPN, Mexico); Nayelli Marsch-Martínez (CINVESTAV-IPN, Mexico)

Gene regulation at the level of transcription is crucial for almost all biological processes in a cell or organism. Transcription factors (TFs) are sequence-specific DNA-binding proteins that are capable of activating and/or repressing transcription. Many mutants affected in development have been associated with altered expression levels of TF genes. Therefore, the analysis of TF genes can be the basis for a better understanding of plant developmental processes. Our lab identified various novel TFs affecting gynoecium and fruit development in Arabidopsis. At the moment, we are studying their genetic interactions and furthermore, to gain a better understanding about how they function on the molecular level, matrix-based yeast two-hybrid screens are performed with known TFs involved in meristem, flower, and fruit development. The latest results will be presented.

Program/Abstract # 21

Asymmetric localization and segregation of Cdx2 transcripts at the 8-cell stage facilitates development of pluripotent cell lineage of mouse embryos

Krzysztof B.Wicher, Maria Skamagki, Agnieszka Jedrusik (Gurdon Inst., UK); Sujoy Ganguly (U of Cambridge, UK); Magdalena Zernicka-Goetz, (Gurdon Inst., UK)

Sub-cellular transcript localization commonly provides spatial regulation of gene expression in metazoan development. However, it is currently unknown whether early mammalian embryos use such a mechanism. Here we demonstrate dynamic localization of transcripts for the trophectoderm transcription factor, *Cdx2*, to the apical cortex of blastomeres about to undertake the first cell fate decision in living mouse embryos. We show that transcript localization depends on a cis-element in the 3' part of the coding-region of *Cdx2*, apical localization of aPKC zeta and an intact microtubule and actin cytoskeleton, a microtubule motor of the kinesin family. Upon differentiative cell division, localized *Cdx2* transcripts are inherited by outside, trophectoderm progenitor cells where they relocalise to the apical cortex. Furthermore, we show that interfering with the localization machinery leads to elevated *Cdx2* in inside cells and, consequently, a significantly diminished number of pluripotent cells. Thus inheritance of *Cdx2* transcripts by outside cells contributes to their differentiation and enables dispossessed inside cells to follow pluripotent development.

Program/Abstract # 22

Involvement of Notch-mediated lateral inhibition and subsequent planar cell migration of Delta1-expressing cells in avian otic placode formation

Yoshio Wakamatsu, Noriko Osumi, Hiroko Shida (Tohoku U, Japan)

Cranial sensory organs arise from cranial placodes, thickenings of ectodermal epithelium. These placodes originate from pre-placodal region (PPR) surrounding anterior neural plate. We examined an expression of *Delta1*, which encodes a Notch ligand, in quail embryos, and found that *Delta1*-positive cells initially emerged as a "salt-and-pepper" pattern at the prospective otic level of the PPR, suggesting Notch-mediated lateral inhibition undergoing to select these cells. Consistently, forced activation of Notch signal in the otic PPR disrupted otic placode formation, and an inhibition of Notch signal resulted in the expansion of the Delta1-positve area of the otic PPR. As development proceeded, it appeared as if these *Delta1*-positive cells were gradually assembled to form the otic placode. Immunofluorescence staining with anti-Delta1 antibody revealed that these Delta1-positive cells rarely contacted with the basal membrane, had long cell processes extending over the apical surface of the PPR, and sometimes contacted each other via these processes. No Delta1-expressing cell has delaminated from the otic PPR. These observations suggest that a subset of the PPR cells may acquire the otic placode fate by the Notch-mediated lateral inhibition and that these cells may subsequently migrate within the PPR epithelium to form the otic placode. Consistently, our time-lapse observation revealed that anti-Delta1-labeled otic PPR cells appeared to migrate in a coalescing direction in cultured quail embryos. This study may provide a cell-biologically unique example of cell migration and sorting within the developing epithelial tissues.

Program/Abstract # 23

BMP signaling in dorsoventral axial patterning

James Dutko, Megumi Hashiguchi, Joe Zinski, Mary Mullins (U Penn, USA)
The vertebrate embryonic dorsoventral (DV) axis is patterned by a bone morphogenetic protein (BMP) activity gradient during blastula and gastrula stages. The BMP morphogen gradient is shaped by BMP antagonists emanating from dorsal regions that generate high signaling levels ventrally and low signaling dorsally. In the zebrafish embryo, BMP signaling requires two ligands, Bmp2b and Bmp7a, that function exclusively as a heterodimer. This heterodimer functions through a heteromeric type I receptor complex composed of Alk3/6 and Alk8. We present results elucidating the requirement for BMP heterodimers in signaling. We also present results examining the dynamics and shaping of the BMP morphogen gradient that patterns DV tissues and its exquisite coordination with anteroposterior patterning.

Program/Abstract # 24
Specification of avian neural crest prior to gastrulation
Martin I. Garcia-Castro (Yale, USA)
We previously reported that neural crest cells are specified during gastrulation, independently from neural and mesodermal tissues, and identified the transcription factor Pax7 as one of the earliest markers of presumptive neural crest cells, and showed that it plays a critical role during neural crest development. Here we report surprising results demonstrating that blastula embryos of Eyal-Giladi stage XII (prior to gastrulation and primitive streak appearance) display clear signs of spatiotemporally restricted neural crest specification. While no neural crest markers are distinguished at this stage, equatorial explants adjacent to the area opaca/area pelucida border go on to express Pax7 after 25 hours of culture in isolation and under non-inducing conditions. Specified explants cultured for longer time express other crest markers (Ap2, Msx1/2, Snail2, Sox9, and HNK-1) and acquire migratory characteristics. Immunofluorescence and qRT-PCR data suggest that these neural crest traits appear independently of mesodermal (Bra, Tbx6L) and neural (Sox2) markers. To verify the *in vivo* contributions made by these territories containing "specified" neural crest we performed fate mapping experiments using DiI and DiO and identify the same lateral territory as poised to contribute to the neural plate border, coexpress Pax7, and generate migratory neural crest cells. Finally we are readdressing the induction mechanism at play, particularly testing the long held tenure of a "classic induction" mediated by cell-interactions. Our results reveal a much earlier specification of neural crest development than previously anticipated with deep implications for the mechanism of neural crest induction.

Program/Abstract # 25
Integrating BMP and SHH signalling with the regulation of the chromosomal landscape of the BMP antagonist GREM1
Aimée Zuniga, Emanuele Pignatti, Frédéric Laurent, Sumit Jaiwal, Javier Lopez-Rios, Rolf Zeller (U of Basel, Switzerland)
We study the key molecular interactions that regulate growth and chondrogenic differentiation of the mesenchymal progenitors that give rise to the limb skeleton. To this aim, we have inactivated *Smad4*, an essential mediator of TGF-β/BMP signal transduction specifically in the mouse limb bud mesenchyme. Our analysis reveals that SMAD4 is required to initiate formation of the SOX9-positive digit ray primordia and aggregation and chondrogenic differentiation of all limb skeletal elements. BMP4 is the main signal transduced by SMAD4 and the BMP antagonist Gremlin1 (GREM1) is key to modulating BMP activity in limb buds. GREM1 functions as a key node in the self-regulatory signaling system that controls distal progression of limb bud development. During limb bud development, *Grem1* expression is triggered by BMP4, which progressively reduces of BMP activity as *Grem1* expression increases in response to SHH signaling. During late limb bud outgrowth and initiation of chondrogenesis, *Grem1* expression is terminated by high AER-FGF signaling in the posterior and the transcriptional regulator GLI3 in the anterior limb bud. We have shown that GLI3-mediated transcriptional down-regulation of *Grem1* promotes the exit of proliferating mesenchymal progenitors toward BMP-dependent chondrogenic differentiation. *Grem1* expression is controlled by a large chromosomal landscape that encompasses several functionally redundant SHH/GLI3-dependent *cis*-regulatory elements. We are using 4C chromatin conformation capture and ChIP approaches to identify all relevant *cis*-regulatory regions with the aim to understand how the BMP and SHH signaling inputs are integrated into the spatio-temporal dynamics of *Grem1* expression.

Program/Abstract # 26
ADMP scales the BMP gradient through enlargement and restriction of the organizer
Abraham Fainsod, Avi Leibovich, Hadas Leibovich-Kot (Hebrew U, Israel); Danny Ben-Zvi, Naama Barkai (Weizmann Inst of Science, Israel)
The size of developing embryos is highly variable depending on genetic polymorphisms and environmental effects. Scaling of tissue pattern with tissue size is required to ensure body plans of reproducible proportions. *Xenopus* embryos develop from different sized eggs that by early/mid gastrula are patterned along the dorsoventral axis by a BMP signaling gradient. This BMP signaling gradient requires modification according to size to ensure that tissues are scaled to keep the right relative size and position. Using mathematical modeling and experimental manipulation, we previously suggested that the dorsoventral BMP gradient is scaled by a contribution of ADMP. ADMP has been attributed a ventral, BMP-like, role although it is expressed in Spemann's organizer. We show by knock-down that ADMP is required during early gastrula for normal organizer size, preceding its ventral activity. In agreement with a dorsal function, organizer gene expression domains are reduced in ADMP morphant embryos. We also identified a receptor mediating the dorsal activity of ADMP within the organizer. ADMP shows higher compensatory expression in larger embryos in agreement with a scaling function as predicted by the computer model. Experimental size manipulation by dissection showed dynamic changes in ADMP expression. These results support novel dorsal and scaling functions for ADMP, suggesting that it performs multiple and

opposed roles during early gastrulation. We propose that ADMP has a very early dorsal function promoting the expansion of the organizer domain and subsequently, it contributes to the BMP gradient to restrict the organizer. These opposed ADMP activities scale the organizer domain and the BMP gradient with embryo size.

Program/Abstract # 27
Stromal-epithelilal crosstalk regulates nephron progenitor cell fate
Amrita Das (UT Southwestern, USA)
The kidney is an essential organ that regulates the chemistry of the blood. It is composed of numerous epithelial tubules known as nephrons. For years, it has been believed that the development of a nephron was soleley dependent upon reciprocal interactions between two embryonic populations, the ureteric bud epithelia and the metanephric mesenchyme. Signals from the mesenchyme promote branching morphogenesis of the bud, while signals from the bud regulate the survival and renewal of a progenitor population within the mesenchyme. The bud also induces a sub-population of the progenitors to undergo a mesenchymal-to-epithelial transition (MET) and differentiate into an epithelial tubule that will form the nephron. We found that Wnt9b, a ureteric bud-derived signal, was necessary for both mesenchymal progenitor renewal and epithelial differentiation. How the same molecule induced two seemingly contradictory processes was unknown. Here, we show that signals from the overlying stromal fibroblasts modify Wnt9b activity promoting progenitor cell differentiation. The atypical cadherin Fat4, encodes at least a part of this stromal signal. Fat4 acts by promoting the removal of the transcriptional regulators Yap and Taz from the nucleus. Nuclear Taz/Yap cooperate with beta-catenin to promote progenitor renewal and repress differentiation. Thus, we have found that opposing signals (Wnt9b from the ureteric bud and Fat4 from the stroma) cooperate to regulate progenitor cell renewal and differentiation. Proper balancing of these signals is required to assure the proper number of nephrons form assuring optimal kidney function.

Program/Abstract # 28
Regulation of Hippo signaling by MAPK pathways
Kenneth Irvine, Venu Reddy, Gongping Sun, Veronica Codelia (HHMI/Rutgers, USA)
The Hippo pathway regulates organ growth; defining mechanisms that regulate this pathway is crucial to understanding how it is utilized to control growth in different contexts. In earlier work we have characterized some of the key upstream regulators of Hippo signaling in *Drosophila*, such as Fat, Expanded, and Merlin. More recently, we have begun to identify and characterize additional inputs into Hippo pathway regulation. Epidermal Growth Factor Receptor (EGFR) signaling plays an important role in growth control, and inappropriate activation of EGFR signaling has been implicated in several cancers. We have identified and characterized a conserved link between EGFR and Hippo signaling pathways. EGFR activates the Hippo pathway transcription factor Yorkie (Yki) through the Ras-MAPK branch of EGFR signaling. Genetic and biochemical experiments implicate the Ajuba LIM protein Jub as a key target of EGFR-Ras-MAPK signaling within the Hippo pathway. An EGFR-Hippo pathway link is conserved in mammalian cells. Wounding, apoptosis or infection can trigger a proliferative response in neighboring cells to replace damaged tissue. Studies in *Drosophila* have implicated Jun kinase (JNK)-dependent activation of Yki as essential to regeneration-associated growth, as well as growth associated with neoplastic tumors. We have found that JNK regulation of Hippo signaling is conserved in mammalian cells, and identified a conserved molecular mechanism by which JNK impinges on Hippo signaling, and which is distinct from EGFR-Ras-MAPK regulation of Hippo signaling.

Program/Abstract # 29
Coordination of patterning and growth in the spinal cord
Anna Kicheva, Ana Ribeiro, Helena Perez Valle, James Briscoe (NIMR, UK)
In the developing spinal cord, several types of neuronal progenitors are specified and spatially arranged along the dorso-ventral axis in response to the morphogen gradient of Sonic hedgehog (Shh). This process of establishment of the neural tube pattern involves the transcriptional specification of progenitor identity, as well as proliferation and terminal differentiation. How these processes are coordinated is poorly understood. To address this problem, we measured the three-dimensional growth rates of each gene expression domain in mouse and chick neural tubes during 3 days of development using immunostainings of sections, clonal analysis, and photoconversion experiments. Independent measurements of the rates of proliferation and differentiation showed that these processes alone can account for the size changes of the gene expression domains for most of the experimental time period. Consistent with this, Olig2 lineage tracing experiments at different developmental stages show that changes of cell identity occur rarely later in development. Our data suggests a 2-phase model of patterning: early domain size depends on switches of cell identity, which are instructed by Shh signaling, whereas the late elaboration of pattern depends on proliferation and differentiation. We are validating this model using different conditions where growth or signaling is altered.

Program/Abstract # 30
From deep water to deep learning: Modeling the teratogenic impacts of the Deepwater Horizon oil spill
Michael Barresi (Smith, USA)
Deep learning in a laboratory course that fosters cross-disciplinary comprehension of key concepts, confidence in experimental design and implementation, and development of critical thinking skills is challenging to achieve. However, engaging students with a novel and relevant research question can reap tangible rewards. The Deepwater Horizon disaster is the largest marine oil spill in history, and

those organisms exposed during their early life stages are the most vulnerable. I proposed that this disaster could provide students a unique opportunity to both learn about and contribute to developmental biology. We obtained Macondo crude oil from the Deepwater Horizon oil spill, and using the zebrafish model system tested whether water accommodated fractions (WAF) of this crude oil could cause teratogenic effects. Students in the "BIO303: Research in Developmental Biology" lab course designed the first experiments, and discovered specific malformations in distinct developmental processes. After preliminary data was obtained in this course, several of these students continued this investigation to show that WAF treatments caused malformations in cranial neural crest cell development leading to defects in craniofacial elements and circulatory function. These and other results provided new insight into the developmental mechanisms of crude oil teratogenesis. By posing a relevant question and providing a tractable research approach, students became fully engaged in designing experiments to adaptively solve problems in developmental biology. Such exposure to real research in a lab course provides students the experiences they need for credible recommendations, the potential for publication, and substantial deep learning.

Program/Abstract # 31
From Silent Spring to Silent Night: A Tale of Toads and Men
Tyrone Hayes (UC Berkeley, USA)
The herbicide, atrazine is a potent endocrine disrupter that chemically castrates and feminizes male amphibians. Further, atrazine exposure induces a hormonal stress response that leads to retarded growth and development, and immune suppression. The immune suppression results in increased disease rates and mortality. Though many factors likely contribute to amphibian declines, pesticides such as atrazine likely play an important role even in populations that appear to decline for other reasons, such as disease. Pesticides like atrazine are ubiquitous, persistent contaminants and, the effects described above occur in all vertebrate classes (fish, amphibians, reptiles, and mammals) examined, via common mechanisms. These observations demonstrate the critical impact that pesticides have on environmental health. Reproductive cancers and birth defects associated with chemical exposure demonstrate that the impact on environmental health is an indicator of negative impacts on public health. Many of the mechanisms are being revealed only now in the scientific literature and agencies are just now beginning to deal with this emergent science and translate it into health-protective policies. In particular, ethnic minorities and lower socio-economic communities are at risk: More likely to live in contaminated communities, work in occupations that increase hazard exposure and less likely to have educational and healthcare access. Given the importance of this science and relevance to public health, there is a strong need to translate this information and provide public access to this knowledge. Command of the science and active involvement by the public in policy decisions is vital.

Program/Abstract # 32
How can you use environmental issues to make Dev Bio relevant to today's students
Diana Darnell (U Arizona, USA)
Students indicate they learn more when they can see the relevance and real-world connections between their classroom science and their lives. We will work together to build and link learning objectives, content and formative assessment tools around mechanisms and evidence for environmental impacts on development. Use examples from the previous talks, or bring examples from your classes. We will discuss and create useful learning strategies with other educator-scientists. I will provide examples relating to diet and epigenetic inheritance, from queen bees to obesity and fertility in humans for those who don't bring their own material. This will be an interactive session so come prepared to participate.

Program/Abstract # 33
Control of leaf size and shape by microRNAs in plants
Ramiro Rodriguez, Juan Debernardi, Uciel Chorostecki, Carla Schommer, ***Javier Palatnik*** *(Inst. Biol. Molec. y Cel. de Rosario, Argentina)*
Plants and other multicellular organisms need precise spatio-temporal control of gene expression, and this regulatory capacity depends, in part, on small RNAs. MicroRNAs are one class of small RNAs that recognize complementary sites in target mRNAs and guide them to cleavage or translational arrest. In plants, most of the ancient microRNAs control transcription factors involved in development and hormone signaling. We have been studying two microRNAs that regulate cell proliferation and differentiation in plants, miR319 and miR396 that regulate transcription factors of the TCP and GRF class, respectively. While miR319 avoids cell differentiation and stimulates cell proliferation, miR396 performs an opposite function by repressing cell division. MiR396 levels steadily increase during the organ development. We found that miR396 antagonizes the pattern of expression of its targets the GRF transcription factors. MiR396 accumulates preferentially in the distal part of young leaves, restricting the expression of GRF2 to the proximal part of the organ, which in turn coincides with activity of cell proliferation. The balance between miR396 and the GRFs is a key element determining the number of cells in leaves. In turn, the miR319-regulated TCP4 induce miR396, so that the two regulatory networks of miR319 and miR396 are interconnected. The functions of the miR319-regulated TCPs go, however, beyond cell division and control several aspects of the plant development. The regulation of the GRFs by miR396, and the TCPs by miR319 is conserved at least in angiosperms and gymnosperms. However, miR396 additionally regulates another transcription factor of the bHLH class but only in Arabidopsis thaliana and closely related species. Interestingly, the regulation of both conserved and new targets is important for leaf development in Arabidopsis. Furthermore, miRNA variants can exist as well in certain species and we found that they can display an a differential activity towards their targets. In summary, we describe how miRNA regulatory networks might expand their

functions by the recruitment of additional targets as well as by slight variations in the small RNA sequences.

Program/Abstract # 34

Tetraspanin18 maintains Cadherin6B protein to antagonize cranial neural crest epithelial to mesenchymal transition

Laura S. Gammill, Corinne L. Fairchild, Joseph P. Conway (U Minnesota, USA)

Unlike their neurepithelial neighbors in the neural tube, neural crest cells undergo an epithelial to mesenchymal transition (EMT) and migrate. We have defined novel post-translational control of cranial neural crest EMT. Premigratory cranial neural crest cells express the transmembrane scaffolding protein tetraspanin18 (Tspan18), which is downregulated prior to migration. Sustained Tspan18 expression prevents cranial neural crest migration and maintains epithelial Cadherin6B (Cad6B) protein despite temporally normal downregulation of Cad6B mRNA. This suggests that Tspan18 antagonizes EMT by post-translationally maintaining Cad6B protein to promote epithelial cell adhesion. In support of this, Tspan18 knockdown leads to premature loss of Cad6B protein from the neural folds. Nevertheless, Tspan18 knockdown is insufficient for precocious migration, at least in part because other steps in EMT take place on schedule. At the onset of EMT, Tspan18 transcriptional repression is independent of the EMT transcription factor Snail-2, but downstream of FoxD3, which is both necessary and sufficient for Tspan18 mRNA downregulation and neural crest migration. Altogether, our results reveal Tspan18-dependent maintenance of Cad6B protein in epithelial cranial neural crest cells that is relieved by FoxD3-dependent downregulation of Tspan18 at the onset of EMT. Thus, in a pathway parallel to Snail-2 transcriptional repression of Cad6B, FoxD3/Tspan18 define a new transcriptional/post-translational input into cranial neural crest EMT that offers mechanistic insight into the timing of neural crest emigration as well as the poorly understood anti-metastatic activity of some tetraspanins.
Supported by NIH F31GM087951 and a U of MN Grant in Aid.

Program/Abstract # 35

The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse

Phillip Grote, Lars Wittler (Max Plunck Inst for Molec. Genetics, Germany); David Hendrix (MIT, USA); Frederic Koch, Bernhard Herrmann (MPI Molec Genet., Germany)

Long non-coding RNAs (lncRNAs) have been shown to influence gene expression by modulating histone modifications and affecting chromatin accessibility. This function involves binding of lncRNAs to the histone modifying Polycomb-repressive complex 2 (PRC2) or Trithorax group (TrxG/MLL) proteins, implicating lncRNAs in lineage commitment, cellular differentiation and embryonic development. We identified Fendrr as a tissue specific lncRNA, which is transiently expressed in the nascent lateral mesoderm, giving rise to the heart and body wall. Inactivation of Fendrr by gene targeting resulted in embryonic death after stage E13.5 due to malfunctioning of the heart, and in rupture of the ventral body wall. The molecular analysis showed that transcription factors controlling lateral plate or cardiac mesoderm differentiation are up-regulated in Fendrr mutant embryos. This was accompanied by an increase in the activating histone H3 Lys4 tri-methylation (H3K4me3) mark either alone or in combination with a decrease of the repressive H3 Lys27 tri-methylation (H3K27me3) mark at their promoters, indicating deregulated PRC2 and TrxG activity at a subset of control genes. Moreover, PRC2 occupancy was decreased at the regulatory regions showing reduced H3K27me3 levels. Fendrr binds to both histone modifying complexes in vivo and to dsDNA derived from target promoters in vitro. Our data identifies a lncRNA that plays an essential role in balancing activating and repressive histone marks at target promoters and is involved in regulatory networks controlling the development of lateral mesoderm derivatives.

Program/Abstract # 36

Identification of a non-coding RNA as a negative regulator of JNK signaling during Drosophila dorsal closure

L. Daniel Ríos-Barrera, Juan R. Riesgo-Escovar (UNAM, Mexico)

Dorsal closure is one of the last steps in Drosophila embryogenesis, whereby the lateral epidermis stretches dorsally to completely wrap the embryo. We have identified a gene, *acal*, whose mutations result in dorsal closure defects. *acal* has low protein coding potential and its transcript is enriched in nuclear preparations. Strikingly, *acal* is processed into fragments in the range of 40 to 100 nucleotides. Hence, we propose that *acal* is a non-coding RNA. During dorsal closure, *acal* is expressed mainly in the central nervous system and in the lateral epidermis. Rescue of *acal* in the lateral epidermis is sufficient to suppress dorsal closure defects. To determine the role of *acal* in dorsal closure, we analyzed activation of the JNK pathway, the dorsal closure trigger, in *acal* mutants. By means of a JNK activity reporter (*puc-lacZ*), we found that *acal* mutants present ectopic activation of JNK signaling. Consistently, reducing the JNK gene dosage partially rescues the *acal* mutant phenotype. These results show that *acal* is a negative regulator of JNK signaling during dorsal closure. The expression pattern of *acal* during dorsal closure is very similar to that of *raw*, a negative regulator of JNK signaling of unknown molecular function. In *raw* mutants, *acal* epidermal expression is strongly reduced, suggesting that *acal* lies downstream of *raw*. To support these results, we analyzed thoracic closure, a process analogous to dorsal closure occurring during metamorphosis. Ectopic expression of *acal* or *raw* alone has no effect on thorax closure; however, co-expression of both genes impairs thoracic closure. Altogether, our results show that *raw* acts at least partially through *acal* to regulate dorsal closure by antagonizing JNK signaling.

Program/Abstract # 37

Regulation of histone mRNA by PIWI homologs in planarian stem cells

Labib Rouhana, Jennifer Weiss, Phillip Newmark (U IL at Urbana-Champaign, USA)

The well-known regenerative abilities of planarian flatworms are attributed to a population of adult stem cells called neoblasts, which proliferate and differentiate to produce all cell types in their bodies. A characteristic feature of neoblasts is the presence of large cytoplasmic ribonucleoprotein (RNP) granules named chromatoid bodies. These organelles are structurally and molecularly similar to RNP granules present in the germline of many organisms. As such, they contain symmetrical dimethylarginine (sDMA) methylation substrates of Protein Arginine Methyltransferase 5, such as PIWI and SmB homologs, as well as Tudor domain-containing proteins that bind to sDMA. This study shows that the *Schmidtea mediterranea* PIWI family members SMEDWI-1 and SMEDWI-3 are required for localization of *germinal histone H4 (gH4)* transcripts to chromatoid bodies. Regulation of histone mRNA by chromatoid body components may go beyond *gH4*, since transcripts of every major histone gene family were also found in these structures. Additionally, *gH4* mRNA levels increased upon inhibition of SMEDWI-1 and SMEDWI-3 levels by RNAi, suggesting the involvement of these PIWIs in histone mRNA turnover. PIWI proteins are better known for silencing transposable elements via piRNA-mediated mRNA turnover and genomic silencing. Similar mechanisms may be involved in the regulation of histone gene expression in planarians, where neoblasts are the only proliferating somatic cells and others are terminally differentiated.

Program/Abstract # 38

System level reconstruction of brain development and function with light-sheet microscopy

Philipp Keller (HHMI- Janelia, USA)

In embryonic development of vertebrates and higher invertebrates, a single cell is transformed into a fully functional organism comprising tens of thousands of cells and more. In a complex process of self-organization, these cells rapidly divide, migrate, differentiate and form tissues and organs able to perform the most challenging tasks. The nervous system is a key component of the developmental building plan that stands out in terms of size, complexity and function. However, very little is known about the developmental dynamics of this complex system, since the technology to comprehensively record and computationally analyze in vivo cell behavior in neural tissues is lacking. The overall objective of our research is to gain such quantitative experimental access, to determine the fundamental rules governing neural development, and to systematically link development to the functional activation of circuits in the nervous system. I will present our experimental approach based on light-sheet fluorescence microscopy, an emerging imaging technology that achieves exceptionally high imaging speed and signal-to-noise ratio, while minimizing light exposure of the specimen. This unique combination of capabilities makes light-sheet microscopes indispensable for the long-term in vivo imaging of entire developing organisms. We are designing advanced implementations of scanned light-sheet fluorescence microscopy, such as the SiMView technology framework for simultaneous multiview imaging [1], to systematically study the early development of entire fruit fly, zebrafish and mouse embryos with cellular resolution. I will furthermore present strategies for automated large-scale image processing, advanced specimen culturing techniques and new transgenic reporter lines. Together, these tools allow us to perform whole-organism functional imaging and quantitatively analyze developmental lineages and their interrelationships in the entire animal [2]. Our goal is to take advantage of these high-resolution data to attain a system-level understanding of cell fate decisions and how they establish the dynamic architecture of neural tissues. In the long-term perspective, we will use this information for the establishment and validation of a computer model of the developing nervous system. I envision that our quantitative approach to the reconstruction of large neuronal system dynamics will provide critical insights into the properties of complex circuits and complement ongoing large-scale electron microscopy analyses of static neuronal network architecture.

Program/Abstract # 39

Regulatory logic of pan-neuronal gene expression

Oliver Hobert, Inés Carrera, Nikolaos Stefanakis (Columbia, USA)

Cell fate decisions in the vertebrate nervous system are particularly complex as the nervous system is composed of a remarkably complex assemblage of cell types. Although a lot is known about how specific transcription factors, or Terminal Selectors (TS), specify different neuronal types by coregulating neuron-type specific terminal differentiation genes, much less is understood about the regulatory programs that control the expression of those neuronal features shared by every neuron, pan-neuronal features. Addressing this question is key to understanding how neuronal fate is determined. Using *Caenorhabditis elegans* as a model system, we are dissecting the *cis*-regulatory logic of broadly expressed neuronal genes and also identifying those *trans*-acting factors that regulate them. Dissectional analysis of *cis*-regulatory control elements of pan-neuronal genes shows a piecemeal regulation of gene expression in different neuronal types as well as redundant elements. We find that TS are also able to regulate expression of isolated *cis*-regulatory modules of some pan-neuronal genes, although in TS mutants full promoters of these genes are not affected. We are currently conducting genetic screens to identify these redundant transcription factors. Analysis of the temporal and spatial expression of broadly expressed neuronal genes by fosmid reporters show expression in the nervous system as well as in other tissues. Progress towards the understanding of how pan-neuronal gene expression is regulated will be presented.

Program/Abstract # 40

Coexpression of the homeogenes barhl2, otx2 and irx3 specifies the identity and properties of the Mid-Diencephalic Organizer

Béatrice Durand, Hugo Juraver-Geslin (IBENS/CNRS, France); José Luis Gómez-Skarmeta (CSIC/ Univ Pablo de Olavide, Spain)

The Mid-Diencephalic Organizer (MDO) secretes the morphogen Sonic Hedgehog (Shh) and controls growth and regionalization of the caudal forebrain. Little is known about how the MDO compartment is established during growth and morphogenesis of the forebrain or what controls MDO competence to express shh. In this study performed in amphibians, using in vivo loss- and gain- of

function validated with explant studies, we describe the network of interactions between the transcription factors barhl2, iroquois, orthodentricle and pax6 that controls establishment of the presumptive MDO (pre-MDO), and we identify the master patterning homeogenes that specify the MDO identity. We establish that the pre-MDO emerges from early neurulation onwards inside the prosomere p2 in a domain that expresses barhl2 and otx2, but is devoid of pax6. The pre-MDO expresses iroquois (irx)3, whereas the prospective thalamus (prothalamus) expresses irx1/2 and early on irx3. The MDO anterior boundary forms at the neurula stage whereas the MDO caudal boundary develops at tailbud stage through cross-repressive interactions between the iroquois factors and the shh-dependent recruitment of irx3 expressing cells from the prothalamus into the MDO. In explants, cells expressing a MDO or a thalamic signature recapitulate in vivo cellular behavior: when in contact with secreted Shh, MDO-cells are competent to express shh and segregate from cells of unrelated lineages, whereas thalamic-cells do not express shh and develop boundaries with a MDO-like territory. Barhl2 function in controlling the levels and activity of ß-Catenin participates in MDO patterning. The specification mechanism we describe provides new insights on caudal forebrain development.

Program/Abstract # 41

Dual placode/neural crest origin of olfactory sensory neurons

Ankur Saxena, Marianne Bronner (Caltech, USA)

The cranial ganglia and sense organs arise from two cell types: neural crest and ectodermal placodes. Both undergo cell migration and/or dynamic cell rearrangements to reach their final configuration. Most cranial peripheral neurons are derived from the placodes, with glia cells coming from the neural crest. In the olfactory system, the classical view has therefore been that the olfactory placode forms all olfactory sensory neurons. In contrast, we show that cranial neural crest cells migrating from the neural tube are the primary source of microvillous sensory neurons. In studying the olfactory expression of a novel protein termed olfactin, we observed significant cell migration into the developing olfactory epithelium. Using photoconversion-based fate mapping and live cell tracking coupled with laser ablation in zebrafish embryos, we determined that neural crest precursors were migrating from the neural tube to surround the olfactory epithelium where they condensed to form the nasal cavity. Eventually, a subset of these cells, coexpressing Sox10 protein and a neurogenin1 reporter, ingressed into the epithelium, intercalated amongst placode-derived cells, and differentiated into microvillous sensory neurons. Timed loss-of-function analysis using a photo-morpholino revealed a critical requirement for Sox10 in microvillous neurogenesis, and we are now investigating the roles of olfactin and other proteins during this dynamic process. Taken together, these findings demonstrate for the first time a Sox10-dependent cranial neural crest migratory contribution to olfactory sensory neurons and provide important insights into the assembly of the nascent olfactory system.

Program/Abstract # 42

The roles of Atoh1 in the developing cerebellum under the influence of multiple organizers

Mary Green, Richard Wingate (King's College of London, UK)

The domain of the developing cerebellum in dorsal rhombomere 1 is specified by signals from two adjacent organising centres, the boundary of the roof plate of the fourth ventricle and the midbrain-hindbrain boundary (isthmus). The cerebellar rhombic lip comprises a dorsal progenitor population, defined by the expression of the transcription factor Atoh1, which sequentially gives rise to both extra-cerebellar and cerebellar populations of glutamateric neurons. Atoh1 expression in the cerebellar rhombic lip is dependent on tissue interaction with the adjacent roof plate boundary. We find that, in chick, the isthmus abuts an expanded region of Atoh1 expression from which extra-cerebellar rhombic lip derivatives are born. Through surgical manipulation of the isthmus and roof plate in cultured explants we demonstrate that this expanded region of Atoh1 is dependent upon the isthmus for its induction and maintenance. We also demonstrate the role of isthmic Fgf8 in the maintenance of this expanded domain through in ovo electroporation of Fgf8 and a dominant-negative FGF receptor. Whilst FGF signalling can modulate the expression of Atoh1 in rhombomere 1, we show that this occurs independently of cell specification at the rhombic lip. Instead we show that loss of isthmic FGF signalling results in a reduction in the overall size of the cerebellum by mediating changes in growth in rhombomere 1. Analysis of FGF receptor knockout mutants and FGF hypomorph mice confirmed the results of dominant negative over-expression in chick. Together this work demonstrates the existence of an FGF-induced Atoh1 population that does not contribute specific rhombic lip derivatives but instead regulates overall cerebellar growth.

Program/Abstract # 43

Evolutionary origin of the turtle body plan from genomic, anatomical and developmental perspectives

Shigeru Kuratani (RIKEN, Japan); Hiroshi Nagashima (Niigata U, Japan); Naoki Irie (RIKEN, Japan)

The turtle shell represents an example of evolutionary novelty, with its unusual topography of musculoskeletal elements achieved by the developmental repatterning of mesodermal tissues. During development, growth of the turtle ribs is arrested in the axial part and allowed to grow laterally towards the turtle-specific carapacial ridge (CR), thereby encapsulating the scapula inside the ribcage. The CR supports the fan-shaped patterning of the ribs concomitant with marginal growth of the carapace by specific expression of some regulatory genes. The developmental background of the turtle shell is also consistent with the recently discovered fossil species, *Odontochelys*. By generating and analyzing draft genomes of Chinese soft-shelled turtle (*Pelodiscus sinensis*) and the green sea turtle (*Chelonia mydas*), we confirmed a close relationship of turtles to the bird/crocodilian lineage, which split ~267.9-248.3 million years ago (Upper-Permian to Triassic period). We also found extensive expansion of olfactory receptor genes in these turtles. Embryonic gene expression analysis revealed an hourglass-like divergence between turtle and chicken embryogenesis, with maximal conservation

around the vertebrate phylotypic period, rather than at later stages that show the amniote-common pattern. Wnt5a expression was found in the growth zone of the dorsal shell, supporting the possible co-option of limb-associated Wnt signaling in the acquisition of this turtle-specific novelty. Our results suggest that turtle evolution was accompanied with an unexpectedly conservative vertebrate phylotypic period, followed by manifestation of their evolutionary novelty.

Program/Abstract # 44
Withdrawn

Program/Abstract # 45
Transcriptional inputs and outputs of reiterative beta-catenin switches in a spiral-cleaving embryo
Stephan Schneider, Benjamin Bastin, Margaret Pruitt, Edward Letcher, Hsien-chao Chou (Iowa St U, USA)
In the spiralian annelid *Platynereis dumerilii* each embryonic cell division oriented along the animal-vegetal axis is accompanied by higher nuclear accumulation of beta-catenin in the vegetal-pole daughter cell. As in the distantly related *C. elegans* where the Wnt/beta-catenin pathway is activated after every anterior-posterior oriented cell division, these observed reiterative asymmetries appear to convey lineage specific cell fate decisions. Ectopic activation of beta-catenin in animal-pole daughters causes the animal-pole daughter cell to adopt the fate of its vegetal-pole daughter cell. However, in contrast to the highly derived Wnt/beta-catenin activation mechanism in *C.elegans*, individual components of the Wnt/beta-catenin signal transduction pathways are highly conserved in *Platynereis*. To gain insights into mechanism and contribution of reiterative beta-catenin asymmetries to segregate cell fates in a spiral cleaving embryo and the formation of the annelid body plan, we (1) defined the stereotyped sister cell asymmetries (as observed by different cell sizes, cell cycle times, and beta-catenin activation patterns) in each cell division cycle until the 220 cell stage, (2) deployed a variety of RNA-seq based approaches to identify genes that comprise the reiterative Wnt/beta-catenin activation mechanisms and potential downstream targets in normal and compromised embryos, and (3) mapped the expression of Wnt pathway components (ligands, receptors, intracellular components, potential target genes) into distinct cell lineages. Our analysis provides the first comprehensive view of spatial and temporal inputs and outputs of Wnt signaling into embryos utilizing a spiral-mode of cell divisions to segregate cell fates.

Program/Abstract # 46
Investigating genomic imprinting in the honeybee methylome
Robert Drewell (Harvey Mudd College, USA)
The Kin Theory of Genomic Imprinting (KTGI) predicts that imprinting will arise when the reproductive interests of parents differ. In colonies of the eusocial honeybee, Apis mellifera, a queen's interests are maximised if she monopolises reproduction in the colony and all her workers are sterile. Males, in contrast, can increase reproductive success if some of their worker offspring are fertile. Honeybees possess a functional DNA methylation system, and methylation is known to play a role in caste determination. Parent-of-origin specific imprinting via methylation provides a candidate mechanism by which a queen may enforce sterility in her worker offspring; by epigenetically modifying genes required for fertility. We examined the genome-wide methylation profile of unfertilized eggs and sperm. 381 genes show significantly differential methylation, with 80% of these genes more highly methylated in eggs. These extensive methylation differences in the germline provide support for the KTGI, showing that the methylome of reproductive males and females differs. Parentally-directed epigenetic modification of genes related to reproduction may therefore be a key mechanism by which eusociality evolves and is maintained.

Program/Abstract # 47
Budgett's frog: a new vertebrate model for morphogenesis at multiple biological scales
Nanette M. Nascone-Yoder, Mandy Womble, Cris Ledon-Rettig, Adam Davis, Mike Dush (North Carolina St U, USA)
Complex morphogenetic processes underlie the emergence of biological form but are often challenging to study in existing vertebrate models. To address this issue, we are capitalizing on the unique developmental features of Budgett's frog, *Lepidobatrachus laevis*. In addition to the experimental advantages that have made amphibian models invaluable, *Lepidobatrachus* embryos also possess exceptional features that are especially powerful for investigating morphogenesis. Their extremely rapid development yields a feeding tadpole in less than two days, reducing to hours morphogenetic events that can take several days or weeks to complete in other models. *Lepidobatrachus* oocytes are eight times the volume of the common laboratory *Xenopus laevis*. Consequently, the large blastomeres of the late blastula can be injected with gain- and loss-of-function reagents to target very specific tissues with high precision. Moreover, because *Lepidobatrachus* morphogenesis proceeds at such a large scale, it offers detailed resolution of organogenesis at even the subcellular level. The remarkable size also enables transcriptional profiling of highly spatiotemporally-defined, universally-applicable morphogenetic events such as tissue folding or organ looping. Finally, *Lepidobatrachus* tadpoles exhibit specializations adapted for a carnivorous diet that provide a new inroad for uncovering the developmental changes that generate phenotypic variation during evolution. Comparative chemical genetic screens can be used to implicate defined molecular pathways and morphogenetic processes in the origin of such novelties. The inimitable features of *Lepidobatrachus* promise to provide an integrated view of morphogenesis at multiple biological scales.

Program/Abstract # 48
Modeling regulatory systems for sea urchin development
Isabelle Peter, Eric Davidson (Caltech, USA)
The formation of distinct cell fate specification domains occurs by means of spatial control of gene expression. At the system level, gene regulatory network (GRN) models attempt to incorporate the complete gene regulatory apparatus which determines a specific developmental process. Yet as these network models include an increasing number of regulatory genes, the outcome of the regulatory interactions between them becomes less easily comprehensible. The sea urchin endomesoderm GRN consists of about 50 regulatory genes and determines the formation of at least five different gene expression domains before the onset of gastrulation. A recently developed Boolean model demonstrates that the experimentally established regulatory interactions which constitute the endomesoderm GRN model are indeed largely sufficient to reconstruct the spatial and temporal regulatory gene expression patterns observed during the first thirty hours of sea urchin development. Furthermore, perturbation of the Boolean model accurately predicted the outcome of experimental perturbation. The Boolean model thus shows that GRN models are in principle sufficient to explain progressive regulatory gene expression during development and it contributes to the formalization of regulatory processes. The insights gained from this analysis are now being applied to a much more complex process of post-gastrular organogenesis, the formation of the embryonic gut.

Program/Abstract # 49
A gene regulatory network for endomesodermal specification in a basal deuterostome.
Veronica Frances Hinman, Brenna McMauley (Carnegie Mellon, USA)
A central pursuit in developmental biology is to understand how maternal processes can generate an embryo with molecularly distinct cell populations. A causal explanation for this requires a comprehensive gene regulatory network (GRN) model that can explain how genes are expressed at exactly the right time and in the right cells. We will discuss our recent work that describes the GRN for one of the most important events in early development, the specification and segregation endoderm and mesoderm, using the sea star, *Patiria miniata*, as our model system . Sea stars are a class of Echinoderms that are considered to represent the basal mode of development of this phylum and possibly of all deuterostomes. The simplicity of sea star early development allows us to provide a detailed explanation of endomesderm specification. Sea stars undergo equal cleavage and generate a holoblastula of only several hundred cells. Endomesoderm fated cells form at the vegetal pole of the early embryo. Later mesoderm fated cells will segregate as central population surrounded by a torus of endoderm. We show that this process is regulated by a spatio temporal gradient of nuclear β catenin. A maternally controlled transcriptional factor based GRN additionally provides timing control for gene activation to ensure a robust response to nuclear β catenin. We will also discuss how these GRN topologies can evolve by altering the β catenin gradient and other signaling pathways.

Program/Abstract # 50
Gene regulatory network of neural crest development
Maneeshi Prasad, (Northwestern, USA)
The Neural Crest (NC) is an embryonic stem cell population that is induced at the neural plate border in vertebrates and later emigrates from the dorsal aspect of the developing neural tube, migrate extensively, and give rise to a large and diverse group of cell types. A network of signaling pathways and transcription factors form the neural crest gene regulatory network (NC-GRN) that regulates the induction, specification and migration of NC. These signaling gradients from the neural and non-neural ectoderm, and mesoderm in the form of Wnt and BMP antagonists induce the expression of early neural crest specifiers, Snail, Twist and Sox9. But not much is known about the downstream targets of these neural crest specifiers and their role during different stages of NC development. However, the reiterative use of these factors during different stages of neural crest development suggests their role in regulating different target genes in a temporally controlled manner. To better understand their role in NC-GRN it is essential to decipher their targets during different stages of NC development. Using genome wide approaches such as ChIP-Seq and RNA-Seq, we have identified new putative regulatory targets of these neural crest specifiers during premigratory stages of neural crest development in Xenopus laevis embryos. These targets include genes involved in regulating EMT as well as maintaining stem cell state. We confirmed the presence of known targets of these factors and also new putative targets that are involved in induction and migration stages of NC development. These Sox9 and Twist targets will provide more insights into the role of these NC specifiers during different stages of NC development and also elaborate their role in NC-GRN.

Program/Abstract # 51
The phosphorylation state of *Drosophila* Mad determines its choice between BMP and Wingless signaling
Edward Eivers, Marlyn Rios, Abigail Aleman, Daniel Lee, Matthew Juarez, Keristineh Vartanpour (CSU Los Angeles, USA)
Bone morphogenetic proteins (BMPs) and Wnts are growth factors that are known to regulate a broad range of cellular events such as stem cell maintenance, cell differentiation and organogenesis, while dysfunctional signaling of either pathway can result in severe developmental abnormalities. Here we describe a new molecular mechanism by which the phosphorylation state of the transcription factor Mad determines its ability to transduce either BMP or Wingless (a Wnt ligand) signals in Drosophila cells. Previously, Mad was thought to function in gene transcription only when phosphorylated by the BMP receptor. We now demonstrate that Mad is required for canonical Wingless signaling specifically when in an unphosphorylated state, by forming a Mad-Pangolin-Armadillo Wnt

transcriptional complex. In contrast, C-terminal phosphorylation of Mad by BMP receptors directed Mad towards BMP signaling, thereby actively competing with the function of Mad in the Wingless pathway. Our results indicate that Mad is a shared component between two unrelated pathways and its distinct signal transduction roles in the BMP and Wnt pathways is controlled by a phospho-serine code on Mad.

Program/Abstract # 52

Gli activators and repressors regulate distinct transcriptional responses through a common cis regulatory module that is required for robust repression of *Gremlin*

Steven Vokes, Qiang Li, Jordan Lewandowski, Marian Powell, Seung Hee Cho, Jacqueline Norrie (UT Austin, USA)

Transcriptional response to the Hedgehog (Hh) pathway is mediated by Gli proteins, which function as context-dependent transcriptional activators or repressors in the respective presence or absence of Hh pathway activation. The mechanism by which Gli proteins activate and repress target genes is poorly understood. In an effort to understand this, we have characterized the response of a Gli-dependent cis-regulatory module (CRM) that regulates *Gremlin* expression in the mammalian limb bud. Contrary to a prevailing model, Gli repression does not modulate the domain of Gli activation-dependent enhancer activity and does not efficiently compete with Gli activation. Instead, Gli repression is detected in regions lacking Gli activator activity where it prevents ectopic transcription driven by additional CRMs. The Gli CRM is not individually essential for regulating *Gremlin*, acting as a shadow repressor that is redundant with additional Gli-dependent CRMs. We propose a model where the properties of Gli activators and repressors are spatially decoupled. Collectively, they regulate transcription through redundant CRMs that act as robust toggle switches to impose Gli-dependent control over transcriptional activity.

Program/Abstract # 53

Modelling and classifying variation in butterfly wings

Filipa Alves (Gulbenkian Inst, Portugal)

The morphological diversity that can be observed on butterfly wings is an excellent example of phenotypic variation. Several butterfly species are becoming established as laboratory model organisms, and a number of natural mutants has already been identified and described. We are using a theoretical modelling approach to study the interplay between the biophysical mechanisms and the gene regulatory networks underlying wing morphology and pigmentation patterning. We are especially interested in how this interplay both generates and constrains the phenotypic variation observed within and among species. Our theoretical models are mainly focused on formulating hypotheses and making testable predictions. The gene regulatory networks are defined by partial differential equations and the spatial gene expression patterns are represented in 2D using finite differences methods. We are testing different possible network topologies and candidate genes by comparing the models' results with the experimental data available. Furthermore, as the models' calibration and validation are strongly dependent on quantifying and estimating the biological parameters involved, we are also developing image analysis tools and databases, and implementing parameter optimization algorithms. Our results provide testable hypotheses for how the observed variation on wing morphology and pigmentation patterns may depend on subtle changes on specific biophysical parameters, opening interesting perspectives to understand the evolution of these mechanisms.

Program/Abstract # 54

Angioblast migration and vascularization of the embryonic cornea are inhibited by lens-derived Semaphorin3A signaling

Peter Lwigale, Chelsea McKenna (Rice, USA)

During eye development, neural crest cells migrate from the periocular region to form the cornea, but it is not clear whether blood vessel precursors (angioblasts) avoid the presumptive cornea resulting its avascularity. Given that periocular angioblasts and ocular blood vessels express neuropilin-1 (Nrp1), a receptor for both vascular endothelial growth factor (Vegf, pro-angiogenic factor) and semaphorin3A (Sema3A, anti-angiogenic factor), we hypothesized that lens-derived Sema3A prevents angioblast migration and vascularization of the developing cornea. We examined the expression of Vegf and Sema3A in the lens by immunohistochemistry and quantified their mRNA by qPCR. We then blocked Sema3A signaling from the region of the presumptive cornea by lens ablation or injection of Sema3A inhibitory peptides. We also investigated whether addition of Sema3A inhibits Vegf induced vascularization of the cornea. Furthermore, we analyzed Nrp1(Sema-/-) mutant mice that lack Sema/Nrp1 signaling for defects in corneal avascularity. Using Tg(tie1:H2B:eYFP) transgenic quail, we show that angioblasts do not migrate into the region of the forming cornea located between the ectoderm and lens. Both Sema3A and Vegf are present in the lens, but the levels of Sema3A transcripts are significantly higher than Vegf during cornea development. Inhibition of lens Sema3A resulted in ectopic angioblast migration and vascularization of the forming cornea. Addition of Sema3A protein inhibits Vegf-induced vascularization of the cornea. We also observed ectopic angioblasts and vasculature in corneas of Nrp1(Sema-/-) mutant embryos. Together, our results show that Sema3A signaling from the lens plays a crucial role in establishing corneal avascularity.

Program/Abstract # 55

An E-cadherin mediated piggyback mechanism drives tissue spreading during epiboly

Miguel Concha, German Reig, Carolina Figueroa, Valeria Larenas, Steffen Hartel (U Chile, Chile)

The spreading of tissues is critical for the building and repair of organisms during morphogenesis and regeneration. One valuable model to study this process is epiboly, whereby cells located in the upper hemisphere of a spherical blastula spread to cover the entire

surface of the embryo. In zebrafish, embryo precursor cells known as deep cells (DCs) spread between the Yolk Syncytial Layer and the overlying surface epithelium (Enveloping layer, EVL) by mechanisms that are still poorly understood. It has been proposed that radial cell intercalation (RCI) drives tissue spreading during DC epiboly. However, DC epiboly and RCI both require E-cadherin mediated adhesion and it is thus possible that RCI is solely a manifestation of a different mechanism of tissue spreading mediated by E-cadherin. Direct assessment of this issue in classical fish models such as zebrafish has been precluded due to the relative high cell density and concomitance of epiboly with other massive cell movements such as gastrulation and convergent extension. Here we report a novel teleost model that offers key advantages for the mechanistic analysis of DC epiboly in vivo. In contrast to zebrafish, epiboly in the annual killifish Austrolebias nigrippinnis occurs in a context of low cell density that precludes RCI, and is temporally dissociated from the cell movements of gastrulation and convergent extension. We found that spreading of DCs during epiboly uses a piggy-back mechanism in the absence of RCL, whereby DCs are passively carried on the surface of the moving EVL due to E-cadherin cell-cell adhesive interactions established between these two cellular domains during epiboly. Sponsors : FONDECYT (1120558, 1120579), Scientific Millennium Initiative (P09-015-F).

Program/Abstract # 56

Dorsal migration and formation of the secondary chain of sympathetic ganglia

Paul M. Kulesa (Stowers, USA); Frances Lefcort (Montana St U, USA); Jennifer C. Kasemeier-Kulesa, (Stowers, USA)

The sympathetic nervous system plays a vital role in the vertebrate organism to regulate involuntary, autonomic functions including breathing and heart rate. Sympathetic ganglia (SG), derived from the neural crest, are discrete structures that form in a beautiful repeating pattern along the vertebrate axis. Previously, we have shown that chemokine signals drive a subpopulation of trunk neural crest cells to the dorsal aorta and separate mechanisms shape the cells into discrete primary SG. Within hours of their formation, sympathetic precursors within the primary SG migrate dorsally towards the ventral surface of the dorsal root ganglia (DRG) and form the permanent, secondary chain of SG. The cellular and molecular mechanisms that mediate dorsal migration and formation of the secondary SG are largely unknown, due to the ventral, less accessible location of this morphogenetic event deep within the embryo. Using transverse slice culture and confocal time-lapse microscopy, we detail the events involved in secondary SG formation. Interestingly, the primary SG move as a cohesive cluster during their dorsal migration. We show extensive cell-cell contacts among SG cells and long filopodial extensions that extend to contact the forming spinal nerve. Extensions from the spinal nerve interact with dorsally migrating SG cells. Tissue studies indicate that when the spinal nerve is ablated or re-located, proper dorsal migration is disrupted. These data indicate extensive cell-cell and cell-microenvironmental interactions are important for proper SG positioning and development.

Program/Abstract # 57

Regulation of cell migration during dorsal appendage morphogenesis

Sandra Zimmerman, Celeste Berg (U Washington, USA)

Cell motility is critical for development and homeostasis. Abnormalities in these processes can produce birth defects or drive cancer-cell metastasis. An excellent model for studying the regulation of cell migration is dorsal appendage (DA) morphogenesis in the Drosophila ovary. Two patches of somatic follicle cells that lie dorsal to the oocyte form the DAs by reorganizing into tubes and crawling over the adjacent, squamous, “stretch” follicle cells. We established that DA-cell migration requires both intrinsic and extrinsic inputs from DA cells and stretch cells respectively. The Sox transcription factor, Bullwinkle (BWK), functions in the nurse cells to regulate DA-cell migration extrinsically, acting through the tyrosine kinases SHARK and SRC42A in the overlying stretch cells. Mutations in bwk lead to DA-cell-adhesion defects, aberrant cell migration, and moose-antler-like DAs. To discover how the BWK-SHARK-SRC42A pathway extrinsically regulates DA-cell migration via the stretch cells, we adapted an established magnetic-bead cell separation protocol for a novel application: mass spectrometry. We purified stretch cells from wild-type vs. bwk egg chambers, compared relative protein expression and phosphorylation, and identified >100 proteins with at least a 2-fold difference in relative abundance, including known cytoskeletal regulators and growth factors. To discern which of these proteins regulate DA-cell migration, we are employing protein and RNA expression analysis, tissue-specific RNAi, and clonal analysis. This approach will identify new factors that regulate DA-cell migration in BWK-SHARK- SRC42A pathway and advance our understanding of how extrinsic signals contribute to coordinated cell migration.

Program/Abstract # 58

p-53 related protein kinase (PRPK) is required for lamellipodia formation and proper cell shape maintenance in *Drosophila* hemocytes

Alvaro Glavic, Vicente Cataldo (U Chile, Chile)

Actin cytoskeleton dynamics is the major determinant of membrane behavior during migration. Dynamic protrusions, specifically lamellipodia and filopodia, are essential for the migration of motile cells. PRPK (p-53 related protein kinase) is an atypical and conserved serine/threonine kinase present from Archaea to humans. In yeast, PRPK (Bud32) has been shown to be part of the KEOPS complex and its mutant displays budding and growth phenotypes. Our previous work showed that Drosophila PRPK is necessary for PI3K/TOR dependent cell growth and proliferation. Here we describe the role of PRPK in lamellipodia formation and cell morphology in hemocytes, Drosophila macrophage-like cells. Using the Gal4/UAS system to express a PRPK tag version revealed its preferential localization at early endosomes. In addition, we found that silencing of PRPK produces hemocytes with abnormal stellate

shape, similar to the Arp2/3 loss of function phenotype, the main actin nucleator in the lamellipodia formation. Accordingly, molecular markers exhibited similar distribution in PRPK and Arpc4 (Arp2/3 complex subunit) knockdown hemocytes. Interestingly, functional interaction assays revealed that Rac1 and Rab35 co-expression, both proteins with demonstrated lamellipodia inducing capacity, attenuated the PRPK knockdown phenotype. Further, Rac1 overexpression phenotype was suppressed by PRPK loss of function. Finally, PRPK deficient hemocytes have decresed phagocytic ability in culture. Our results suggest that PRPK is critical in lamellipodia structuration, a necessary protrusion to right immune cell function. Funding: FONDECYT 1100366, FONDAP 15090007

Program/Abstract # 59

Alterations in Ptch1 Cis-Regulation underlie loss of antero-posterior identity and digit reductions in bovine limbs

Rolf Zeller (U Basel, Switzerland); Amandine Duchesne (Jouy en Josas, France); Sepziale Dario (Basel, Switzerland); Guillaume Andrey (Lausanne, Switzerland); Erkan Uenal, Christian Basel (Basel, Switzerland); Benoit Robert (Paris, France); Carol Wicking (Brisbane, Australia); Denis Duboule (Lausanne, Switzerland); Javier Lopez-Rios (Basel, Switzerland)

Bovine limbs exemplify the skeletal diversification and digit reductions that occurred during evolution of the artiodactyl clade. Comparative functional analysis of mouse and bovine embryos reveals the limb-specific molecular alterations that underlie this morphological variation. No overt differences are detected in establishment of the anterior-posterior (AP) asymmetry and the morphoregulatory signaling system interactions during the onset of limb bud outgrowth. Subsequently, the mesenchymal expression of key genes becomes rather apolar, which indicates that the initial AP asymmetry is lost during progression of bovine limb bud development. Genetic analysis has established that up-regulation of *Ptch1* expression in the mesenchymal cells responding to SHH is crucial to normal mouse limb development. In spite of the increase in *Shh* expression, *Ptch1* remains very low in the mesenchyme of bovine limb buds. Furthermore, *Gli1* expression in bovine limb buds is consistent with a failure in *Ptch1*-mediated sensing of graded SHH signaling in the mesenchyme. 4C-Seq analysis of mouse limb buds and sequencing of the *Ptch1* locus from different artiodactyl species identified a candidate limb bud cis -regulatory region, which is significantly and specifically divergent when comparing artiodactyl and pentadactylous species. Transgenic analysis shows that the mouse *cis*-regulatory region drives expression of a *LacZ* reporter into the posterior-distal mesenchyme. The functional importance of this transcriptional alteration was evidenced by analysis of mouse limb buds lacking mesenchymal *Ptch1*, which results in molecular changes strikingly similar to the expression patterns observed in bovine limb buds.

Program/Abstract # 60

The development and evolution of oxygen-sensing cells

Dorit Hockman (U Cambridge, UK); Alan Burns (Univ College London Inst of Child Health, UK); Alessandro Mongera (Max-Planck Institut für Entwicklungsbiologie, Germany); Shannon Fisher (U Pennsylvania, USA); Knapik, Ela (Vanderbilt, USA); Robert Kelsh (U of Bath, UK); Clare Baker (U Cambridge, UK)

Oxygen-sensing cells involved in the respiratory reflex develop in association with the embryonic pharyngeal arch arteries and gut endoderm. In the adult, oxygen-sensing cells are located in the carotid body and lungs of amniotes, and in the gills and orobranchial cavity of anamniotes. These cells respond to hypoxia in the blood and surrounding air/water, triggering increased ventilation via the respiratory reflex. Despite their physiological importance, little is known about their development or evolution. The oxygen-sensing cells of the gills, which develop in association with pharyngeal arch arteries, are hypothesised to be evolutionarily related to those of the carotid body, which are neural crest-derived and develop in association with the third pharyngeal arch artery. However, this relationship has never been tested. Using neural fold grafts in the chick and genetic lineage-tracing in the mouse, we confirm the neural crest origin of the carotid body, and also show that the oxygen-sensing cells of the lungs are not neural crest-derived. Using vital dye labelling, neural fold grafts, genetic lineage-tracing and analysis of zebrafish mutants lacking all neural crest cells, we show that the oxygen-sensing cells in the gills and orobranchial cavity of lamprey, zebrafish and frog are not neural crest-derived. Hence these cells cannot share an evolutionary origin with carotid body glomus cells. Our results suggest that oxygen-sensing cells in the lungs, not the carotid body, are homologous to those found in the gills and orobranchial cavity of anamniotes, and, furthermore, that the importance for the respiratory reflex of hypoxia-sensitive neural crest-derived cells seems to have evolved in association with air-breathing.

Program/Abstract # 61

The evolution and development of leaves in lycophytes and ferns

Barbara A. Ambrose, Alejandra Vasco, Tynisha Smalls, Robbin Moran (NY Botanical Garden, USA)

There is a vast amount of leaf morphological diversity and leaves are a common feature found across the land plants. However, not all land plant leaves are considered homologous. The fossil record and anatomical evidence suggest that leaves evolved at least 4 times independently in vascular land plants. Molecular genetic studies have unraveled the network underlying the development of flowering plant leaves and some comparative studies have been performed in lycophytes. However the experimental results in the lycophytes have led to diametrically opposed conclusions about the conservation of a common leaf developmental network between lycophytes and flowering plants. These studies focused on Class III HD-Zip and Class I KNOX genes. To better understand the role of these genes in leaf evolution and development we are cloning and analyzing genes from these 2 key transcription factor families across the lycophytes and ferns . In addition, we are analyzing key leaf genes in a monophyletic group of fern species, Elaphoglossum section Squamipedia , to better understand the evolution and development of leaves in this group that have widely divergent leaf

morphologies. Our analyses not only provide insights into leaf development in lycophytes and ferns but also are essential for understanding leaf evolution and development across the land plants.

Program/Abstract # 62

Transcriptome sequencing reveals heterochronic shift of chordate gene networks in *Molgulid* ascidians

Billie J. Swalla (U Washington, USA); Elijah Lowe (Michigan St U, USA); Max Maliska, Ceri Weber (U Washington, USA); Kanchan Pavangadkar, C. Titus Brown (Michigan St U, USA)

We are investigating the complex mechanisms by which evolutionary novelty evolves through changes in gene networks during animal development. One of the most insightful models for investigating chordate evolution makes use of two closely related ascidian species that have dramatically different larval body plans. *Molgula oculata* eggs develop into free-swimming chordate tadpole larvae, whereas a closely related sister species, *Molgula occulta* , have eggs that develop into an anural, or tailless ascidian. Fertilization and cleavage in *M. occulta* are remarkably similar in timing and pattern to its sister species, *M. oculata*. However, the anural M. occulta embryo fails to differentiate several chordate features, including an otolith (gravity sensing vesicle), notochord and tail muscle cells, which are characteristic of ascidian tailed tadpole larvae. We have been investigating the cellular and molecular basis of these tailless ascidians by comparing the transcriptome of the hybrid and the two species embryos at several different developmental stages. Work in *Ciona intestinalis* has allowed identification of many of the genes in the notochord network. Surprisingly, the notochord specification gene network is intact and expressed in *Molgula occulta*, in spite of the tailless embryo lacking a notochord. However, metamorphosis genes are also expressed early, much earlier than has ever been seen in other ascidian species. We believe that this early expression of the metamorphosis gene network is contributing to the tailless phenotype. We are using this system to investigate how the evolution of gene networks influences larval morphology.

Program/Abstract # 63

Withdrawn

Program/Abstract # 64

Pluripotency versus differentiation during the first cell fate decision in the mouse embryo

Magdalena Zernicka-Goetz, Ivan Bedzhov (Gurdon Inst, UK)

The first cell fate decision in the mouse embryo is initiated at the 8 to 16-cell stage transition when asymmetric cell divisions generate precursors of pluripotent and differentiated lineages. Despite the importance of this event, it remains undetermined how daughter cells of such asymmetric divisions differ in gene expression and whether they can be regarded as truly distinct cell types. In order to gain insight into the molecular mechanisms underlying the first steps of lineage segregation, we have applied full transcriptome sequencing to reveal global differences between these precursor cells. This has revealed an unexpected complexity of differential expression of transcriptional networks and signalling pathways as cells first enter distinct paths and provides a wealth of information about the possible requirements for specification of unique cell types. Functional assays have revealed several novel, differentially expressed genes that are critical for setting apart the first cell lineages in the developing embryo. This dataset will be valuable for the discovery of further novel genes and pathways governing the balance between pluripotency and differentiation in early mouse development.

Program/Abstract # 65

Mechanical signals and development: investigating the structure behind the architecture in plants

Olivier Hamant (U de Lyon, France)

Changing shape is changing structure. This implies that at any given time point, a shape can be associated with a pattern of tension and compression, i.e. a pattern of mechanical stress. As shown in animal single cells mainly, mechanical cues can affect important cell processes such as cell polarity, cell fate or cell division. Plants are ideal systems to investigate how mechanical signals control development in a tissue context, for technical reasons but also as mechanics in plants mainly relies on the balance between cell wall stiffness and turgor pressure. Focusing on the shoot apical meristem, the plant stem cell niche, we found that mechanical signals control the orientation of cortical microtubules, which guide the deposition of cellulose and thus control the mechanical anisotropy of plant cell walls. This in turn supports morphogenetic events, such as tissue folding, which further consolidates the stress pattern. We also found that this mechanical feedback loop promotes growth heterogeneity in tissues. We propose that the maintenance of a basal level of growth heterogeneity potentiates organogenesis. Scaling up, we have analyzed the link between mechanical stress and the pattern of organ initiation at the shoot meristem. Whereas this pattern seems relatively independent from mechanical inputs during initiation, a novel and robust post-meristematic pattern was obtained when microtubules are genetically uncoupled from the cellulose deposition machinery. This provides a didactic example of the interplay between growth and patterning, and suggests a contribution of mechanical stress in the robustness of patterns. Prospects for this work are numerous and will be discussed in the talk.

Program/Abstract # 66

Planar Cell Polarity directs Septin-mediated compartmentalization of cortical actomyosin

Asako Shindo, John Wallingford (UT Austin, USA)

Convergent Extension (CE) is a fundamental collective cell movement critical for morphogenesis of various embryonic tissues. The Planar Cell Polarity (PCP) pathway is well known as a main regulator for CE. However, it is still unknown how the PCP pathway

communicates with downstream machinery such as actomyosin to coordinate cell behaviors. We approached the question by focusing on CE during notochord formation in *Xenopus laevis* embryos. We found that actomyosin predominantly accumulates at specific topologies of cell-cell junctions along the medio-lateral axis. Live imaging shows that the actomyosin accumulation pulses synchronously with cell edge shrinkage; and we found that the shrinking edges have higher cortical tension than other edges. Interestingly, tracking photoactivated actin biosensors revealed that actin localization is spatially restricted by septin, a cytoskeletal element that acts as a partition protein in various cell types. Knockdown of septin disrupts the selective accumulation of actomyosin at specific cell junctions, leading to a disturbance of the spatially-restricted cortical tension needed for medio-lateral intercalation. Finally, we found that the PCP pathway is required for septin localization and polarized actomyosin function. Together, we conclude that the PCP pathway regulates septin to generate discrete domains of active actomyosin along cell edges to coordinate medio-lateral intercalation during CE.

Program/Abstract # 67

The dynamic puzzle of cell shape and polarity in plant morphogenesis

Yara Elena Sanchez Corrales, Matthew Hartley (John Innes Ctr, UK); Jop van Rooij(Utrecht U, Netherlands); Enrico Coen, Stan Marée, Verônica Grieneisen, (John Innes Ctr, UK)

Although considerable progress has been made in identifying genes that control cell polarity, it is still unclear how they work together to generate cells with particular shapes. We also have limited understanding about how cell polarity, cell shape and cell growth are temporally and spatially related to ensure a correct organ development. Plant cells offer an excellent system to address these questions because, in contrast to animal cells, they cannot move during development; therefore, plant cells are restricted inside an organ. To understand cell shape and polarity coordination within a growing tissue, we here focus on the development of pavement cells, an intricate system in which the full complexity of polarity and shape change comes together at many levels. Pavement cells show a characteristic jigsaw puzzle-like shape in a non-homogenous spatial pattern within a growing leaf. Combining live-imaging techniques and novel quantitative shape analytic tools with computational and mathematical modelling techniques we have captured cell size and shape changes over time and extracted quantitative information that will be directly compared with our modelling efforts to capture cell polarity and multicellular behaviour. Our results show that local changes at individual cell-level can lead to global patterns, and we hypothesize on the mechanisms of this regulation.

Program/Abstract # 68

Sp6 and Sp8 transcription factors are necessary mediators of WNT/ß-Catenin function in the limb ectoderm

Marian Ros, Endika Haro, Irene Delgado (U de Cantabria, Spain); Yoshihiko Yamada (NIH, USA); Ahmed Mansouri (MPI for Biophysical Chemistry, Germany); Kerby Oberg (Loma Linda U, USA)

The apical ectodermal ridge (AER) is a specialized epithelium located at the distal dorso-ventral (DV) rim of the developing limb that is crucial for limb bud development. The induction of the AER is a complex process that relies on intricate interactions among the FGF, WNT, and BMP signaling pathways operating within the ectoderm and between the ectoderm and mesoderm of the early limb bud. Furthermore, induction of the AER is linked to the establishment of DV patterning. Sp6 and Sp8 are two members of the Specificity Protein family of transcription factors that are expressed in the limb bud ectoderm and function downstream of WNT/ β Catenin signaling and upstream of *Fgf8*. Their individual genetic inactivations result in a mild syndactyly phenotype for *Sp6* and limb truncation, due to the premature regression of the AER, for *Sp8*. To investigate a possible functional redundancy between *Sp6* and *Sp8*, we generated double *Sp6*;*Sp8* null mutants. We also generated *Sp6*-null; *Sp8*-conditional mutants using a *Sp8* floxed allele with the *Ap2-Cre* and with the *Msx2-Cre* deleter lines. Our results show that double *Sp6*;*Sp8* mutants are tetra-amelic. Initial budding occurs, but *Fgf8* and *Bmp4* are not activated in the limb ectoderm and the dorsal marker *Wnt7*a persists throughout the limb bud ectoderm. The phenotype of mutants bearing a single functional copy of *Sp6* (*Sp6* $^{+/-}$;*Sp8*$^{-/-}$) is indistinguishable from that of the double mutants, whereas the presence of a single functional allele of *Sp8* (*Sp6*$^{-/-}$;*Sp8*$^{+/-}$) results in a Split Hand Foot Malformation phenotype. We conclude that Sp6 and Sp8 work in a redundant manner as indispensable mediators of WNT/ β Catenin signaling in the limb ectoderm and that their function links the Proximo-distal and DV axes.

Program/Abstract # 69

Coordinating organogenesis: Insights from the Drosophila eye

Tiffany Cook, Mark Charlton-Perkins, John Mast (Cincinnati Children's Hosp., USA); Elke Buschbeck (U Cincinnati, USA)

The Drosophila compound eye is a powerful system to study molecular networks controlling the generation of a complex organ. This is due to its regular crystalline architecture and its easy electrophysiological assessment, which allow ready detection of morphological abnormalities and visual dysfunction. We previously showed that two direct cell-restricted Ras and Notch (N) target genes, the transcription factors Prospero (Pros) and dPax2, antagonize each other to define neuronal vs non-neuronal (R7 photoreceptor [PR] vs lens-secreting cone cells [CCs]) fates, but cooperate during CC specification and lens formation. We further demonstrated that Pros and dPax2 mediate these functions through positive feedback with Ras and Notch signaling, respectively. Combining genetic, transcriptomic, cell biological, and electrophysiological approaches, we have now defined two novel non-autonomous CC functions that also require Pros and dPax2. Early in eye development, differential Ras/Pros and N/dPax2 activities oppositely control the formation of the two essential retinal pigmented epithelia (RPE) cell types: 2° and 3° pigment cells. Later, Pros and dPax2 in CCs cooperate to regulate PR structure and function, respectively. These new CC functions involve interplay with both cell adhesion and

junctional proteins (RPE patterning and PR morphogenesis, respectively), as well as homeostatic glia-related factors (PR function). Combined, our studies reveal that CCs not only generate the fly corneal lens, but also serve as patterning hubs - integrating intrinsic (Pros and dPax2) and extrinsic (Ras and N) factors - to ensure the coordinated development of a functional visual system.

Program/Abstract # 70

A nervous embrace: WNT signaling initiates the neuronal-epithelial communication essential for submandibular gland organogenesis.

Wendy Knosp (NIDCR/NIH, USA); Sarah Knox, Gail Martin (UCSF, USA); Matthew Hoffman (NIDCR/NIH, USA)

Communication among multiple progenitor cell types at distinct locations and times during embryogenesis initiates and exquisitely coordinates organogenesis. During submandibular gland (SMG) development cholinergic signals from the parasympathetic ganglia (PSG) maintain the epithelial progenitor cells as a reservoir for organogenesis, and the epithelial progenitors in turn produce neurotrophic factors for PSG survival and axon outgrowth. However, the signals that establish the initial interaction between the neuronal progenitors and the epithelial duct are unknown. Here, we identify WNT signals from the epithelial duct promote neuronal progenitor cell survival and proliferation, and establish the initial nervous embrace of the duct by the PSG. We also demonstrate that when WNT expression is reduced in the duct, there is a concomitant reduction in WNT signaling and a striking loss of PSG formation. This absence of the PSG results in a loss of epithelial progenitor cells and disrupted SMG development. Activation of WNT signaling in vivo restores PSG formation and reestablishes the association of the PSG with the duct, which rescues epithelial development. These results demonstrate that WNT signaling controls the development and arrangement of the PSG around the duct. This WNT-dependent initial nervous embrace of the duct by the PSG is essential to establish the neuronal-epithelial communication that is required for progenitor cell maintenance and organogenesis.

Program/Abstract # 71

The fate and behavior of Ret-expressing tip cells in kidney development

Paul N. Riccio (Columbia, USA); Hideki Enomoto (RIKEN, Japan); Frank Costantini (Columbia, USA)

Signaling through the receptor tyrosine kinase RET is required for the normal progression of mammalian kidney development. While a complete portrait of the transcriptional changes effected by RET activation has emerged through microarray profiling, comparatively little is known about the fates of *Ret*-expressing tip cells, themselves, and of the cell-level manipulations required to sustain branching of the ureteric bud. We demonstrate that *Ret*-expressing tip cells are multipotent progenitors that give rise to the entirety of the renal collecting system. Pulse labeling of *Ret*-expressing tip cells confirms that they form a self-renewing niche that gives rise to the "trunk" regions of the collecting system, including both principal and intercalated cell types. Loss of *Ret* from a portion of the tip cells severely disrupted normal branching, yielding hypomorphic kidneys with dysplastic tips. This occurred even upon later stage *Ret* deletion, suggesting a continued role for RET in maintaining the branching program. Mosaic analysis with double markers (MADM) was utilized to follow the fates of individual cells that lost *Ret*. This genetic tool proved incredibly powerful, revealing that cells that lose Ret are near completely excluded from the tip domain. This sorting behavior is fairly rapid, and we hypothesize that RET activity might confer an ability to undergo cell rearrangements, or migration-like movements, that keep a cell positioned at the tip. Collectively, these findings augment our appreciation of the Ret -expressing tip domain as a special compartment within the branching ureteric bud.

Program/Abstract # 72

The role of Pitx2c and Par3/aPKC in Left/Right asymmetric gut curvature

Adam Davis, Nanette Nascone-Yoder (North Carolina St U, USA)

Intestinal malrotation is a potentially lethal disorder that occurs in 1 in 500 newborns. Normal gut morphogenesis and directional rotation is dependent, in part, on *Pitx2c*, a transcription factor that is exclusively expressed on the left side of the gut tube. However, little is known of the downstream targets of *Pitx2c* that mediate the cellular morphogenetic properties integral for left-right (LR) asymmetric gut morphogenesis. Par-3 is a morphogenetic scaffolding protein that recruits aPKC to the apical region of cells to promote apical-basal polarization, a process that is crucial for tissue morphogenesis, epithelial maturation and the development of epithelial organs. We found that, prior to and during overt foregut curvature, Par-3 and aPKC are apically enriched within *Pitx2c*-expressing cells in the left gut endoderm. These cells also exhibit apical enrichment of downstream effectors of Par-3/aPKC polarity, including E-cadherin, γ-tubulin, and α-tubulin. These cellular asymmetries suggest that *Pitx2c* controls asymmetric foregut morphogenesis by inducing the apical enrichment of Par-3 in the left, but not right, foregut endoderm, leading to precocious epithelial morphogenesis on the left side, which drives topological curvature. In support of this hypothesis, ectopic expression of *Pitx2c* on the right induces ectopic apical enrichment of Par-3, aPKC, E-cadherin, γ-tubulin, and α-tubulin in the right endoderm, resulting in a lack of curvature. Our results suggest that *Pitx2c* generates morphological asymmetry in the gut tube by controlling the apical localization of Par3/aPKC to promote epithelial polarization in the endoderm. This work provides a new inroad to understand the etiology of intestinal malrotation.

Program/Abstract # 73

Pax3 and Pax7 regulate cranial neural crest cell growth and maintenance through an unexpected environmental stress response pathway

Antoine Zalc, Revital Rattenbach, Frédéric Relaix (UPMC-Paris, France)
Exposure to environmental pollutants, such as Dioxins, during pregnancy leads to teratogenic defects. It was shown that exposure to TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) can cause reproductive and developmental defects such as craniofacial malformations. However the molecular link between these teratogenic compounds and developmental pathways remain poorly understood. Here we show that mice deficient for Pax3 and Pax7, two related paired-homeobox transcription factors essential for neural crest cells development, display severe craniofacial defects. We identified downstream molecular pathways linked with Pax3/7 function in craniofacial morphogenesis. Strikingly, w e have demonstrated that not only impairment in the functions of these proteins leads to the formation of facial clefts reminiscent of that seen upon TCDD exposure, but also that these defects are mediated by ectopic activation of the Aryl Hydrocarbon Receptor (AhR) signalling , the receptor to TCDD. Importantly, we show that blocking AhR function rescues facial closure in Pax3/7-deficient embryos. These results demonstrate that Pax3 and Pax7 have a novel and unexpected role in regulating environmental stress response pathways during development.

Program/Abstract # 74
The molecular basis of development and diversification of beetle horns.
Teiya Kijimoto, Armin Moczek (Indiana U, USA)
One of the most fascinating themes in evolutionary developmental biology is the molecular and developmental basis of diversity in form and shape. Horned beetles in general, and the genus *Onthophagus* in particular, provide excellent opportunities to address this fundamental question given the dramatic diversity that exists both within and between species in important aspects of horn development, including location, number, size, and shape of horns. Since beetle horns do not share obvious homology with traditional insect structures, they offer additional opportunities to investigate the development and evolution of a novel trait. Lastly, many aspects of horn development are influenced variably by both genetic and environmental factors (in particular nutrition), thus making horns useful traits with which to explore the developmental basis of plasticity. Here we report on the role of sex-determination and appendage patterning genes, and their potential interplay, in the regulation of shape and size of beetle horns. Specifically, (i) we show that sex determination gene *doublesex* (*dsx*) plays a fundamental role in sex- as well as nutrition-dependent horn development and its diversification, including the secondary loss of plasticity and the rapid evolution of reversed sexual dimorphism (Kijimoto et al. 2012). (ii) Second, we present our most recent findings on patterning genes that specify important aspects of horn development, and whose activation may be regulated at least in part by *dsx*. We discuss the most important implications of our results for our understanding of the regulation and diversification of shape and the role of cooption in evolutionary innovation.

Program/Abstract #75
Withdrawn

Program/Abstract # 76
Changing the way we teach: how we should and why we must! (Viktor Hamburger Prize Lecture)
Bill Wood (U Colorado-Boulder, USA)
Several efforts over the past decade have sought to increase the use of research-based pedagogy in college-level biology courses. Although the resulting movement toward a more scientific approach to teaching is slowly gaining ground, change is difficult; there is still a long way to go, and time may be running out. If we cannot increase the added value of the residential college experience by providing more effective instruction to a more diverse population of students, the rapid evolution of low-cost online alternatives poses a serious threat to existing institutions of higher education.

Program/Abstract # 77
Evolutionary origin and functional diversification of retinoid signaling in development
Michael Schubert (Lab Biol Dev de Villefranche sur Mer, France)
Retinoids constitute a group of fat-soluble morphogens related to retinol (vitamin A) that play crucial roles in early development, organogenesis and tissue homeostasis by regulating cell proliferation, differentiation and apoptosis. In vertebrates, most retinoid functions are mediated by retinoic acid (RA) binding to heterodimers of the retinoic acid receptor (RAR) and the retinoid X receptor (RXR). Retinoid signaling was thought to be vertebrate-specific, but studies in invertebrate chordates have revealed RA functions that are conserved in all chordates. To obtain further insights into the evolutionary diversification of retinoid signaling in the chordate lineage, we dissected its time-dependent functions during development of two models located at key positions of the chordate tree: amphioxus (a cephalochordate) and the lamprey (an agnathan vertebrate). Comparison of these species revealed an ancestral role for retinoid-FGF antagonism in patterning the chordate brain, a mechanism that has been further modified in the gnathostome vertebrate lineage. Outside chordates, however, evidence for functional roles of retinoid signaling remains scarce, although bioinformatic analyses indicate that the genes encoding the main mediators of retinoid signaling in chordates are also present in other metazoan phyla, including xenambulacrarians (e.g. sea urchins, hemichordates) and lophotrochozoans (e.g. annelids, mollusks). These in silico results suggest that the retinoid pathway has already been present in the last common ancestor of protostomes and deuterostomes. Altogether, this work thus emphasizes the importance of comparative approaches for understanding the evolution of developmental mechanisms.

Program/Abstract # 78

***In vivo* receptor localization and expression screen identifies a novel mechanism of EGFR regulation through Ezrin/Radixin/Moesin proteins**

Juan Miguel Escobar-Restrepo, Andrea Haag, Peter Gutierrez, Alessandra Bühler (U of Zurich, Switzerland)

C. elegans let-23 is the homologue of the EGFR family of receptor tyrosine kinases and is required for the formation of the hermaphrodite vulva. Baso-lateral localization of EGFR in the Vulval Precursor Cells (VPCs) is required for efficient receptor activation by LIN-3 EGF. We performed an in vivo RNAi screen for defects in receptor localization and/or expression using a functional EGFR::GFP reporter. We identified ERM-1, the homologue of mammalian Ezrin, Radixin and Moesin, as a negative regulator of the EGFR/RAS/MAPK pathway. We find that ERM-1 sequesters and stabilizes EGFR in an inactive compartment at or near to the baso-lateral plasma membrane of the VPCs: (1) *erm-1(lf)* or RNAi against erm-1 causes a reduction in the baso-lateral EGFR::GFP signal. (2) *erm-1(lf)* suppresses the Vulvaless phenotype of reduction-of-function mutations in components of the EGFR/RAS/MAPK pathway and enhances the Multivulva phenotype in a gain-of-function mutation in *let-60 ras*. (3) ERM-1::mCherry co-localizes with EGFR::GFP at the baso-lateral plasma membrane of the VPCs. (4) Recombinant ERM-1::GST interacts with EGFR from worm extracts independent of LIN-7 or the PDZ binding motif of EGFR, suggesting a different complex to the LIN-2/LIN-7/LIN-10. (5) FRAP showed a significantly faster recovery of basal EGFR::GFP signal in *erm-1(lf)*. Thus ERM-1 inhibits the internalization at the baso-lateral plasma membrane and/or the lateral diffusion of EGFR within the plasma membrane. We propose that ERM-1 retains a fraction of EGFR in an inactive compartment to prevent the immediate activation of the entire pool of baso-lateral EGFR by EGF and thus allow the generation of a prolonged signal.

Program/Abstract # 79

The role of PTEN in the regulation of ciliogenesis

Iryna Shnitsar, Miriam Barrios-Rodiles, Mikhail Bashkurov, Eduardo Aguiar, Laurence Pelletier (Mount Sinai Hospital, Canada); Rudolf Winklbauer (U of Toronto, Canada); Jeffrey Wrana (Mount Sinai Hospital, Canada)

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a dual activity phosphatase, which plays a pivotal role during cell migration, survival and apoptosis. It was initially discovered as phosphatidylinositol (3,4,5)-trisphosphate phosphatase and one of the main regulators of PI3/Akt signaling. Subsequently PTEN was shown to dephosphorylate protein targets and affect the function of other signaling proteins, including p53, BAD, FAK and RhoA. Mutations and deletions in the PTEN gene are frequently associated with a variety of cancers, including prostate, breast and brain, establishing its role as a tumor suppressor. In the present work we addressed the function of PTEN during early *Xenopus* embryo development by knocking down its expression with morpholino oligonucleotides. We observed a dramatic disruption of anterior-posterior fluid flow due to a severe decrease in skin cilia formation. Both confocal and transmission electron microscopy studies demonstrated defects in apical docking of basal bodies upon down regulation of PTEN expression. Moreover, staining of endogenous PTEN revealed that the protein is localized in both the cilia axoneme and in the area adjacent to the basal body. We are currently analyzing PTEN function in mammalian ciliated cells to determine the signaling pathways regulated by this protein during cilia formation.

Program/Abstract # 80

Mechanisms and roles of Hedgehog signaling in the zebrafish

Phil Ingham (IMCB, Singapore)

Discovered exactly 20 years ago, the *Sonic Hedgehog* (*Shh*) gene is now well known as one of the key regulators of vertebrate embryonic development. In addition, dysfunction of the Hedgehog signaling pathway has been implicated in a variety of cancers as well as congenital disorders, stimulating a concerted effort to discover drugs that modulate the pathway. Analysis of the mechanisms and functions of Shh in the zebrafish have revealed both conserved and divergent aspects of Hedgehog signaling. I will review some of these findings, focusing in particular on our recent studies of the vertebrate orthologue of *Drosophila* Costal2, the kinesin family member Kif7, mutations of which are associated with a number of human conditions including Acrocollosal and Joubert syndromes. Our results highlight the complexity of the Hedgehog signal transduction pathway and underline the value of a multi-systems approach to its analysis.

Program/Abstract # 81

CCDC28B is a novel protein involved in ciliogenesis that modulates mTORC2 function and interacts with SIN1 to control cilia length

Magdalena Cardenas-Rodriguez, Florencia Irigoín (Inst. Pasteur de Montevideo, Uruguay); Daniel P.S Osborn (Univ College London, UK); Cecilia Gascue (Inst. Pasteur de Montevideo, Uruguay); Nicholas Katsanis (Duke, USA); Philip L. Beales (Univ College London, UK); Jose L. Badano (Inst. Pasteur de Montevideo, Uruguay)

Primary cilia are cellular structures that play a key role in sensing, transducing and integrating both mechanic and chemical signals. Thus, these organelles are critical in the interaction of cells with other cells and the environment. Consequently, it is not surprising that cilia dysfunction has been causally linked to a number of phenotypes and human conditions termed ciliopathies. CCDC28B was originally identified as a second site modifier of the ciliopathy Bardet-Biedl syndrome (BBS) and we have shown previously that is a novel protein involved in the regulation of cilia length. However, the mechanism by which CCDC28B achieves this biological role is unknown. Here we present data showing that CCDC28B interacts with SIN1, a component of the mTOR complex 2 (mTORC2), and

acts as a positive regulator of this complex, participating in its assembly/stability and modulating its activity. We show that Ccdc28b regulates cilia length in vivo, at least in part, through its interaction with Sin1 and that depletion of Rictor, another core component of mTORC2, does not result in shortened cilia. Thus, we describe a previously unknown role of SIN1 in cilia biology, which is independent of its mTORC2-related function. Taken together, our findings implicate CCDC28B in the regulation of mTORC2 and provide mechanistic insight to understand the role of CCDC28B in the regulation of cilia length uncovering a novel function of the core mTORC2 component SIN1 in this process.

Program/Abstract # 82

Molecular Mechanism of Gprk2-dependent Smoothened Regulation in Drosophila

Dominic Maier, Shuofei Cheng, David Hipfner (IRCM, Canada)

Hedgehog (Hh) signaling plays a conserved and essential role in regulating development and homeostasis of numerous tissues. Cytoplasmic signaling is initiated by Smoothened (Smo), a G-protein-coupled receptor (GPCR) family member, whose levels and activity are regulated by the Hh receptor Patched (Ptc). In response to Hh binding to Ptc, Smo accumulates at the membrane in a hyper-phosphorylated, active state. cAMP-dependent Protein kinase A (Pka) and Casein kinase I (CkI) are crucial kinases in this step. However, phosphorylation of Smo by Pka and CkI accounts only for a fraction of total Smo phosphorylation suggesting that other kinases might also be involved in Smo regulation. G-protein-coupled receptor kinases (GRKs) are known to phosphorylate activated GPCRs, leading to the termination of G-protein-dependent signaling and receptor internalization. We have shown that Gprk2, a member of the GRK family in *Drosophila melanogaster*, promotes Smo phosphorylation, most likely directly. By using two independent approaches we have mapped multiple Gprk2-dependent phosphorylation sites in the Smo C-terminus. Because Hh target gene expression is reduced in *gprk2* mutant flies, we hypothesized that phosphorylation of Smo by Gprk2 is required for full activation of Smo and consequently of Hh target genes. Consistent with this, we find that depletion of Gprk2 in *Drosophila* S2-R+ cells also decreases Hh target gene expression as measured with a transcriptional reporter assay. Interestingly, expression of Smo constructs in which Gprk2 phosphorylation sites have been mutated to alanine also decreases Hh target gene expression, mimicking Gprk2 depletion. Based on these findings we propose that complete Smo activation depends on at least three kinases: Pka and CkI as well as Gprk2.

Program/Abstract # 83

Identification and regulation of adrenocortical stem cells

Ed Laufer, Salma Begum, Alex Goldberg, Alex Paul (Columbia, USA)

The adrenal cortex is an endocrine organ that produces steroid hormones critical for regulating ionic balances and blood volume, and modulating stress responses. We previously defined both Shh-expressing cells within the zona glomerulosa at the cortical periphery and Shh-responsive, Gli1+ cells in the overlying capsule mesenchyme as progenitors of all steroidogenic cell types. We have used long-term genetic lineage tracing techniques to ask whether either population contains bona-fide adrenocortical stem cells. We find that Shh lineage cells persist throughout life, and continuously contribute to all steroidogenic cell types, while the Gli1 lineage generates steroidogenic cells only during embryogenesis. Thus only the Shh population contains cells with adrenocortical stem cell properties. The canonical Wnt pathway has been implicated as a potential regulator of adrenocortical stem cells at the gland periphery. We find that Shh-expressing cells have elevated levels of beta catenin, and are also marked by a transcriptional reporter of canonical Wnt signaling. To address the relationship of Wnt signaling and maintenance of the Shh population, we deleted a conditional beta catenin allele from the cortical population using cre recombinase expressed under the control of promoters expressed throughout the steroidogenic lineage. If we delete beta catenin prior to the condensation of the gland, then the cortical cells disperse, and adrenal formation fails. However if we delete beta catenin after gland formation, then the cortex shrinks over a few weeks, with a progressive outer to inner loss of cortical populations. These data are consistent with canonical Wnt signals helping define the adrenocortical stem cell niche.

Program/Abstract # 84

Early insights into the morphogenesis of the activated zone for regeneration or repair in the axolotl and in the mouse

Saori L. Haigo (UCSF, USA); Aida Rodrigo-Albors, Akira Tazaki, Elly M. Tanaka (DFG Ctr. for Regenerative Therapies Dresden, Germany); Jeremy F. Reiter (UCSF, USA)

Despite a recent renaissance of interest in regenerative biology, we still have a limited understanding of basic cellular mechanisms that underlie tissue repair in animals. Among vertebrates, urodele amphibians have the remarkable ability to regenerate damaged organs, while mammals show restricted tissue repair with scarring of damaged organs. To begin to elucidate shared and divergent mechanisms utilized by regenerating or repairing vertebrates, we sought to identify the activated zone of cells surrounding a full-thickness wound in the mouse skin. Similar to the axolotl spinal cord, which activates a 500 micrometer zone adjacent to the amputation plane sufficient to reconstruct the lost spinal cord following tail amputation, our preliminary observations suggest that the mouse skin may have a similar 500 micrometer zone adjacent to the wound that is activated to repair the epidermis following injury. Moreover, this activated zone maintains this 500 micrometer distance from the wound edge despite changing the size of initial injury. We also present data on real time imaging of the initial morphogenesis of spinal cord regeneration in the axolotl. Finally, we provide a comparative perspective on the initial cellular events that underlie regeneration or repair in vertebrates.

Program/Abstract # 85

Guts and gastrulation: cell dynamics and the morphogenesis of the early mouse embryo

Anna Katerina Hadjantonakis (Sloan Kettering Inst., USA)

Gastrulation is a paradigm for tissue morphogenesis, as it involves the coordination of cell fate specification and cell movement, which together drive tissue formation and segregation. In the mouse, gastrulation transforms a cup-shaped structure comprising two tissue layers (epiblast and visceral endoderm) and separated by a single basement membrane, into one comprising three tissue layers (epiblast, mesoderm and gut endoderm) and two basement membranes. Our studies are focused on understanding the coordinate events driving this transformation. Live imaging combined with genetic labeling studies suggest that morphogenesis of gut endoderm in the mouse embryo involves a dynamic widespread intercalation between two endodermal populations, definitive and visceral. This morphogenetic event results in the formation of an epithelium on the surface of the embryo, comprising cells of two distinct origins. By investigating mutants in which gut endoderm morphogenesis is perturbed we are developing a mechanistic understanding of the cell behaviors regulating this process. Definitive endoderm cells adopt a trajectory aligned with the wings of mesoderm as they exit the primitive streak. From there they emerge on the surface of the embryo, by egressing into the visceral endoderm epithelium. In doing so they execute a program of mesenchymal-to-epithelial transformation (MET) involving the acquisition of apico-basal polarity and resulting in the co-ordinate assembly of a basement membrane at the gut endoderm / mesoderm interface, a fundamental feature of gastrulation. Progress with these studies will be discussed.

Program/Abstract # 86

Pitx2 regulates proliferation in skeletal muscle cells and modulates muscle regeneration

Estefania Lozano-Velasco, Alejandra Contreras, Daniel Vallejo, Ana Soriano, Diego Franco, Amelia Aránega Jimenez (U Jaen, Spain)

Regeneration of skeletal muscle mainly depends on adult muscle progenitors, named satellite cells. Some of these satellite cells are capable of both proliferate/self-renewal and differentiate along the skeletal muscle lineage, defining them as stem cells. Pitx2 expression has been detected in myotomes, putative migrating myoblasts during development as well as in adult satellite cells. We have previously documented that c-isoform of Pitx2 plays a pivotal role modulating proliferation vs differentiation during myogenesis, balancing Pax3+/Pax7+ myogenic population *in vivo*. Here we demonstrate that Pitx2c increase myoblast proliferation by repressing miR-1, miR-15b, miR-23b, miR-106b and miR-503. Moreover, Pitx2c overexpression in isolated mouse satellite cells also increases cell proliferation and leads to Pax3 up-regulation but Pax7 down-regulation suggesting that Pitx2c play a role balancing symmetric vs asymmetric cell division in satellite cells. Interestingly, we have observed that Pitx2c is up-regulated after muscle injury indicating a putative involvement of this transcription factor in muscle regeneration. In this context, we have developed a strategy based on in vivo cell transplantation experiments to further investigate the Pitx2c implications on regenerative myogenesis. Preliminary results show that Pitx2c-overexpressing satellite cells injected into limb muscles of cardiotoxin treated or Dmd/mdx mice can enhance muscle regeneration as well as increase the expression of dystrophin by modulating miR-31 expression. These results place Pitx2 as a new player on skeletal muscle satellite cell biology and will help us to identify unknown functions of Pitx2 during regenerative myogenesis.

Program/Abstract # 87

Renal repair post mechanical injury in *Xenopus laevis* tadpoles

Shoshoni Caine, Kelly McLaughlin (Tufts, USA)

For centuries researchers have puzzled over how body parts are created and patterned during development and, in some instances, are able to regenerate after damage, loss, or injury. Despite much progress in these areas of study, the underlying mechanisms mediating the coordination of cellular behavior continues to be central to scientific discussions. In order to gain insights into the regeneration of renal structures, our lab investigates renal tissue renewal utilizing the simple, embryonic kidney found in *Xenopus laevis* tadpoles. For this work we developed a unilateral nephrectomy technique designed to specifically excise renal tubules in tadpoles that allows us to study tubule repair after injury in a versatile vertebrate organism. We observed that these nephrectomized animals not only have the ability to regenerate properly patterned tissue, but the restored tissue was also functional. Thus, we now focus on the regulation of cell behaviors occurring during regeneration, especially the cellular, molecular, and biophysical mechanisms used to govern renal tissue renewal. In addition recent findings suggest that tubule restoration occurs via a process involving both an early apoptotic event and via the regulation of matrix metalloproteinase (Xmmp-9) expression. This work is a necessary first step in a coherent research program focused on fundamental aspects of kidney regeneration control at multiple levels of inquiry.

Program/Abstract # 88

A synergistic role for chemokine and FGF signaling in directing collective migration of the lateral line primordium- insights from models and experiments

Ajay Chitnis, Damian Dalle Nogare, Katherine Somers, Swetha Rao (NICHD/NIH, USA)

Chemokine signals steer leading cells during collective migration of the posterior lateral line primordium (PLLp) in zebrafish. We developed agent-based computer models to explore how a chemotactic response to CXCL12a, mediated by Cxcr4b in leading cells and prevented by Cxcr7b in trailing cells, could determine unidirectional migration of the PLLp when it is coupled with local degradation of CXCL12a. Our models of chemokine signaling-based migration were able to recapitulate the complex behaviors of the

leading cells of the PLLp after a leading fragment was severed from the remaining PLLp by laser ablation. However, the models were unable to account for the polarized protrusions of trailing cells toward a leading fragment. This behavior suggested that leading cells may also be a source of chemoattractive cues for trailing cells. Inhibiting FGF signaling prevented this behavior, while providing an exogenous FGF source confirmed a chemoattractive role for FGF in trailing cells. While leading cells steer PLLp migration by following a local chemokine gradient generated with the help of trailing cells, our studies suggest trailing cells play follow the leader, at least in part, by migrating toward FGFs secreted by leading cells. Our study illustrates how a combination of modeling and experiment has led to a better understanding of the emergent behavior of the PLLp system.

Program/Abstract # 89

Aquaporin3b is required during neural tube closure and gastrulation

Christa Merzdorf, Daniel Van Antwerp, Kelly Christensen (Montana State, USA)

We identified an aquaglyceroporin, aqp3b, with a unique expression pattern during *Xenopus laevis* early development. It is found in the superficial-most cells at the edges of the neural plate (which become the neural folds). In general, aquaporin proteins are essential for the maintenance of cellular water balance and a growing body of evidence suggests a role of aquaporins in cell migration. The passage of water and other small solutes through aquaporin channels can change the size and shape of cells, which may facilitate the sculpting of tissues. Specifically, morpholino-based knockdown of aqp3b expression lead to delayed neural tube closure. Closer inspection of the affected areas showed significant changes to cell shape, including a lack of apical constriction, and a loss of cell polarity markers. The affected cells are neighbors of aqp3b-expressing cells and not the expressing cells themselves, suggesting an action-at-a-distance mechanism for aqp3b activity. While examining earlier embryonic stages, *in situ* hybridization analysis of gastrula embryos revealed specific aqp3b expression in the inner cell layer of the blastocoel roof and in the future mesodermal tissue in the marginal zones. Accordingly, higher levels of aqp3b morpholino treatment resulted in defects during gastrulation. The blastocoel roof appeared somewhat disorganized and the normally sharp line between involuting and noninvoluting mesoderm was disordered. These changes may be the result of impaired cell size regulation, cell migration, adhesion or intercalation and appear to be cell autonomous in gastrula embryos. Our studies continue to identify the mechanisms by which aqp3b plays essential roles during Xenopus neural tube closure and gastrulation.

Program/Abstract # 90

Mitotic cell rounding accelerates invagination of the *Drosophila* tracheal placode

Takefumi Kondo, Shigeo Hayashi (RIKEN, Japan)

Animal cells change their shape into sphere upon mitotic entry. Mitotic cell rounding is a conserved process governed by extensive rearrangement of actin cytoskeleton and increasing osmotic pressure. During development, since epithelial morphogenesis is controlled by cell shape changes through the regulation of cytoskeletal structure in interphase, inappropriate mitosis is known to interfere with tissue morphogenetic event. Invagination is one of the key morphogenetic processes, which converts flat epithelial sheets into three-dimensional structures. To understand cellular mechanisms of epithelial invagination, we performed live imaging of *Drosophila* tracheal placode as a model system, and found that this morphogenetic event is divided into two distinct phases by speed. Invagination begins with a slow phase under the control of EGFR signaling; in this process, the central apical-constricted cells, which are surrounded by intercalating cells, form a shallow pit. This slow phase is followed by a fast phase that is coincided with mitotic entry of central cells, leading to the internalization of all the cells in the placode. We found that mitotic cell rounding, but not cell division, of the central cells in the placode is required to accelerate invagination, in conjunction with EGFR-induced Myosin II contractility in the surrounding cells. We propose that mitotic cell rounding causes the epithelium to buckle under pressure and acts as a switch for morphogenetic transition at the appropriate time.

Program/Abstract # 91

Actomyosin cables and tissue fusion promote epidermal spreading during *Drosophila* head involution

Natalia Czerniak, Arturo D´Angelo (CRG, Spain); Julien Colombelli (IRB, Spain); Jerome Solon (CRG, Spain)

How morphogenetic processes are coordinated in space and time? During late *Drosophila* embryogenesis, dramatic tissue remodeling is required to cover the whole organism with an epidermal layer. A striking coordination occurs between two main processes: dorsal closure (DC), a wound healing related process, and head involution (HI), a complex movement leading to reorganization of head structures. In this study, we describe the coordination between these two processes and mechanisms driving tissue progression during HI. With high resolution imaging, we observed the presence of actomyosin cables regularly spaced along the epidermis. Interestingly, these actomyosin cables appear to bundle and to reinforce at the leading front of the epidermis during HI. Laser dissection of the cables allowed us to measure tension generated in the cables throughout the process and to confirm their role as a major driving force. Specific mutants affecting DC progression result in impaired HI, suggesting an original mechanical feedback between both processes. By impairing zipping during DC, we show that both processes are mechanically coupled via a “zip-slip” mechanism, by which zipping and epidermal fusion during DC allows tissue spreading during HI by releasing the tension in the epidermal tissue. Consequently, the epidermis progression over the head during HI promotes the zipping at the anterior canthus of DC. In summary, we reveal the first evidence of the mechanisms by which epidermis is spreading during HI and of the coordination of two major morphogenetic processes, DC and HI.

Program/Abstract # 92

The *C. elegans* RB protein LIN-35 induces germ cell apoptosis under starvation

Rosa Navarro, Laura Láscarez-Lagunas, Carlos Silva-García, Tzventanka Dinkova (UNAM, Mexico)

In *Caenorhabditis elegans*, physiological germ cell apoptosis eliminates more than half of the germ cells in the hermaphrodite gonad to support gamete quality and germline homeostasis by a still unidentified mechanism. External factors that act through different pathways can also affect germ cell apoptosis. The BH3-only protein EGL-1 induces germ cell apoptosis when animals are exposed to pathogens or agents that cause DNA damage. DNA damage-induced germ cell apoptosis also requires the nematode p53 homolog CEP-1. Previously, we found that environmental conditions such as heat shock, oxidative and osmotic stress induce germ cell apoptosis through an EGL-1 and CEP-1 independent mechanism that requires the MAPK pathway. However, we observed that starvation increases germ cell apoptosis by an unknown pathway. Using polysomal gradients to compare mRNA translation levels from well-fed to 6h starved adult hermaphrodites; we found that general mRNAs translation is inhibited under these conditions. Among the translational arrested mRNAs is *ced-9*, which encodes for the anti-apoptotic Bcl2 homolog in *C. elegans*. However, the mRNA of *lin-35*, the *C. elegans* RB homolog, avoids this general translational arrest condition and its protein accumulates considerably. It has been previously shown than LIN-35 represses *ced-9* transcription under normal conditions to partially induce physiological germ cell apoptosis. We observed that under starvation *ced-9* expression decreases dramatically in a LIN-35 manner. We proposed a model where under starvation germ cell apoptosis is triggered due to higher LIN-35 accumulation and a reduction of *ced-9* transcription and translation levels.

Program/Abstract # 93

Caspase-8 regulates hematopoiesis at two distinct stages during embryonic development

Christopher P. Dillon (St. Jude Children's Res Hosp, USA); Andrew Overst (Seattle, USA); Ricardo Weinlich, Laura Janke, Douglas Green (St. Jude Children's Res Hosp, USA)

Caspase-8, the initiator caspase of the death receptor pathway of apoptosis, is essential for embryonic development. Caspase-8 knockout animals die at E10.5 due to a failure of yolk sac vascularization. Ablation of RIPK3, a kinase that promotes necrosis, a form of programmed cell death, rescues caspase-8-deficient mice, suggesting a dynamic interplay between these two death pathways during development. While the tissues affected and the signals that lead to death in the Casp8-/- embryos are still poorly characterized, the specific ablation of casp8 in endothelial or hematopoietic tissues provides evidence that Casp8 may play a key role in the development of the hematopoietic system. To investigate whether TNF triggered the embryonic death of Casp8-/- deficient animals at E10.5, we crossed these mice onto a Tnfr1-/- background. Intriguingly, Casp8-/-Tnfr1-/- mice had normal yolk sac development and progressed appropriately until ~E15.5 before the embryos died. Histological examination of the embryos suggested that this later embryonic lethality might be the result of liver defects. As the fetal liver is an important site of hematopoiesis, we sought to test the hematopoietic potential of cells from these embryos. Casp8-/-Tnfr1-/- fetal livers failed to reconstitute the immune system of lethally irradiated recipients. Together, these results demonstrate two essential windows during development where caspase-8 regulates hematopoiesis. These observations further suggest that caspase-8 mediated cell death likely regulates ongoing perinatal hematopoiesis and additional investigation could provide important insights for enhancing immune response against pathogens.

Program/Abstract # 94

Characterization of Integrins function in specific cells for cell-corpses engulfment in *Caenorhabditis elegans*

Tsung-Yuan Hsu, Hsiao-Han Hsieh (National Taiwan U, Taiwan)

Clearance of apoptotic cells by engulfment plays an important role in the homeostasis and development of multicellular organisms. Despite the fact that the recognition of apoptotic cells by engulfment receptors is critical in inducing the engulfment process, the molecular mechanisms are still poorly understood. Here, we characterize a novel cell corpse engulfment pathway mediated by the integrin α subunits INA-1 and PAT-2 in *Caenorhabditis elegans* and show that they specifically function in hypodermal and muscle-mediated engulfment during embryogenesis. Inactivation of *ina-1*or *pat-2* resulted in a defect in apoptotic cell internalization. We first identified that the extracellular region of INA-1 and PAT-2 recognize and then binding to the surface of apoptotic cells *in vivo*, and the intracellular region mediate specific signaling for engulfment. We identify essential roles of the integrin α subunit INA-1 acts upstream and directly interaction of SRC-1, non-receptor tyrosine kinase, and CED-2 (CrkII) to transmit engulfment signaling which through the conserved signaling molecules CED-5 (DOCK180)/CED-12 (ELMO)/CED-10 (RAC) respectively, preferentially act in epithelial cells to mediate cell corpse removal during mid-embryogenesis. Moreover, in contrast to INA-1, small GTPase CDC-42 and its activator UIG-1, a guanine-nucleotide exchange factor, in PAT-2–mediated cell corpse removal. The PAT-2 and CDC-42 both function in muscle cells for apoptotic cell removal and are co-localized in growing muscle pseudopods around apoptotic cells. Our results demonstrated that PAT-2 functions through UIG-1 for CDC-42 activation, which in turn leads to cytoskeletal rearrangement and apoptotic cell internalization by muscle cells. Take together, basis of our results demonstrated that provided the non-canonical regulatory through alpha subunite, but not beta subunite for cell-corpse engulfment. Furthermore, different engulfing cells utilize distinct repertoires of receptors for engulfment at the whole organism level.

Program/Abstract # 95

Morphogenetic apoptosis/compensatory proliferation at the borders of DPP expression in the genital disc of *Drosophila*

Ana Macias, Gimena Fussero, Carolina Arias, Marcelo Zacharonok (U Nacional de Cordoba, Argentina)
The relation among proliferation and death during growth is explained by the competition mechanism. This sees, the cells growth, in relation with their neighbors; if the rhythms of growth are not coincidental, the lower growing cells are removed by apoptosis.There is a special type of cell competition called morphogenetic apoptosis.This occurs if discontinuities in the growth /surviving signals Dpp/Wg are induced.Under these circumstances death mediated by the JNK mechanism is observed at the borders of the discontinuities. A tissue-physiologycal scenario for such discontinuities could be the time/place when Dpp and Wg expressions start. The differences in the amount among, expressing and non expressing cells should be high at the beginning but should decreased as development progress. In accordance, morphogenetic apoptosis must occurs at the borders of Dpp/Wg expressions. We tested this idea in the genital disc of *Drosophila* where the activation of Dpp occurs late in development, there is a cell division arrest up to that time, and death plays roles. We center our analysis in the activities of Dpp, JNK, the pro-apoptotic genes RGH and the enzymes caspases. Our main results indicated there is cell competition/morphogenetic apoptosis at the borders of Dpp expression, revealed principally not by the death but for its compensatory proliferation. The net balance among death/proliferation appeared cero, so physiologycal cell competition does not vary the size. The levels of active JNK and caspases are important for: the execution of death, the control of proliferation, and the occurrence of compensatory proliferation.The JNK/capases activity over proliferation determines them as factors of cell competition.

Program/Abstract # 96
"Audio, Video Disco" - Establishing the cellular pattern of the organ of Corti
Andy Groves (Baylor Coll Med, USA)
The organ of Corti is a highly specialized sensory structure running along the length of the mammalian cochlear duct. It contains a precisely organized array of mechanosensory hair cells, surrounded by glial-like supporting cells. The exquisite mechanical sensitivity of this structure is necessary for the high frequency hearing that is a characteristic of most mammals. The almost crystalline arrangement of cells in the organ of Corti is established by tightly orchestrated sequence of patterning signals. First, a gradient of BMP signaling is established across the cochlear duct that positions the progenitors for the organ of Corti in the middle of the duct. Second, local signaling centers established by this BMP gradient interact to define the boundaries of the organ of Corti progenitor domain. For example, expression of Notch ligands and Fringe glycosyltransferases on one side of the progenitor domain set up an asymmetrical pattern of Notch signaling that precisely defines the position and numbers of hair cells that differentiate at this boundary. Third, FGF signaling from differentiating hair cells acts locally to precisely define the number and position of particular classes of supporting cells. It does this by regulating transcription factors such as Hey2 that are normally considered to be targets of Notch signaling. We speculate that this co-option of new regulatory pathways may have contributed the evolution of this highly derived sensory organ.

Program/Abstract # 97
Role of Abdominal-B and Planar Cell Polarity in controlling Left-Right asymmetry establishment and morphogenesis in *Drosophila*
Stéphane Noselli (Inst. Signal. Dev. Biol. & Cancer Res., France); Jean-Baptiste Coutelis, Charles Geminard, Nicanor Gonzalez-Morales (Inst. de Biologie Valrose, France)
Breaking left-right (L/R) symmetry in Bilateria embryos is a major event in body plan organization. The establishment of L/R asymmetry is essential for handedness, directional looping of internal organs (heart, gut...) and differentiation of the heart and brain. Defects in L/R asymmetry during embryogenesis can lead to a variety of defects including congenital heart diseases, spontaneous abortion, asplenia, polysplenia, etc. In vertebrates, L/R asymmetry can be set up at distinct embryonic stages, and involves distinct mechanisms including the nodal flow, ions flux and asymmetric cell migration. In order to better understand how L/R asymmetry is established, we initiated the study of L/R asymmetry in *Drosophila*. We identified the rotation of genitalia as a suitable L/R phenotypic marker: during metamorphosis, genitalia undergo a stereotyped 360° clockwise (or dextral) rotation leading to the coiling of the spermiduct around the gut. The myosinID gene (myoID, aka Myo31F) was identified as a major L/R determinant in flies, required for dextral coiling of organs including genitalia and gut. In the absence of myoID gene activity, flies show a situs inversus phenotype and organs undergo sinistral morphogenesis. A modifier genetic screen was performed to identify potential myoID interacting genes during L/R patterning. We show that the Abdominal-B (Abd-B) gene controls early establishment of L/R asymmetry, distinct from its function in anterior-posterior patterning. Abd-B mutant flies develop symmetrically, with Abd-B controlling the expression of the myoID dextral determinant, as well as the activity of the opposite sinistral pathway. Therefore, Abd-B acts as a symmetry breaking factor controlling the transition from symmetry to asymmetry. We will also present new results showing how MyoID and Planar Cell Polarity control the coiling of the hindgut. We characterized a specific L/R organizer for the adult hindgut and show that MyoID acts cell non-autonomously in this organizer to direct PCP-driven directional torsion in the adjacent tissue. Altogether, these data provide new information on the upstream mechanisms breaking symmetry in Drosophila as well as downstream pathways executing asymmetric morphogenesis. Publications : Adam et al., Development 2003 ; Spéder et al., Nature 2006; Hozumi et al., Nature 2006 ; Spéder & Noselli, Curr. Opin. Cell Biol. 2007 ; Spéder et al., Curr. Opin. Genet. Dev. 2007 ; Coutelis et al., Sem. Cell Dev. Biol., 2008 ; Suzanne et al., Curr. Biol. 2010 ; Petzoldt et al., Development 2012 ; Coutelis et al., Developmental Cell, 2013.

Program/Abstract # 98

A gradient of sulfated proteoglycans is required for dorsal-ventral skeletal patterning in sea urchin embryos

Cynthia Bradham, Finnegan Hewitt, Michael Piacentino, Christy Li, Jia Yu, Evan Bardot, David Lee, Hajerah Hameeduddin, Arlene Reyna, Oliver Chung, James Chaves, Patrick Ferrell, Ian Murray, Matthew Tse, Ah Ra Cho, Amanda Core, Jasmin Coulomb-Huntington (Boston U, USA); Albert Poustka (Max-Planck Inst. Molec Genet, Germany)

The sea urchin larval skeleton is secreted by primary mesenchyme cells (PMCs) in response to instructive ectodermal cues. During gastrulation, PMCs become organized in a vegetal ring of cells with additional cells forming ventrolateral cords that extend animally, in response to these cues. This PMC pattern gives rise to the skeletal pattern. We identified mutliple ectodermal genes responsible for skeletal patterning using an RNA-seq-based screen. Among them is SLC26a2 (SLC), a sulfate transporter required for synthesis of sulfated proteoglycans (sPGs). LvSLC loss of function (LOF) results in loss of the ventral and animal (VA) skeletal elements, and perturbs PMC positioning in VA regions. SLC is expressed in the VA ectoderm, and its expression profile correlates well with sPGs, which are enriched both ventrally and animally. The ventral-to-dorsal sPG gradient is strongly diminished in SLC LOF embryos. These results indicate that sPGs function to attract PMCs to VA positions. Interestingly, LOF for Notch2, another validated candidate, promotes the opposite distribution of PMCs, such that they accumulate in VA regions at the expense of dorsal, despite the ventral expression domain for LvNotch2. This suggests that Notch2 signaling promotes the opposite effect, directing a subset of PMCs dorsally, away from sPGs. Consistent with this interpretation, combined LOF for both SLC and Notch2 strongly perturbs PMC localization and blocks skeleton formation. Together these data suggest a model in which VA-expressed SLC promotes local sPG synthesis that in turns serves as an attractive cue for PMCs, while VA-expressed Notch2 functions as a switch that induces a subset of PMCs to instead migrate dorsally, away from sPG cues.

Program/Abstract # 99

Nodal Morphogen Interpretation

Alex Schier (Harvard, USA)

Morphogens are long-range signaling molecules that pattern developing tissues in a concentration-dependent manner. The graded activity of morphogens within tissues exposes cells to different signal levels and leads to region-specific transcriptional responses and cell fates. To determine how morphogen gradients are established and interpreted, we study morphogens belonging to the Nodal family. Nodal signals induce and maintain cell fates in embryos and embryonic stem cells. It is poorly understood how dynamic Nodal signaling is interpreted by responding cells to generate different cell types. I will discuss our recent studies that determine how signal concentration, signaling duration, and cellular history underlie the interpretation of Nodal signals.

Program/Abstract # 100

Bucky ball interacts with RNA binding proteins to pattern the oocyte and follicle cells in zebrafish

Florence L. Marlow, Amanda Heim, Odelya Hartung, Sophie Rothhämel, Andreas Jenny (Albert Einstein Coll. Med., USA)

In vertebrates the first asymmetries are established during oogenesis along the animal-vegetal axis, but the underlying molecular mechanisms are poorly understood. Bucky ball (Buc) was identified in zebrafish as a novel vertebrate specific regulator of oocyte polarity, acting through unknown molecular interactions. Our studies show that endogenous Buc protein localizes to the Balbiani body, a conserved, asymmetric structure in oocytes that requires Buc for its formation and that asymmetric distribution of Buc in oocytes precedes Balbiani body formation, defining Buc as the earliest marker of oocyte polarity in zebrafish. Through a transgenic strategy, we determined that proper localization of Buc is essential for oocyte polarity. Moreover, analyses of mosaic ovaries indicates that oocyte pattern determines, likely via a close range signal or direct cell contact, the number of animal pole specific micropylar cells that are associated with an egg. We demonstrate Buc protein interacts with Rbpms2, a conserved RNA binding protein localized to the Balbiani body. Cumulatively, our results are consistent with a model whereby Buc establishes oocyte polarity through interactions with RNA binding proteins, initiating a feedback amplification mechanism in which Buc protein recruits RNA binding proteins that in turn recruit buc RNA and other RNAs to the Balbiani body.

Program/Abstract # 101

Target-specific robustness to Hedgehog production levels in the *Drosophila* wing disc

Marcos Nahmad, Arthur Lander (UC Irvine, USA)

Developmental patterns of gene expression are generally highly precise and reproducible despite variability of certain genetic and environmental parameters. Self-enhanced ligand degradation has been proposed as a general mechanism to provide robustness of morphogen gradients to changes in ligand production. The Hedgehog (Hh) signaling pathway self-limits its range of signaling by transcriptionally activating its own receptor, Patched (Ptc), and thereby self-promoting ligand degradation. Previous theoretical studies suggest that signal-dependent-upregulation of Ptc may provide robustness to variability in Hh levels, but such studies presume that Hh target genes are a direct readout of the steady-state distribution of the Hh gradient. However, as was recently shown in the wing imaginal disc of *Drosophila*, not all target genes respond to steady-state Hh levels; dynamics of the Hh gradient also play a role in conveying positional information in this system. Here we show experimentally that steady-state-determined Hh targets (e.g. collier and ptc) are more robust to changes in Hh dosage than transient-determined Hh targets (such as dpp). Using mathematical modeling, we show that this result is in agreement with a dynamic model of Hh signal interpretation. Surprisingly, we provide quantitative evidence that the observed lack of robustness in determining the width of the dpp pattern does not affect the phenotype of the adult

wing, suggesting that additional mechanisms ensure that wing patterning is also robust to changes in the width of the dpp expression domain.

Program/Abstract # 102

Deciphering the cis-regulatory grammar behind enhancer architecture using a dynamic mathematical model

Jacqueline Dresch, Daniel Bork, Adam Brown (Harvey Mudd, USA); Chichia Chiu, David Arnosti (Michigan St U, USA); Robert Drewell (Harvey Mudd, USA)

With new high throughput techniques leading to large data sets of increased quality, mathematicians are no longer making theoretical hypotheses about biological processes. Genome sequencing and transcriptome analysis has provided a quantitative basis for detailed modeling of gene expression in eukaryotes. Combining the strengths of differential equation models, which incorporate important temporal changes during development, and thermodynamic descriptions, which allow the exploration of the cis -regulatory grammar of transcriptional enhancers, we have developed a novel model that provides a dynamical description of gene regulatory systems. Our modeling approach uses detailed DNA-based information, as well as spatial transcription factor concentration data. A thermodynamic model is used as the mRNA synthesis term in our differential equation, while the differential equation incorporates all other terms, representing translation, decay and diffusion. By incorporating data on DNA sequence, we are able to successfully model context-specific features of enhancers, as well as replicate the dynamic expression of a simple *Drosophila* gene regulatory circuit that drives development in the blastoderm embryo. Where protein and cis -regulatory information is available, our model provides a powerful method to recapitulate and predict dynamic aspects of eukaryotic transcriptional systems that will greatly improve our understanding of gene regulation at a global level.

Program/Abstract # 103

Regulation of neural precursor cell plasticity during neuronal dopaminergic differentiation in the mouse midbrain

Luis Covarrubias, José-Manuel Baizabal (IBT- UNAM, Mexico); Omar Collazo-Navarrete (IFC-UNAM, Mexico); Celina García (IBT- UNAM, Mexico); Magdalena Guerra-Crespo, (IFC-UNAM, Mexico), Gilda Guerrero-Flores (IBT-UNAM, Mexico); Maya-Espinosa, Guadalupe (IFC-UNAM, Mexico); Jorge Landgrave-Gómez, Niurka Trujilloo-Paredes, Concepción Valencia (IBT-UNAM, Mexico)

Stem cells must restrict their differentiation potential in order to generate specific cell types. Initial restrictions result from the cell positional identity acquired during early development. Then, within defined domains, cells are instructed to follow specific differentiation pathways, each running through distinct phases such as specification, determination and terminal differentiation. Specification and determination stages can be estimated in vivo by the expression and/or requirement to reach the final fate of certain genes; however, the actual plasticity of a cell population needs to be determined by functional assays. We have designed a system to evaluate the differentiation potential of neural precursor cells (NPCs) within the mesencephalic dopaminergic lineage. Specific neuronal differentiation must consider two coordinated processes: (a) neurogenesis, and; (b) neuronal specification, which defines the neurotransmitter produced and the interneuronal connectivity of the mature neuron. E10.5 and E12.5 ventral mesencephalic NPCs differentiate into neurons within the neurogenic environment of the embryo, but produce mostly astrocytes when cultured in vitro in the presence of Fgf2, suggesting that most dividing NPCs are not committed to become neurons; neuronal determination appears to be controlled by the Notch/Delta-like 1 signaling. On the other hand, NPCs from E9.5 and E10.5 produce neurons at a similar efficiency but only those from the latter stage behave as committed giving rise to TH+ neurons even out the natural differentiation niche. Our work contributes to identify the intrinsic and extrinsic requirements for specific differentiation and disclose the epigenetic plasticity of NPCs. (CONACyT 131031).

Program/Abstract # 104

Vertebrate phylotypic period as a source of basic body plan

Naoki Irie (RIKEN, Japan); Guojie Zhang (Shenzhen, China), Shigeru Kuratani (RIKEN, Japan)

Why do vertebrates have basic body plan in common? Is it merely an issue of how we group the animals for descriptive purposes? Or is there any inevitability that arises from unknown features of their developmental system? Although the exact answer remains to be clarified, recent comprehensive, transcriptome analysis highlighted the conserved mid-embryonic period in vertebrate embryogenesis, called vertebrate phylotype. However, the vertebrate phylotypic period hypothesis has only been supported by comparison among model organisms that have rather common body plan (e.g. mouse, chicken, zebrafish, xenopus). Here we determined the draft genomes of two turtle species and further investigated the temporal position of phylotypic period by comparing the RNAseq based transcriptome between turtle and chicken embryos genesis. Our results, not only demonstrating the hourglass-like divergence during turtle chicken embryogenesis, in terms of gene expression profiles, but also provided a basis for “molecular adjustment” of embryonic timetables among different animals.

Program/Abstract # 105

Withdrawn

Program/Abstract # 106
Developing a research-based molecular biology course for freshman students
Merzdorf, Christa (MSU - Bozeman, USA
This newly developed molecular biology course directly integrates the instructor's research, allowing students to gain research experience along with thorough instruction. The research is based on a microarray screen that was conducted in the instructor's laboratory. The screen identified direct target genes of Zic transcription factors in *Xenopus laevis* embryos. The students select from among these genes for further study. The genes are subcloned to generate probes for in situ hybridization to determine expression patterns; overexpression and knockdown experiments in *Xenopus* embryos will help determine the roles of these genes during development; promoter regions will be cloned and reporter constructs will be generated and tested; successful reporter constructs will be studied by deletion analysis. The course design allows successive courses to build on each other, thereby advancing the research each semester. During spring semester 2013, the students cloned their genes. Probe generation and in situ hybridization is planned for the next semester. This teaching lab is run like a research lab to maximize the research experience. The students make their own buffers, design and plan everything they do. They learn to formulate hypotheses, trouble shoot, keep excellent records. At the same time, they acquire deep theoretical knowledge, give presentations, learn to find and read literature, use web-based tools, and write journal-style articles. After this experience, students join research laboratories early during college with a thorough tool kit. Students, who desire to continue working on their selected genes can join my laboratory for undergraduate research projects. The course received extremely favorable reviews.

Program/Abstract # 107
Evolution of vertebrate animal design, from the top down
Thorn, Judith M. (Knox College, USA)
Many introductory developmental biology courses examine how changes in *Hox* gene expression can explain differences in vertebrate form. Many undergraduate students are not familiar with the axial skeletal anatomy of various vertebrates, and are not readily able to visualize how changes in *Hox* gene expression results in different vertebral structure. This laboratory exercise asks students to work backwards from the adult anatomy to deduce embryonic gene expression patterns. Students determine the number and axial identity of each vertebra in several articulated skeletons (one example group: *Canis lupus familiaris* (dog), *Crotalus sp*. (rattlesnake), *Rana catesbeiana* (American bullfrog). After analyzing their observations (e.g. are their equal numbers of lumbar vertebrae? do the lumbar vertebrae from each organism look the same?), students generate hypotheses about anterior/posterior patterning for each skeleton. As support for their model, students are asked to include a figure with relevant hypothetical in situ hybridizations of embryos. Initial assessment indicates that this assignment promotes the integration of the understanding of the developing embryo, the adult animal and the regulation of gene expression.

Program/Abstract # 108
Exploiting Nematode Diversity to Teach Advanced Techniques in Bioinformatics, Molecular Evolution and Fluorescence and Electron Microscopy
Howell, Carina (Lock Haven University, USA)
Nematode worms account for the vast majority of the animals in the biosphere. They are colossally important to global public health as parasites, and to agriculture both as pests and as beneficial inhabitants of healthy soil. Students at Lock Haven University, a primarily undergraduate institution, are examining the internal and external morphology and anatomy of a variety of soil nematode wild isolates collected locally and obtained from the Caenorhabditis Genetics Center (CGC). We are using DIC microscopy, fluorescence microscopy (DiI staining of amphid and phasmid neurons) and scanning electron microscopy to gain an understanding of nematode morphological diversity, and to teach undergraduates these sophisticated microscopy techniques. We are using both classical systematics (e.g. diagnostic keys) and molecular markers (e.g. ribosomal RNA) to classify these wild isolates. Our aim is to build a detailed anatomical database in order to dissect genetic pathways of development and function across phylogeny and ecology, while providing students with uncommon technical training for graduate study and the workforce.

Program/Abstract # 109
The History of Biology and Medicine in Britain: A Study Abroad Course at Central Michigan University
Hertzler, Philip Lamar; Swanson, Bradley (Central Michigan Univ, USA)
Undergraduate study abroad courses are enlightening and memorable, providing important historical context for topical material and perspective on another culture. Science courses are often underrepresented as study abroad options, reducing international experiences for science students. To address the university's goal of greater participation in study abroad experiences, we created a course on the development of biological and medical concepts in the UK. Two versions of this course ran during the summers of 2009 and 2012. Five students participated in the 10-day 2009 class, focused on the theory of evolution, during Darwin's 200 th birthday year. Visitations included museums in London, Darwin's birthplace in Shrewsbury, the glacial landscape of Snowdonia, Wales, Cambridge University, the Natural History Museum at Tring, and Darwin's adult home at Downe. Active learning experiences included a boulder distribution study in Snowdonia, an evaluation of character traits used to designate botanical taxa at Cambridge University Botanic Gardens, and a coevolution of flower and pollinator study in Downe. Six students participated in the 14-day 2012 class, visiting science and medical museums in London, the home of the Royal Navy at Portsmouth, Bath and Berkeley, the home of Edward Jenner,

Usk, Wales, the birthplace of Alfred Russell Wallace, Snowdonia and Shrewsbury. Students presented biographical overviews of Hippocrates, Joseph Priestly, John Hunter, Florence Nightingale, John Snow, and Rosalind Franklin at pre-course meetings, and an integrative essay on the significance of the theory of evolution after the course. The next offering of the course in summer 2014 will be 2-3 weeks, combining elements of the previous versions.

Program/Abstract # 110
Regulation of the Meis2 homeobox gene
Barrett, Cody; Nelson, Kyle; Zerucha, Ted (Appalachian State University, USA)
Homologues of the *Meis* genes family have been identified in all animals studied. *Meis* genes are a member of the TALE (three amino acid loop extension) superclass of atypical homeobox genes, whose products are haracterized by an additional three amino acids between helix 1 and helix 2 of their homeodomain. The products of the *Meis* genes appear to function as cofactors, interacting with other transcription factors and DNA to facilitate transcriptional regulation. Most importantly, they appear to act as cofactors with the evolutionarily conserved Hox proteins as well as other various homeobox genes. The vertebrate homeobox-containing *Meis* gene family contains at least four members and while little is known about their regulation, they are expressed in conserved patterns throughout embryonic development of those vertebrates examined. Using phylogenetic footprinting to search for regulatory elements in association with the *Meis* family of homeobox-containing genes, we identified a highly conserved element located downstream of the *Meis2* gene. This putative enhancer is very well-conserved in sequence and relative position amongst the genomes of all vertebrates examined, including human, mouse, chicken, zebrafish and the pufferfish *Takifugu rubripes*. Furthermore, this element contains several putative transcription factor binding sites for proteins that could be regulating *Meis2* expression. We have demonstrated the ability of this element to drive reporter gene expression in the developing brain of zebrafish embryos consistent with the expression of *Meis2*.

Program/Abstract # 111
Identification and embryonic expression of a highly conserved Meis-linked gene
*Williams, Zachary Scott; Cochrane **Anna**; Carpenter, Brandon; Graham, Brantley; Zerucha, Ted (Appalachian State University, USA)*
We have identified a novel and previously uncharacterized gene, *zgc:154061* that is located directly downstream of the zebrafish *meis2.2* gene. Putative orthologs of this gene have been identified in all animals for which publicly available genome data is available and it is always found directly downstream of *Meis2*. The *zgc:154061* gene and its vertebrate orthologs are organized in a convergently transcribed manner with respect to the *Meis2* gene in all species we have examined (*meis2.2* in teleosts). During zebrafish development, transcripts of *zgc:154061* are observed in every cell of the embryo from the earliest stage through the shield stage. Expression of *zgc:154061* gradually decreases from its peak value at 0 hpf until 8 hpf and then is observed to be activated again at 12 hpf as determined by quantitative real time PCR. This later expression is observed throughout the neural tube before becoming restricted to the retina and tectum opticum by 48 hpf. Whole mount and cross-section immunohistochemistry, using an antibody raised against a peptide portion of the predicted zebrafish protein product, has revealed that the gene is actively translated into protein and highly localized to the retinal area and optic nerve. Western blots have shown the protein to be expressed during all stages of development and as early as 2hpf.

Program/Abstract # 112
Dual activator and repressor roles for NKX2-5 in heart development
Dupays, Laurent; Shang, Catherine; Wilson, Robert; Kotecha, Surendra; Towers, Norma; Mohun, Timothy (London, UK)
The homeobox transcription factor NKX2-5 is essential for vertebrate heart development and play an essential role in the physiology of the human adult heart. However, despite extensive analyses, the downstream effectors mediating NKX2-5 function during heart development remain largely unknown. We used a combination of genome-wide chromatin immunoprecipitation and high-throughput transcriptome analyses (ChIP-seq and RNA-seq) to identify hundreds of direct targets of NKX2-5 during mouse heart development. We found that NKX2-5 is binding on two DNA motifs of different affinity. The binding of NKX2-5 on that new motif is mutually exclusive of another cardiac transcription factor. Both transcription factors present partially overlapping expression pattern during cardiac differentiation suggesting that cardiac progenitors are sequentially experiencing high level of their expression. Shared transcriptional targets suggest spatial and temporal synchronization of a common pool of targets between those two factors. Finally, we show that reduced expression of NKX2-5 lead to an increase in glycolysis suggesting a shift in metabolism occurring with a shift in troponin isoform in the failing NKX2-5 hypomorphic heart.

Program/Abstract # 113
Regulatory specificity of different Sox transcription factors during neuro- and gliogenesis
Klum, Susanne (KI, USA); Zaouter, Cecile (Solna, Sweden); Ramsköld, Daniel; Bergsland, Maria (Stockholm, Sweden)
To better understand how neural stem cells (NSCs) are regulated to generate neuronal and glial progeny, we have used ChIP-Seq to characterize and compare the binding profile of Sox transcription factors in NSCs (Sox1-3), neurons (Sox11) and oligodendrocytes (Sox10). These analyses have revealed an unexpected sequential binding of Sox proteins to neuronal and glial genes. We show that the vast majority of genes bound by Sox11 in neurons has previously been bound by Sox1-3 in NSCs. Hence, Sox1-3 that are expressed in

NSCs, but not in neurons, bind not only to genes that are expressed in NSCs, but also to silent genes that will become activated in Sox11-positive neurons. Vice versa, Sox11, which is expressed in neurons but not in NSCs, binds to active neuronal genes as well as to silent NSC genes that were previously targeted by Sox1-3. Moreover, in NSCs Sox1-3 also pre-bind genes that are specifically expressed in Sox10-positive oligodendrocytes. Despite these broad binding profiles of Sox proteins in the developing nervous sytem, in vitro studies show that Sox1-3 can specifically activate cis-regulatory elements (CREs) of NSC genes, while Sox11 and Sox10 specifically activate CREs of neuronal and oligodendrocyte genes, respectively. To examine how this functional specificity of Sox proteins is regulated at the gene level, we are simultaneously analyzing thousands of Sox bound CREs in vitro using a multiplex reporter assay. The goal of this project is to reveal binding motifs of potential partner factors, which confer functional specificity to Sox proteins during the differentiation of neurons and oligodendrocytes from NSCs.

Program/Abstract # 114
Investigating direct targets of mSOX3 in neural progenitor cells
McAninch, Dale (University of Adelaide, Australia); Rogers, Nick; Thomas, Paul (Adelaide, Australia)
Intellectual disability (ID) affects 2-3% of the population, of which approximately 30% of cases are thought to be due to mutations in genes on the X-chromosome. X-linked hypopituitarism is a form of syndromic ID that is caused by mutations and duplications of the central nervous system transcription factor gene *Sox3*. In mice, *Sox3* is expressed in neural progenitor cells (NPCs) throughout embryogenesis and is important for maintaining them in their progenitor state. Published ChIP-seq data for mSOX3 in embryonic stem (ES) cell-derived NPCs has identified more than 9000 mSOX3 binding sites across the genome, however little is known regarding the function of these binding sites. To identify SOX3-regulated genes, genome-wide expression profiling was performed comparing wild type and Sox3 null NPCs. 24 differentially expressed (DE) genes were identified in *Sox3* null NPCs, 5 up regulated and 19 down regulated, many of which have unknown functions and expression patterns. Next we performed a cross platform comparison of the two datasets which revealed an 85% enrichment of mSOX3 binding sites neighbouring DE genes, compared with a 34% enrichment of binding sites near non-DE genes, suggesting that many of the DE genes are direct targets. Dbx1 is homeobox gene that is down regulated in *Sox3* knockout NPCs and expressed within a subset of *Sox3* expressing NPCs in the developing mouse embryo. We investigated *Dbx1*'s potential as a direct target by analysing five neighbouring evolutionary conserved mSOX3 binding sites by ChIP-PCR analysis. All five sites were shown to enrich by ChIP-PCR, which, taken together with the down regulation in *Sox3* null NPCs, suggests *Dbx1* is a direct target of mSOX3.

Program/Abstract # 115
SOX9 directly modulates cell cycle regulators during post-EMT heart valve development.
Garside, Victoria C.; Cullum, Rebecca; Hoodless, Pamela (BC Cancer Agency, Terry Fox Labs, Canada)
The correct formation of the heart valves is critical for establishing proper blood flow during embryonic life as the load on the heart is increasing. Abnormal heart valve formation leads to one third of all cardiovascular birth defects. These heart valve defects can have detrimental effects on heart function throughout life and can lead to an increase in susceptibility to disease as an adult. A transcription factor called SOX9, has a critical role in heart valve development, specifically in the proliferation and diversification of the heart valve progenitor cells. The loss of SOX9 in the mouse heart valves leads to major valve abnormalities and results in embryonic lethality. Although SOX9 is known to play critical roles in many organ systems, to date, there are few known transcriptional targets of SOX9. To identify genome-wide transcriptional targets of SOX9 in E12.5 atrioventricular canal (AVC, valve forming region) and limb, we performed chromatin immunoprecipitation coupled with sequencing (ChIP-Seq). For the first time, we have identified thousands of potential SOX9 transcriptional targets in the E12.5 AVC and limb with 2607 and 9092 SOX9 sites, respectively. Interestingly, Gene Ontology (GO) analysis on common SOX9 peaks revealed that SOX9 directly binds genomic regions of genes required for cell cycle during valve development and, although a role for SOX9 in proliferation has been suggested, we have now identified SOX9 target genes involved in this process. Additional studies are aimed at understanding the specific role of select SOX9 target genes involved in cell cycle in developing heart valves and will help to elucidate the role they play in heart valve regulatory networks.

Program/Abstract # 116
Modulation of SoxE function in the Neural Crest by the SoxD family protein, Sox5
Nordin, Kara Marie (Northwestern University, USA)
SoxE transcription factors are essential Neural Crest (NC) regulatory factors. They act reiteratively throughout NC development to promote the formation, maintenance and differentiation of NC cells. Interestingly, SoxE factors induce the formation of derivatives in a context-dependent manner through a related Sox protein, Sox5, which is known to modulate SoxE function. We show that *Xenopus* Sox5 is expressed in NC cells as well as in the paraxial mesoderm and pre-placodal ectoderm. We provide evidence that Sox5 plays an early and essential role in patterning the ectoderm that is independent of SoxE factors. Loss of function experiments suggest that Sox5 promotes epidermal formation at the expense of both the neural plate and the neural plate border regions of the ectoderm. To investigate later roles for Sox5 in the neural crest we generated a hormone inducible form of Sox5. Using this tool we find that while Sox5 can cooperate with SoxE factors to activate the collagen type II (Col2a1) promoter, it inhibits SoxE mediated activation of dopachrome tautomerase (Dct). To further investigate the context dependent functions of Sox5, we examined which protein domains were required for its different functions. We show that dimerization is required in order for Sox5 to cooperate with SoxE factors on

the Col2a1 promoter whereas DNA binding is required for Sox5 to inhibit SoxE activation of Dct. These findings shed important mechanistic light on NC cell formation and diversification.

Program/Abstract # 117

The Levels of Sox21 Alter its Function in Neurogenesis

Whittington, Niteace C.; Cunningham, Doreen; Casey, Elena Silva (Georgetown University, USA)

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

Program/Abstract # 118

Transcriptional Elongation is Important in the Regulation of Neural Crest Development.

Hatch, Victoria; Ford, Chris; Barber, Amanda; Tomlinson, Matt; Wheeler, Grant (Norwich, UK)

We have carried out forward chemical genetic screens using *Xenopus laevis* embryos to identify compounds disrupting pigment cell development. Phenotypes showing changes in cell migration, morphology and pigmentation were observed. One compound Leflunomide, led to defects in neural crest and subsequently pigment cell development. Leflunomide is an inhibitor of Dihydroorotate Dehydrogenase, which is part of the pyrimidine synthesis pathway. Inhibiting the pool of pyrimidines in the cell leads to inhibition of transcriptional elongation. To further characterise the molecular mechanism of Leflunomides action we are looking in more detail at the effect of inhibiting transcriptional elongation on neural crest development using *Xenopus* whole embryo and animal cap assays. In addition we have begun to look at the Super Elongation Complex, which regulates transcriptional elongation in the cell. Knockdown of components of this complex lead to similar phenotypes to those described for leflunomide. Our studies have led to the identification of control of transcriptional elongation as playing an important role in regulating neural crest development.

Program/Abstract # 119

MiRNA regulators of prickly pear fruit development

Rosas Cárdenas, Flor de Fatima (LANGEBIO, CINVESTAV-IPN, Mexico); Cruz Hernandez, Andres (Universidad Autonoma de Querétaro, Queretaro, Mexico); Marsch Martinez, Nayelli (Cinvestav-IPN, Irapuato, Mexico); de Folter, Stefan (LANGEBIO, CINVESTAV-IPN, Irapuato, Mexico)

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression in animals and plants (Khraiwesh et al., 2010). MiRNAs are involved in the control of many developmental processes, including fruit development. With increasing amounts of information in miRNA, their expression, abundance and conservation between various species and tissues, provides a new opportunity to study the role of miRNAs in non-model species, such as *Opuntia ficus indica.* MiRNA analysis allow an approach to key genes identification in the prickly pear fruit development and, get an explanation of the possible control in fruit development process. Northern blot and tissue printing analyses were used in order to identify miRNAs involved in prickly pear fruit development. 34 miRNAs were detected in at least one developmental stage. Comparative profiling of our identified miRNAs revealed five different expression patterns during prickly pear fruit development. We further observed considerable variability in miRNA expression within varieties of prickly pear. Our results provide the first resource of miRNAs involved in the regulation of prickly pear fruit development, and show the dynamics and complexity of miRNA expression in this species.

Program/Abstract # 120

Role of miRNAs in *Drosophila melanogaster* during development and under stress.

Narvaez Padilla, Veronica, (UAEM, USA); Sanchez Diaz, Ivan; Peregrina Garcia, Jose Emmanuel; Reynaud, Enrique (Instituto de Biotecnologia, UNAM, Cuernavaca, Mexico)

MicroRNA or miRs are small, non-translatable RNAs (~22nt) which induce downregulation of gene expression either by inducing mRNA degradation or by translational repression. Since their first description in 1993, a lot of evidence has accumulated showing the importance of these genes in the correct development and functioning of organisms. Mis-regulation of miRs has been linked to many diseases, including cancer, diabetes, Alzheimer, etc. We have used *Drosophila melanogaster* to study the function of a cluster of miRs (mir-310/311/313/313) during development and under stress conditions. We have found that null individuals for these miRs have

abnormalities in the development of wing bristles, but have found no developmental abnormality in individuals that over-express these genes. On the other hand, we found that over-expression of these miRs affects only when the over-expression is during early development, having no effect in later stages. However, both over-expression and null mutants show altered, although distinct response to different types of stress. For example, flies that over-express miRs have an enhanced sensibility to nicotine whereas null mutants respond normally. Meanwhile, null mutants show a higher sensitivity to heat stress and have a shorter life expectancy, while flies over-expressing these miRs respond normally to heat and have a normal life expectancy. These observations underlie the complex and fine regulation that the miRs exert over the pattern of expression of other genes.

Program/Abstract # 121

Shared cis-regulatory modules regulate transcription of evolutionarily conserved and bidirectionally transcribed miRNA-opsin gene pairs in the medaka retina

Daido, Yutaka; Kusakabe, Takehiro G. (Kobe, Japan)

In a vertebrate retina, there are two major types of photoreceptor cells, rods and cones, which use distinct types of opsins. Cones can be further divided into several different types based on their wavelength sensitivity and morphology. Although photoreceptor development has been extensively studied in a variety of vertebrate species, the mechanism by which cone subtypes are established is poorly understood. As an attempt to elucidate the mechanism of cone subtype specification, we have been conducting comparative analysis of transcriptional regulation of photoreceptor-specific opsin genes using medaka as a model species. During the course of the study, we have found two miRNA genes, *miR-726* and *miR-729* each of which is located in the upstream region of a cone opsin gene in an opposite direction. Here we report the evolutionary conservation of the miRNA-opsin gene pairs and their transcriptional co-regulation in a specific cone subtype by a common cis-regulatory module. *miR-729* is located in the upstream of a UV-sensitive opsin gene in a variety of teleost species, whereas the pair of *miR-726* and a red-sensitive opsin gene is conserved not only in teleosts but also in amphibians and reptiles. The results of fluorescent reporter assays in vivo suggest that the paired miRNA and opsin genes are co-expressed in photoreceptor cells under the control of the common *cis*-regulatory modules. Coupling between transcriptional and post-transcriptional regulations through the miRNA-opsin gene pairs may be an evolutionarily conserved mechanism to confer and/or maintain photoreceptor subtype specificity.

Program/Abstract # 122

microRNAs expression during the development of the external ear in mouse.

Juárez Figueroa, Ulises; Torres Maldonado, Leda (Instituto Nacional de Pediatría, Mexico); García Segura, Laura; Miranda Rios, Juan (Instituto de Investigaciones Biomédicas, UNAM, Mexico); Frias Vazquez, Sara (Instituto Nacional de Pediatría/Instituto de Investigaciones Biomédicas, UNAM, Mexico)

External ear development in mice is regulated by many genes, such as *Pact, Wnt5, Fgf8, Fgf10, Bmp5, Eya1, Tbx1, Hoxa1, Hoxa2, Six1, Hoxb6, Hoxa7, Fgfr 1-3,* that have been described in animals with abnormalities in the external ear development. The expression of these genes could be regulated by microRNAs (miRNA), that are small noncoding RNAs involved in diverse physiological, developmental and pathological processes by regulating the expression of target messenger RNAs (mRNAs). During embryogenesis and organogenesis many miRNAs show spatially and temporally restricted expression patterns, to describe these patterns during the external ear development in mice we predicted the miRNAs that can regulate the genes involved in the development of the external ear using the databases DIANA-microT, TargetScanS and mirDB. For each gene we obtained a catalog of miRNAs, for example, for *Hoxa1* the predicted miRNA are: mmu-miR-10a/b, mmu-miR-let7, mmu-miR- 30, mmu-miR-216b, mmu-miR-181, mmu-miR-99, mmu-miR-377-3p, mmu-miR-3088-3p and mmu-miR-511-3p. In order to corroborate the theorical data, we performed microarray GeneChip ® miRNA 3.0 Array Affymetrix using samples of external ear tissues from mice embryos of 13.5 and 14.5 dpc.

Program/Abstract # 123

Several Hedgehog responsive enhancers contribute to patched expression

Lorberbaum, David S.; Ramos, Andrea; Barolo, Scott (University of Michigan, Ann Arbor, MI, USA)

Generating the cellular diversity required for all organisms hinges on the transcriptional output of surprisingly few cell signaling pathways. Hedgehog (Hh) signaling is one of these pathways and is essential for activation and repression of several target genes. All gene targets of the Hh pathway are directly regulated by Cubitus interruptus (Ci), the lone *Drosophila* effector of Hh signaling. Conserved in vertebrates as the Gli family of transcription factors (TFs), Ci functions through binding to enhancers, or *cis*-regulatory regions of DNA that contain binding sites for TFs. These elements are the key to the spatial and temporal regulation of all 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 activate *ptc* transcription in the developing wing, but this enhancer does not respond to Hh signaling in embryos, another developmental stage at which Hh signaling is critical. In our search for the embryo enhancer, we identified several temporally and spatially regulated Hh responsive enhancers that contribute to embryonic *ptc* expression and suggest a role for non canonical Ci binding sites for interpreting the Hh signaling pathway.

Program/Abstract # 124

Analysis of the space/time sonic hedgehog expression using luciferase as a dynamic reporter

Bastidas-Ponce, Aimée; Covarrubias, Luis; Wood, Christopher (Instituto de Biotecnología-UNAM, Mexico)

In the embryonic developing midbrain, the dopaminergic neurons (mDAN) form, at the ventral midline, from floor plate cells that express the morphogen Sonic Hedgehog (Shh). The niche for differentiation is formed and maintained by Shh in concert with other morphogens, whose expression levels vary dynamically over time. In this study, we designed a strategy to characterize the dynamics of *shh* transcription through monitoring the activity of bioluminescent reporter genes coupled to transcriptional regulatory elements (enhancer/promoter) of *shh* gene. We used the SBE1 and SFPE1 enhancers to direct expression of reporter genes to specific regions of the neural tube (midbrain and caudal regions, respectively). We analyzed the expression of these vectors in HEK293T, ESCs during differentiation into mDAN and cells isolated from the embryonic mesencepahlon (E10.5), using population bioluminescence assays and monitoring single cells on a microscope. For all the cell types, our results indicate that luciferase activity was generally extremely low, although in primary cells, regulatory elements did direct and limit expression of luciferase to the ventral mesencephalic-derived cells in a dynamic manner. During the *in vitro* differentiation of ESCs we observed changes in the transcriptional activity of *shh*. These lines could be used to generate transgenic mice to analyze *in vivo* the dynamic transcription of *shh* during embryonic development. Supported by CONACyT 132478.

Program/Abstract # 125

The transcriptional regulatory logic of Sonic Hedgehog target genes in the mouse limb

Lewandowski, Jordan; Powell, Marian (University of Texas at Austin, USA); Ji, Hongkai (Johns Hopkins Bloomberg School of Public Health, USA); Vokes, Steven (University of Texas at Austin, USA)

The Sonic Hedgehog (Shh) pathway is required to properly form the vertebrate limb by directing a complex transcriptional response that is mediated by GLI transcription factors. Although Shh signaling has been extensively studied, little is known about how the transcriptional output is regulated. Previous work using microarray gene expression data and GLI3 chromatin immunoprecipitation identified 205 putative Shh-target genes and their associated GLI binding regions (GBRs). The goal of this study is to identify additional factors enriched in GBRs, and determine if the factors contribute to regulate Shh-target genes. In order to identify genes likely to be regulated by Shh signaling we performed whole-mount in situ hybridization on the 205 genes, and identified 42 expressed in the posterior limb. To determine if these genes are regulated by a common set of transcription factors we searched for enriched DNA motifs in GBRs associated with the 42-gene group. One DNA motif of particular interest is Sp1, which is enriched in GBRs associated with 23/42 posterior genes. Inhibiting Sp1-mediated transcription with mithramycin A in mouse limb bud cultures inhibits 15/23 genes associated with GBRs containing Sp1 and Gli motifs. We propose a model a model where GLI and SP1 function to regulate the transcription of a subset of Shh-target genes in the developing limb bud.

Program/Abstract # 126

Characterization of Spry2 Cis-Acting Elements Responsive to FGF Signals

Zhang, Ying; Lewandoski, Mark (National Cancer Institute, USA)

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

Program/Abstract # 127

Mechanisms of Tbx4 action on hindlimb development

Luxey, Maëva (IRCM, Canada); Nemec, Stephen; Drouin, Jacques (Montreal, Canada)

Limb development is governed by a genetic program common to all appendages. Morphological and functional differences between forelimbs (FL) and hindlimbs (HL) appear to result from modulation of this developmental program. Mechanisms for specification of limb identity have focused on three transcription factors with limb-restricted expression patterns: Pitx1 and Tbx4 that are expressed in HL, and Tbx5 expressed in FL. Characterization of the transcriptional properties of these factors revealed that Pitx1 acts upstream of

Tbx4 and contributes to Tbx4 repression. Tbx4 and Tbx5 share a conserved transcriptional activation domain that may control limb bud growth as Tbx5-/- mice fail to develop FLs. In addition, Tbx4 has a unique C-terminal repressor domain, the activity of which is correlated with the role of Tbx4 in HL identity. Interestingly, the Tbx4 repressor activity appears to be context-dependent, suggesting a role for critical corepressor(s). We use biochemical approaches to investigate the putative Tbx4 coregulators, in particular through the purification of protein complexes and GS/MS analyses. Further, we have implemented genomic approaches (RNA-Seq on FL and HL from Wt and Pitx1 -/- buds and ChIP-Seq for Pitx1, Tbx4 and chromatin marks) t o identify targets of Tbx4 and Pitx1 action. Collectively, these analyses will define the gene regulatory networks and mechanisms reponsible for Tbx4 function(s) in HL development.

Program/Abstract # 128

Hoxa5 Function in Organogenesis is Controlled by a Complex Regulatory Network Involving YY1 Transcription Factor

Berube-Simard, Felix-Antoine; Olivier, Boucherat; Jeannotte, Lucie (CRC-HDQ, Canada)

The development of the fertilized egg into a multicellular organism depends on a hierarchy of molecular events requiring the specific spatio-temporal expression of multiple genes. Among the key players are *Hox* genes encoding transcription factors essential in specifying the regional identity along the embryonic axes and orchestrating morphogenesis. *Hox* mutant mice present homeotic transformations affecting the skeleton and anomalies in organogenesis that can impair viability. We are interested in deciphering the function and the regulation of Hox genes using as a model the Hoxa5 gene. The characterization of the Hoxa5 mutant mouse line generated in the laboratory has led to a better understanding of the role of Hoxa5 in several pathologies including COPD and cancer. Moreover, our work on Hoxa5 developmental regulation allowed us to position Hoxa5 in the molecular cascade controlling lung development. Hoxa5 mutant mice present a panoply of phenotypes indicative of the broad range of Hoxa5 actions throughout life. Most of Hoxa5$^{-/-}$ mice die at birth from respiratory distress due to tracheal and lung dysmorphogenesis and surviving mutants display lung airspace enlargement and goblet cell metaplasia. Hoxa5 expression is confined to the mesenchyme of the entire respiratory tract suggesting that it provides regional cues to the contiguous epithelia and participates to cell fate determination. Indeed, lung goblet cell metaplasia results from Clara to goblet cell transdifferentiation, a process accompanied by an increased activity of Notch signaling. Hoxa5 expression in the developing lung is under the control of cis-acting sequences located in the Hoxa4-Hoxa5 intergenic region that specifically bind the Zn finger transcription factor YY1. Our findings unveil Hoxa5 as a micromanager controlling not only organ formation and axial patterning but also details of cell morphogenesis and function. Hoxa5 function is intimately linked to an accurate regulation of its expression.

(Financial support from CIHR and NSERC)

Program/Abstract # 129

Protein-protein interaction of Antp/Scr Homeodomains in the genetic control of development in D. melanogaster

Elizondo-Rodríguez, F. Salomé; Reséndez-Pérez, Diana (FCB-UANL, Mexico)

Hox genes encode for a group of transcription factors involved in the regulation of diferentiation networks that lead to the proper development of *Drosophila*. Homeodomains in addition to its DNA binding ability, also have functions of RNA binding, secretion, penetration and protein-protein interactions. These broad functions of HD-containing genes are essential for development suggesting that HD-containing proteins cross react with other HD-containing partners. This cross-regulation of HDs interactions prompted us to investigate further protein-protein interactions of Hox HDs. Since previous work showed that Sex comb reduced (Scr) repression is mediated by Antennapedia in vivo , we focused on HD protein interaction of Antp and Scr *Drosophila* Hox genes. Here we describe protein-protein interaction of Antp/Scr using Bimolecular fluorescence complementation and transcriptional activation assay. Our results indicated that Antp HD has a crucial role in protein-protein interaction of Antp/Scr in which YPWM was not absolutely required. We also found Extradenticle (Exd) is a requisite cofactor for the HD interactions of Antp and Scr. Currently, we are also analyzing residues in the HDs involved in this interaction and transcriptional activity using cofactos as Exd. In addition, we are verifying the presence of trimeric interactions and participation of Exd to the Scr repression by Antp. These results indicate that HD-containing genes f orm protein complex that regulates downstream genes through protein-protein interaction modifying DNA binding affinity of transcription factors that may explain epistatic relations of Hox genes in *D. melanogaster*.

Program/Abstract # 130

Protein-protein interaction of Antennapedia with TFIIE-ß in *Drosophila melanogaster*

Altamirano Torres, Claudia Dalila; Reséndez-Pérez, Diana; Cárdenas-Chávez, Diana L.; Elizondo-Rodrígiuez, F. Salomé (FCB-UANL, San Nicolás de los Garza, Mexico)

Segmental identity along the anteroposterior axis of bilateral animals is specified by Hox genes. Antennapedia homeoproteín (Antp) is responsible for thoracic segments and head formation in *Drosophila*. Antp acts by DNA binding through the conserved homeodomain (HD) and by protein-protein interactions of YPWM motif with the transcription factor BIP2 linking this homeoprotein with the basal transcription machinery. Therefore, we analyzed if other general transcription factors (GTFs) are involved in Antp functional specificity. Here we describe protein-protein interactions of Antp HD with the GTF TFIIE-β by Bimolecular Fluorescence Complementation (BiFC) and transactivation assays. Our results indicated that helix II of the Antp HD has a crucial role in this protein-protein interaction with TFIIE-β and is not mediated by YPWM motif . We also found that aminoacids Iso-32 and His-36 of helix II of Antp HD are required for this interaction. Transcriptional activity of Antp was analyzed by the presence of TFIIE-β and we

are currently determining the functional relevance of this Antp interaction in embryonic and adult development in *D. melanogaster* by homeotic transformations and in vivo BiFC assays. Taking in account the conservation of the Antp HD and its expose location in helix II, the functional role of the residues 32 and 36 could be extrapolated to other hox proteins in the functional specificity by protein-protein interaction with GTFs in the genetic control of *Drosophila melanogaster*.

Program/Abstract # 131

Dynamic of p8 and p52 during early embryonic development and spermatogenesis of *Drosophila melanogaster*

Mandy; Cruz, Grisel; Zurita, Mario (Instituto de Biotecnologia (UNAM), Mexico)

The transcription/DNA-repair factor TFIIH is composed of ten subunits (XPD, XPB, p44, p34, p52, p62, p8, Cdk7, CycH and MAT1) and is involved in three fundamental processes that are DNA repair, transcription and cell cycle control. It's known that p8 and p52 proteins physically interact, however, the relevance of this interaction is unknown. In previous studies we have observed that p8 null organisms are affected in embryonic mitotic divisions and sperm differentiation is arrested at primary spermatocyte stage. Likewise organisms with hipomorfic p52 present the same spermatogenesis defects. In this work we describe for the first time the in vivo dynamics of p8 and p52 during the first embryonic mitotic divisions and spermatogenesis of *Drosophila melanogaster*, by using transgenic flies expressing p8 and p52 fused to CFP and YFP respectively. In the syncytial blastoderm we observed that both subunits remain around the chromosomes in the nucleus during prophase, metaphase and anaphase and go to the cytoplasm during telophase for their subsequent re-joining to the nucleus in the interphase of the next cycle. These observations and the mitotic defects could suggest a possible role of these proteins in mitosis during embryogenesis. However more studies are necessary to confirm this hypothesis. Moreover, in testes p8-CFP and YFP-p52 are located at nucleus and nucleolus of primary spermatocytes and seem to co-localize with bivalent chromosomes during meiosis in these cells. In a p52 mutant background the localization of p8-CFP in testes is affected and using western blot assays it was observed that levels of others TFIIH components are decreased, not happening the same in the opposite case. This suggests that p52 and p8 interaction is important to maintain the proper p8 localization and TFIIH stability in these cells in the fly

Program/Abstract # 132

Paused Pol II Coordinates Tissue Morphogenesis in the *Drosophila* Embryo

Bothma, Jacques; Lagha, Mounia; Juarez Esposito, Emilia; Ng, Samuel (Univ of California-Berkeley, USA); Stefanik, Laura (Philadelphia, USA); Tsui, Chiahao (Univ of California-Berkeley, USA); Johnston, Jeffrey; Chen, Kai (Stowers Institute for Medical Research, USA); Gilmour, David (Philadelphia, USA); Zeitlinger, Julia (Stowers Institute for Medical Research, USA); Levine, Michael (Univ of California-Berkeley, USA)

Paused RNA Polymerase (Pol II) is a pervasive feature of Drosophila embryos and mammalian stem cells, but its role in development is uncertain. Here, we demonstrate that there is a spectrum of paused Pol II, which determines the "time to synchrony"--the time required to achieve coordinate gene expression across the different cells of a tissue. To determine whether synchronous patterns of gene activation are significant in development, we manipulated the timing of *snail* expression, which controls the coordinated invagination of ~1000 mesoderm cells during gastrulation. Replacement of the strongly paused *snail* promoter with moderately paused or nonpaused promoters results in stochastic activation of *snail* expression and the progressive loss of mesoderm invagination. Computational modeling of the dorsal-ventral patterning network recapitulates these variable and bistable gastrulation profiles, and emphasizes the importance of timing of gene activation in development. We conclude that paused Pol II and transcriptional synchrony are essential for coordinating cell behavior during morphogenesis.

Program/Abstract # 133

Cis-acting transcriptional repression establishes a sharp boundary in chordate embryos

Imai, Kaoru; Daido, Yutaka; Kusakabe, Takehiro; Satou, Yutaka (Kyoto, Japan)

The function of Bone morphogenetic protein signaling system in dorso-ventral (DV) patterning of animal embryos is widely conserved among the Bilateria. In vertebrates, the BMP ligand anti-dorsalizing morphogenetic protein (Admp) is expressed dorsally and moves to the opposite side to specify the ventral fate. Here we show that Pinhead is an antagonist specific for Admp with an essential role in establishing the sharp boundary of the ascidian epidermis along the DV-axis. *Pinhead* and *Admp* exist in tandem in the genomes of a wide range of animals. This genomic configuration is important for mutually exclusive expression of these two functionally opposed genes through cis-acting transcriptional repression. Our data suggest that this dual negative regulatory mechanism is widely conserved in a wide range of animals.

Program/Abstract # 134

Role of the MADS-box gene AGL19 in the cellular homeostasis of *Arabidopsis thaliana* root: its cellular and molecular functions and epigenetic regulation

Hernández Marroquín, Víctor Rogelio; Garay Arroyo, Adriana (Instituto de Ecología, UNAM, Mexico)

MADS-box genes code for transcription factors involved in *Arabidopsis thaliana* development. Three genes of this family with clear effects in root development have been characterized in our laboratory – *AGL12* (*XAL1*), *AGL14*, and *AGL17*. We also have preliminary data suggesting that *AGL19* is also involved in the proliferation-differentiation equilibrium of the root apical meristem (RAM). It is well known that *AGL19* plays a role during transition to flowering in the shoot apical meristem, that its expression is

downregulated by polycomb-group proteins, and that such regulation is suppressed after a vernalization treatment. Polycomb-group protein CLF methylates chromatin in the *AGL19* locus, with the first intron being the most frequent target. Thus, it has been proposed that CLF jointly with other PRC2 proteins silences *AGL19* expression by methylation. It is not known if this silencing occurs in the root, the organ where *AGL19* shows the highest expression. On the other hand, our data from fusions of *AGL19* promotor region and reporter genes suggests that *AGL19* is upregulated by auxin. Studies on CLF mutants show that its role in the RAM is not linked to changes in auxin concentration. Based on this data, we ask ourselves whether the *AGL19* regulatory pathway involving polycomb-group proteins and the one involving auxin are connected. In this work we characterized *AGL19* molecular and cellular functions through analysis of a loss-of-function mutant. We found that *AGL19* silencing by polycomb-group proteins also occurs in the root. Finally, we propose experiments involving auxin induction of *AGL19* expression in CLF and LHP1 mutants in order to find out whether its epigenetic regulation is independent to auxin regulation.

This work is supported by CONACyT (180098; 180380; 167705; 152649; 105678) and DGAPA, UNAM (IN204011-3; IN203113-3; IN226510-3; IB201212-2) grants.

Program/Abstract # 135

The SWI2/SNF2 Chromatin Remodeling Factor CHR9 Regulates Floral Meristem Identity in Cooperation with LEAFY

Lamb, Rebecca S.; Kovach, Jeffrey; Habina, Matthew; Siriwardana, Nirodhini (Ohio State University, USA)

In angiosperms the flower is the unit of reproduction and is considered to be a modified shoot. The floral meristem identity genes are required to specify meristems as flowers rather than as shoots. In the model plant species *Arabidopsis thaliana* as well as other flowering plants the LEAFY (LFY) transcription factor is a key regulator of floral development, regulating expression of other floral meristem identity genes and floral homeotic genes that control floral organ identity. We have identified CHR9, a member of the SWI2/SNF2 family of chromatin remodeling factors, as an LFY-interacting protein. *chr9* loss-of-function mutations have mild meristem identity defects while *CHR9* overexpression lines flower early with shoots converted to flowers. Physical and genetic interactions between LFY and CHR9 indicate that these factors act together. Evidence will be presented that CHR9 acts with LFY to regulate expression of floral meristem identity genes, including *TERMINAL FLOWER 1 (TFL1)*, and thus CHR9 is involved in the control of plant reproductive architecture.

Program/Abstract # 136

Paternal contributions to early embryogenesis of *Arabidopsis thaliana*: A functional genetic approach

Del Toro, Gerardo Del Toro; García-Aguilar, Marcelina; Gillmor, Stewart (CINVESTAV-IPN, Mexico)

In animals, zygotic genome activation and maternal product decay are prerequisites for transfer of developmental control from the mother to the zygote, a phenomenon known as the Maternal to Zygotic Transition (MZT). In plants, the existence of a MZT is controversial. Two recent studies have reported allele-specific transcriptome profiles of early embryogenesis, one providing support for maternal dominance with significant paternal input (Autran et al., Cell 2011), and the other study arguing for equivalent parental contribution (Nodine and Bartel, Nature 2012). However, neither study tested the biological relevance of this phenomenon. Here we report the first systematic functional genetic analysis of paternal gene activity in early plant embryogenesis. Our assay consists of testing the timing of the ability of wild type paternal alleles to complement pre-globular embryo phenotypes conditioned by mutant maternal alleles. In our analysis of 50 embryo defective mutants (*emb*), we found that 82% of genes showed delayed complementation, while 18% showed immediate complementation. In addition, we used *GCT/MED13* and *CCT/MED12*, two genes that show delayed phenotypic complementation, to correlate delayed paternal function with delayed paternal expression. Thus, activation of many paternal alleles in early embryogenesis is gradual, while a significant proportion of paternal alleles show immediate activity. Our results demonstrate that plants do not have discrete step for zygotic gene activation, and suggest that the maternal and paternal genomes make unequal contributions to early plant embryogenesis.

Program/Abstract # 137

Expression of Aminopeptidase N Genes During Sea Urchin Development

Ingersoll, Eric P.; Drab, Diana L. (Penn State, USA)

Aminopeptidase N (APN) is an exopeptidase that has been shown to play a role in the development of nematodes and fruit files. Recent advances in genome sequencing have made available information allowing the identification of genes in the sea urchin genome. We have searched the sea urchin genome and found a number of APN genes. In this study, we are determining which of these APN genes is expressed during embryonic development. We used reverse transcription PCR to detect gene-specific mRNAs throughout development. Our studies have identified several APN genes that are expressed in embryos. We will present data on the temporal expression of these genes throughout the entirety of sea urchin embryonic development. In the future, we hope to determine the spatial expression patterns and developmental functions of these genes.

Program/Abstract # 138

Repulsive guidance molecules expression pattern during chicken embryogenesis suggested new roles for these molecules during notochord formation, somitogenesis and myogenesis

Jorge, Erika; Ahmed, Mohi (Mount Sinai School of Medicine, USA); Bothe, Ingo (Sloan Kettering Institute, USA); Coutinho, Luiz (Universidade de São Paulo, Brazil); Dietrich, Susanne (University of Portsmouth, UK)

Repulsive guidance molecules (RGM) are ligands for Neogenin receptor which play a number of roles during nervous system development, including neural tube closure; neuronal and neural crest cell differentiation and axon guidance. RGM molecules were also implicated in BMP signaling, which regulates a variety of processes during development. Given that new roles for Neogenin outside the nervous system are recently emerging, and that BMP signaling controls the development of neural as well as non-neural tissues, we were wondering to which extent RGM molecules may control non-neural developmental processes. Chicken embryos were used to investigate possible new biological roles for RGM genes in these processes. The current edition of the chicken genome predicts the existence of only two RGM genes (among the four members of the family) in this species: RGMa and RGMb. The expression pattern of RGMa and RGMb was determined by in situ hibridization, from gastrulation (HH3) to organogenesis (HH27) stages of chicken embryonic development. Our data confirmed RGMa expression in the neural plate and neural tube, and RGMb expression in differentiating neurons and cranial and dorsal root ganglia. New expression patterns were observed for RGMb in the notochord. Moreover, both RGMa and RGMb were expressed in the somite, with RGMa predominantly labeling muscle precursor and muscle stem cells and RGMb labeling differentiating muscle. Therefore, our expression pattern data suggested so far unknown roles for RGM molecules in notochord, somite and skeletal muscle development.

Program/Abstract # 139

The transcription factor cScratch2 is an early marker for post-mitotic neural precursors

Vieceli, Felipe; Kanno, Tatiane (Universidade de São Paulo, Brazil); Simões-Costa, Marcos; Bronner, Marianne (California Institute of Technology, USA); Yan, Irene (Universidade de São Paulo, Brazil)

The Scratch (Scrt) genes code zinc-finger transcription factors that participate in neural development. Functional studies in different animal models indicate that Scrt promotes survival of neural cells. In addition, gain-of-function studies in the mouse embryonic cortex suggest a role in neuronal migration. In this work, we have investigated the embryonic expression of the chicken Scrt2 ortholog (cScrt2) and its relationship with other neural factors. Sequencing of RACE-PCR products from HH19 and HH24 libraries suggests the existence of at least four alternate spliced transcripts. In situ hybridization in whole mounts using a probe that should detect all the identified transcripts show that cScrt2 is first expressed in few cells of the hindbrain and in the nasal placode by HH15, and later in the hindbrain, spinal cord, cranial ganglia and dorsal root ganglia (DRG). Double labeling in sections indicate that cScrt2-positive cells in the spinal cord are post-mitotic and express NeuroM, and that cScrt2 is coexpressed with Islet1 in motoneurons and DRG neurons. Our results suggest that cScrt2 is one of the first genes expressed after cell cycle arrest of spinal cord and DRG neurons, and may act in conjunction with NeuroM. In silico analysis of genomic sequences to identify potential regulatory non-coding regions revealed the presence of a conserved intronic element in the cScrt2 locus that contains putative transcription factors binding sites. Additionally, in silico search for post-translationally regulated sites identified candidate phosphorylation targets in the predicted aminoacid sequence. These data raise new questions on the different levels of regulation acting on the Scrt2 gene in vertebrates.

Program/Abstract # 140

Cell-type specific analysis of chromatin modifications at the Drosophila shavenbaby gene

Preger-Ben Noon, Ella; Preger-Ben; Lemire, Andrew; Stern, David (Howard Hughes Medical Institute, USA)

Pattern formation involves the integration of multiple inputs from gene regulatory networks through *cis*-regulatory regions, leading to cell-type specific gene expression. Many developmental genes possess large regulatory regions composed of many individual enhancer modules. Each of these modules contributes some features of the expression pattern which together coordinate the overall pattern. Such modularity allows the formation of complex gene expression patterns as well as the evolution of these patterns. The drosophila *shavenbaby* (*svb*) gene provides a superb model for studying *cis*-regulatory structure, function, and evolution. The svb gene encodes a transcription factor that regulates the development of cuticular hair-like projections called trichomes. Svb is expressed in a complex pattern in the embryonic ectoderm and acts to switch epidermal cells between naked cuticle and trichome bearing cells. At least six enhancer modules located in the *cis*-regulatory region of svb together recapitulate the entire svb embryonic expression pattern. This collection of individual *svb* enhancers, each regulated in a cell-specific manner, provides an opportunity to examine how varied transcriptional inputs regulate chromatin structure. To generate a comprehensive view of the regulatory landscape of the svb gene we have used a new method of cell-type specific analysis of chromatin states. Briefly, embryonic nuclei expressing fluorescent reporters under the control of different *svb* enhancers are sorted and histone modification patterns at the *svb* loci are determined by ChIP-seq. Here we report the current state of our analysis.

Program/Abstract # 141

Zac1 controls cell cycle exit of neural progenitors through direct regulation of cyclin-dependent kinase inhibitor expression along the entire rostrocaudal axis of the developing central nervous system

Rraklli, Vilma (Ludwig Institute for Cancer Research, Sweden)

The central nervous system (CNS) is characterized by a sophisticated architecture where different cell types support neuronal functions. During CNS development neurons, astrocytes and oligodendrocytes are generated from a pool of neural progenitors located in the ventricular zone (VZ) and subventricular zone (SVZ). Proper development and functionality of CNS is achieved via regulatory mechanisms that dictate when neural progenitors should proliferate or exit the cell cycle. Despite the crucial importance of coordination between cell cycle exit and differentiation, such mechanisms remain poorly understood. Here we show that the zinc finger transcription factor Zac1 regulates cell cycle exit through control of expression of cyclin-dependent kinase inhibitors (CKIs).

Zac1 expression in the developing cortex and spinal cord is restricted to the dividing neural progenitors in the VZ and SZ. *Zac1* overexpression, in cortex and spinal cord, elicits cell cycle exit and expulsion from the germinal zones. In the cortex this is accompanied by upregulation of the CKI *Cdkn1c*, whereas in the spinal cord *Cdkn1b* expression is induced. The premature cessation of proliferation is, however, not accompanied by precocious acquisition of differentiated neuronal characteristics. *In vitro*, we show that, Zac1 directly binds to the promoter region of the *Cdkn1c* gene and forcefully induces its expression. Our results show that Zac1 is a key regulator of cell cycle exit in cycling neural progenitors through the induction of CKIs. Furthermore, this study reveals a molecular pathway that specifically regulates cell cycle exit without affecting other aspects of differentiation indicating that these processes are not inextricably linked.

Program/Abstract # 142

Transcription regulation of anterior hypothalamic development

Mahmud, Abdullah Al (University of Montreal, Canada); Michaud, Jacques (CHU Sainte Justine, Canada)

The paraventricular nucleus (PVN) of the anterior hypothalamus regulates several processes that are critical for survival, including the regulation of energy balance and of blood pressure. SIM1 directs the terminal differentiation of at least five types of PVN neurons identifiable by the production of OT, AVP, CRH, SS and TRH. Whereas *Sim1*-/- mice die shortly after birth, *Sim1*+/- mice survive but develop hyperphagia and early-onset obesity. We have shown that *Sim1* functions along a physiological pathway in the PVN for the control of food intake. *Sim1* thus regulates the development of the PVN as well as its function. The objective of this project is to identify novel regulators of PVN development. We have identified a regulatory element that specifically directs expression in all cells of the developing PVN. Using this element, we have generated transgenic mice that express gfp in these cells. We next collected the domain expressing gfp at different developmental stages (E11.5, E12.5, E13.5, E14.5 and E16.5) as well as the immediate posterior domain of the developing hypothalamus. We are currently comparing the transcriptomes from these samples by performing RNA-seq. We have also used the same strategy to collect hypothalamic samples from *Sim1* mutant and wild-type embryos. By comparing the transcriptomes of these different sets of embryos, we will be in a position to identify the factors that are directly or indirectly regulated by SIM1. As shown by our work on *Sim1*, regulators of PVN development have the potential of influencing physiological processes. The factors that will be identified in the course of this project may thus play a role in the pathophysiology of common disorders of homeostasis.

Program/Abstract # 143

Do disruptions of a ZIC2 Non-coding Conserved Element cause Holoprosencephaly?

Barratt, Kristen S. (The Australian National University, Australia); Hu, Ping (National Human Genome Research Institute, USA); Garrett, Lisa (Transgenic Core Facility, NIH, USA); Roessler, Erich; Muenke, Maximillian (National Human Genome Research Institute, NIH, USA); Arkell, Ruth (The Australian National University, Australia)

Holoprosencephaly (HPE) is the most common structural malformation of the forebrain, sometimes presenting as cyclopia. Only 25% of human HPE cases with normal chromosomes have been attributed to mutation of one of nine HPE associated genes, leaving 75% of HPE cases with an unknown origin. Germline mutation of the transcription factor *Zic2* causes HPE in both man and mouse and recently variants in non-coding regions of ZIC2 have been considered a putative cause of HPE. Comparative genomics identified a 540bp Non-coding Conserved Element (NCE) in the ZIC2 3'UTR and screening of 528 human HPE probands isolated six variant sequences in this NCE. None were identified in ethnically matched control populations, excluding that they are polymorphisms and suggesting a contributing role in the development of HPE. We hypothesize that the identified NCE acts as an enhancer; a cis-acting DNA regulatory element that stimulates transcription independent of its position or orientation. Interrogation of the NCE sequence identified multiple transcription factor (TF) binding sites, some of which are altered in the six identified variants. Furthermore, ZIC2 HPE mutations are known to be loss of function, suggesting an enhancer role for the NCE in promoting ZIC2 expression during early embryogenesis. We have shown that this NCE acts as an enhancer in transgenic zebrafish and mammalian cells and it is currently being tested in transient transgenic mice. We aim to demonstrate that the ZIC2 NCE drives LacZ reporter expression during murine embryogenesis, and that introduction of the identified variants alters reporter expression. We are also examining candidate TFs to determine if they bind and regulate the ZIC2 NCE during gastrulation.

Program/Abstract # 144

A novel approach to study modulators of Wnt/beta-catenin pathway using Wnt reporter transgenic *Xenopus* and tailored-TALENs mutagenesis

Tran, Hong Thi; Van Imschoot, Griet; Van Roy, Frans; Vleminckx, Kris (Ghent University, Belgium)

The Wnt/beta-catenin signaling pathway plays a crucial role during embryonic development and throughout adult life. Its aberrant activation in human often results in the development of cancer. Although many domains of Wnt expression have been identified during embryogenesis in *Xenopus*, the dynamics and the identification of the responding cells have been mostly unexplored due to the lack of appropriate reporter tools. Previously, we have generated *X. tropicalis* transgenic reporter lines that express a dGFP variant under control of a multimeric beta-catenin/TCF responsive element (Tran et al., 2010). dGFP is expressed in the transgenic lines in a dynamic pattern throughout embryogenesis and reports general known domains of active Wnt signaling. Moreover, GFP patterns identified a number of tissues as novel sites revealing previously unrecognized cell populations with beta-catenin/TCF dependent transcriptional activity. These transgenic lines were proven to be reliable tool to study regulators of Wnt signalling. Recently, we have

also generated several of such transgenic lines in *X. laevis*. Remarkably, the expression patterns of dGFP in *X. laevis* in general resemble those in *X. tropicalis*. To further explore the potential usage of these reporter lines, we make use of the tailored-TALENs (Transcription activator-like endonucleases) to direct gene disruption of few known modulators of the Wnt/beta-catenin pathway on the transgenic embryos and analysing the gene knockdown effects on the reporter expression. Our preliminary results showed that this combination approach holds a great potential for further investigating functions and uncovering new regulators of the Wnt/beta-catenin signaling.

Program/Abstract # 145

Ets-1 is an Essential Regulatory Factor of Neural Crest Formation in *Xenopus*

Geary, Lauren (Northwestern University, USA)

Neural crest cells are a hallmark of vertebrate embryos, and an excellent system in which to address questions about how stem cell attributes are acquired and how cell behavior is integrated with changes in cell fate. Neural crest cells arise at the neural plate border in regions of intermediate BMP signaling, and Wnt signals are essential for their induction. FGF signals have also been implicated in neural crest induction, although the precise contributions FGF signals make to neural crest formation has been controversial. A full understanding of the upstream regulation of this important cell population requires better insight into the respective roles of these signaling pathways. Members of the ETS family of transcription factors are common downstream mediators of receptor tyrosine kinases such as the FGF receptor. A number of ETS proteins have been implicated in neural crest induction, migration, and differentiation, yet when they are active and how they are regulated remains elusive. Here, we show that in *Xenopus*, the founding ETS family member Ets-1 is expressed in neural crest forming regions of the ectoderm, and that its expression is maintained in the neural crest through migratory stages. Ets-1 is an essential component of the neural crest gene regulatory network (NC-GRN) as morpholino-mediated depletion of this factor disrupts the expression of neural crest specifier genes. We present evidence on the role and regulation of Ets-1 in the formation and subsequent development of the neural crest.

Program/Abstract # 146

Tgfß3 signals through Twist1 and then Snail1 to down regulate E-cadherin expression during epithelial-mesenchymal transition (EMT) in the palate

Svoboda, Kathy K. (Texas A&M Univ Baylor College of Dentistry, USA); Yu, Wenli (University of California-San Francisco, USA); Ruest, L-Bruno (Texas A&M Baylor College of Dentistry, Dallas, USA)

Palatal fusion is a tightly controlled process which comprises multiple cellular events downstream of cell surface signaling, including cell movement and differentiation. Midline epithelial seam (MES) degradation is essential to palatal fusion. One feature of MES degradation is the down regulation of E-cadherin. Snail1 is expressed in the MES and is a known repressor of E-cadherin. In this study, we asked if Snail1 was necessary for palatal fusion. We also determined a possible mechanism regulating the expression of the *Snail1* gene in palatal shelves. Whole mount in situ hybridization combined with organ cultures of mouse palatal shelves, with ChIP and luciferase assays in cultured cells and RT-PCR experiments were used to study transcriptional regulation of E-cadherin. Mouse palatal explants treated with Snail 1 siRNA did not degrade the MES and E-cadherin was not repressed leading to failure of palatal fusion. We had previously shown that transforming growth factor beta 3 (Tgfb3) activated *Twist1* expression in the MES cells before degradation. In this study, Tfgβ3 regulated *Snail1* mRNA, as *Snail1* expression decreased in response to Tgfb3 neutralizing antibody and a PI3K inhibitor (LY294002). *Snail1, Twist1* and *E2A* genes were expressed in the E13.5 mouse palate in overlapping expression patterns. Luciferase assays were used to determine that Twist1, in collaboration with E2A transcription factors, regulated the expression of *Snail1*. Twist1/E47 dimers bond the *Snail1* promoter to activate expression. Without E47, Twist1 repressed *Snail1* expression. These results support the hypothesis that Tgfβ3 signals through Twist1 and then Snail1 to down regulate E-cadherin expression during palatal fusion.

Program/Abstract # 147

The Transcriptional Regulation of Muscle Development in *Drosophila melanogaster*

Brunetti, Tonya; Duong, Sandy; Cripps, Richard (University of New Mexico, USA)

Muscle development in *Drosophila* is beneficial due to the diverse and unique set of muscle types in the thorax. Using specific muscle type enhancer elements and a reverse genetic screen has allowed us to recognize regulators of muscle development. We have identified new roles for two transcription factors that have human orthologs and function in either the regulation of muscle identity or in the proper fusion of myoblasts. These two transcription factors are extradenticle (exd) and Myocyte enhancer factor 2 (MEF2). Exd has been shown to control muscle identity by working in concert with homothorax. The two genes are expressed in all muscle types except the tergal depressor of the trochanter in the thorax. The absence of either gene results in a fiber type switch from fibular to tubular. Further investigation has shown these genes bind to Actin88F, a structural gene found in fibular muscles. The binding of these genes results in proper expression of structural genes which allows for correct muscle identity. Without these genes, the muscles lose their identity and begin to atrophy. In contrast, Mef2 has been shown to regulate muscle formation. The absence of Mef2 results in the lack of muscle formation, ultimately leading to pupae death. Upon the analysis of the fusion gene, singles bar (sing), RT-PCR revealed MEF2 as a potential regulator of sing expression. Knockdowns of sing result in lethality due to the inability for myoblasts to fuse together to form syncytial muscle fibers. This finding has thus provided a novel function for MEF2 in myoblast fusion. Thus,

understanding the mechanisms that underlie the transcription control of muscle development can provide insight into human myopathies and ailments.

Program/Abstract # 148
Withdrawn

Program/Abstract # 149
Alteration of MMP9 and TIMP1 expression by high glucose in mouse blastocysts is dependent on oxidative stress
Sánchez Santos, Alajandra; Martínez Hernández, María Guadalupe; Baiza Gutman, Luis Arturo (FES Iztacala, UNAM, Mexico)
Hyperglycemia during diabetes has been associated with enhanced oxidative stress and metabolic alterations that lead to changes in gene expression. High concentration of glucose induce the formation of reactive oxygen species (ROS), which can alter the expression of matrix metalloproteinases (MMPs), producing changes in the synthesis and degradation of the extracellular matrix (ECM). MMP9 is secreted by the mouse blastocyst during implantation, and it has been involved in the invasive behavior of trophoblast *in vivo* and *in vitro*. However, few studies have been focus in MMP9 expression on blastocyst during implantation. In the present work we determined the effect of oxidative stress caused by high concentration of glucose on the expression of MMP9 and TIMP1 in mouse blastocysts developing in culture. Gestational day (GD) 4 blastocysts were cultured in HAM-F10 and treated after 2 days of culture (GD7), with glucose 25 mmol/L or H_2O_2 10 µmol/L. The antioxidant N-acetylcysteine (NAC) or ROS inhibitors (apocynin, rotenone) were added to some groups of glucose treated embryos. *Mmp9* and *Timp1* mRNAs were measured using real time RT-PCR. MMP9 levels were analyzed by zymography in SDS-PAGE-gels co-polymerized with gelatin. Glucose 25 mmol/L and H_2O_2 10 µmol/L induced higher levels of MMP9 protein and mRNA. Both conditions also cause diminished levels of *Timp1* mRNA. NAC and ROS inhibitors restored *Mmp9* mRNA, MMP9 protein and *Timp1* mRNA concentration to levels similar to control group (embryos cultured with glucose 6 mM). We concluded that oxidative stress caused by high concentration of glucose may regulate the invasiveness of trophoblast during embryo implantation, through the control of MMP9 and TIMP1 expression. Supported by PAPIT, DGAPA, UNAM, grant IN230611.

Program/Abstract # 150
Spalt major directly regulates seven-up expression in Drosophila oenocytes
Ryan, Kathryn M.; Mason, Grace; Cripps, Richard (University of New Mexico, USA)
Oenocytes are hepatocyte-like cells oriented bilaterally in the Drosophila embryo and larva. These cells function in lipid metabolism as well as pheromone secretion. The focus of our work is to understand the developmental genetic program controlling the formation of these cells. We have identified a minimal enhancer responsible for expression of the *seven-up (svp)* gene in the oenocytes. Svp is orthologous to the vertebrate COUP-TF proteins (Chicken Ovalbumin Upstream Promotor Transcription Factors. These proteins have been shown to be important in vertebrate development. Here we demonstrate that Spalt major (Salm) is a direct regulator of the *svp* enhancer. We also show that Svp is upstream of *mirror*, another important gene in oenocyte development. Understanding the relationship of these genes in the context of the simpler Drosophila model may lead to a better understanding of the genetic regulation of COUP-TFs, which have been shown to be involved in many of the same biological functions in vertebrates, as flies.

Program/Abstract # 151
A Genetic and Chemical Genetic Approach to Study Cell Fate Decisions via JAK/STAT Attenuation
Monahan, Amanda J.; Seley-Radtke, Katherine; Starz-Gaiano, Michelle (Univ of Maryland-Baltimore, USA)
Throughout development cells undergo precise spatio-temporal patterning governed genetically, which generally involves a signal transduction cascade, such as the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway. Border cell migration during Drosophila oogenesis provides a powerful system to study JAK/STAT regulation. The egg chamber is composed of the germ line encased by somatic epithelial follicle cells. At mid-oogenesis, a subset of anterior follicle cells (AFC) receive graded STAT activity. The 4-6 AFC with the highest level of STAT signaling form motile border cells. STAT activation must be precisely regulated as too few or too many motile cells results in migration defects. We have utilized two approaches to study STAT regulation. In the first, we establised *Socs36E* is necessary to limit STAT signaling in the AFC. We found STAT and the STAT feedback inhibitor Apontic (APT) regulate *Socs36E*. Genetic interactions suggest Socs36E functions with Cullin-2 in an E3 ubiquitin ligase complex. Thus, APT attenuates STAT at the protein level - via *Socs36E* - to limit migratory cell fate and post transcriptionally via *miR-279* to facilitate detachment. In our second approach, we have modified the synthetic scheme of a known JAK ATP-competive inhibitor and are synthesizing novel analogs that have altered interactions in the catalytic domain. *In vitro* and *in vivo* assays allow us to interrogate the importance of specific interactions between JAK and its substrates, as well as study the transcriptional and developmental effects of inhibiting STAT signaling. Through fly and chemical genetics our work demonstrates the importance of proper JAK/STAT regulation in developmental and disease contexts.

Program/Abstract # 152
Comparative transgenic analysis of enhancers near the human and mouse short-stature genes SHOX and Shox2
Cobb, John A.; Rosin, Jessica; Abassah-Oppong, Samuel (University of Calgary, Canada)

Disruption of presumptive enhancers downstream of the human short stature homeobox gene (*hSHOX*) is a frequent cause of the zeugopodal limb defects characteristic of Léri-Weill dyschondrosteosis (LWD). The closely related mouse gene *mShox2* is also required for limb development, but in the more proximal stylopodium. In this study we used transgenic mice in a comparative approach to characterize enhancer sequences in the *hSHOX* and *mShox2* genomic regions. We first analyzed the regulatory potential of a genomic region containing a duplicated conserved noncoding element (dCNE) downstream of *mShox2* and *hSHOX*. We identified a strong limb enhancer directly adjacent to the *mShox2* dCNE that recapitulates the expression pattern of the endogenous gene. Interestingly, in order to drive strong limb expression this enhancer requires sequences only conserved in the mammalian lineage, whereas the more deeply conserved sequences of the dCNE function as a neural enhancer. In addition we found that DNase I hypersensitive sites in limb bud samples (available through the ENCODE project) showed better correlation with limb enhancer activity near *mShox2* than did evolutionary conservation. Similarly, we found that a conserved element downstream of *hSHOX* (CNE9) also functions as a neural enhancer in transgenic mice. However when the CNE9 transgenic construct was enlarged to include adjacent, non-conserved sequences frequently deleted in LWD patients, the transgene drove expression in the zeugopodium of the limbs, as would be expected for a *hSHOX* enhancer. These data demonstrate that transgenic mice can be used as tools for characterizing the enhancer deletions that cause LWD and other *SHOX*-deficient phenotypes.

Program/Abstract # 153

Importance of inhibitory mechanisms for deriving specific somatic lineages from the epiblast

Hisato Kondoh, Kazunari Matsuda, Tatsuya Takemoto (Osaka U, Japan)

Somatic development initiates from the epiblast in post-implantation embryos. Whereas ES cells are frequently used as a model for studying somatic cell development, ES cells must actually go through the epiblast state before entering in somatic lineages, as evidenced by the transient expression of the epiblast hallmark *Fgf5*. We utilized an epiblast stem cell (EpiSC) line, for the purpose of characterizing gene regulatory networks that promote specific pathways of somatic development. It was found that when epiblast cells select a single pathway of development, mechanisms to inhibit other developmental pathways are activated. Anterior neural plate develops under the condition where endodermal, mesodermal and posterior neural development is inhibited. Endoderm development characterized by *Sox17* and *Eomesodermin* expression is permitted when the endoderm-inhibiting, and neuro-mesoderm-promoting activity of Sox2 and Zic2/3 is suppressed. The paraxial mesoderm develops not directly from the epiblast but from an intermediate, the axial stem cells, which have neuro-mesodermal bipotentiality, when the function of pro-mesodermal Tbx6 inhibits the expression of pro-neural Sox2.

Program/Abstract # 154

Single-cell RNA-Seq reveals dynamic, random monoallelic gene expression in mammalian cells

Deng, Qiaolin, (, Sweden), Ramsköld, Daniel; Reinius, Björn; Sandberg, Rickard (Stockholm, Sweden)

In diploid eukaryotic organisms, the maternally and paternally derived copies of each gene are often assumed to be expressed simultaneously at similar levels in the cell. Known exceptions to this include genes undergoing parent-of-origin imprinting, X-chromosome inactivation, and the recently reported clonally inherited random monoallelic expression of around 10% of autosomal genes. Here, we present genome-wide transcriptome analyses on 267 single cells derived from crossed mouse preimplantation embryos (CAST/EiJ x C57BL/6J), containing oocytes to late blastocyst stages. This allowed us perform hitherto most comprehensive analyses of allele-specific transcriptions in single mammalian cells by SNP (single nucleotide polymorphism) calling. Surprisingly, we discovered that most autosomal genes are expressed monoallelicly in each cell. This monallelic expression is highly dynamic, random, and not inherited after cell division. Our study has thus uncovered a novel mode of allelic gene expression characterized by independent and random allelic transcription events, a phenomenon that is likely consequent to the stochastic nature of transcriptional bursting. Moreover, our discovery has a fundamental implication for phenotypic variability and disease penetrance as well as severity.

Program/Abstract # 155

Molecular mechanisms underlying sex determination and reprogramming in the mouse

Sekido, Ryohei; Lovell-Badge, Robin (MRC National Institute for Medical Research, UK)

In mammals, sex relies strictly on the presence of the Y chromosome-linked testis-determining gene *Sry*. In mice, its transient expression between E10.5 and E12.5 triggers the differentiation of Sertoli cells from the supporting cell precursor lineage, which would otherwise give granulosa cells in ovaries. Our work has shed light for the first time on the role of SRY in the direct activation of *Sox9* expression. SRY synergistically acts with NR5A1/SF1 through a testis-specific enhancer of *Sox9* (TES) to promote Sertoli cell differentiation. SOX9 expression, once activated, is maintained in Sertoli cells throughout life.

On the other hand, ovarian development is established by an active repression of the testicular pathway rather than it depending entirely on a passive default pathway. For example, FOXL2 directly binds to TES and represses its enhancer activity in granulosa cells. Therefore, de-repression of TES activity by *Foxl2* ablation in XX mice causes somatic sex reprogramming and eventually transdifferentiation of ovaries into testes. To elucidate the intrinsic differences between Sertoli cells and granulosa cells by characterising gene expression profiles, we have taken advantage of the transgenic mouse lines expressing the *ecfp* reporter gene, which allow pure populations of Sertoli cells and granulosa cells to be isolated by FACS. We have identified a number of genes showing sexual dimorphic expression by RNA-seq analyses, and are currently investigating the role(s) of those genes in supporting cell differentiation.

Program/Abstract # 156

Systematic Identification of Ftz and Ftz-F1 Responsive Target Genes and Their Enhancers

Field, Amanda; Anderson, Ray; Xiang, Jie; Pick, Leslie (College Park, USA)

Fushi tarazu (Ftz) and its obligate cofactor Ftz-F1 cooperatively bind to DNA and co-regulate the transcription of genes responsible for segmentation in the early embryo. Ftz is a homeodomain protein which binds DNA promiscuously, whereas Ftz-F1 is an orphan nuclear receptor with a well characterized DNA binding sequence. More information is needed about the binding site before targets can be found computationally. Towards this end, a microarray analysis was performed comparing blastoderm/gastrulation stage wild type and *ftz-f1* mutant embryos. This resulted in a short list of candidate targets that were downregulated in the absence of Ftz-F1 protein. The microarray data was combined with the blastoderm staged ChIP-chip data sets, generated by the BDTNP, showing where Ftz protein is bound in the genome. This combination produced a testable list of novel candidate Ftz/Ftz-F1 target enhancers near the genes of interest from the microarray. To test whether these regions correspond to Ftz/Ftz-F1-dependent enhancers, reporter genes were constructed in which these genomic regions are fused upstream of a basal promoter and *E. coli lacZ*. Reporter gene expression were analyzed in wild type, *ftz* and *ftz-f1* mutant transgenic *Drosophila*. Once Ftz-responsive enhancer regions are well defined, this will be used to computationally extract the code for Ftz/Ftz-F1 DNA binding.

Program/Abstract # 157

E74A overexpression induces BhC4-1-lacZ expression in the salivary gland of transgenic *Drosophila*

Monesi, Nadia (FCFRP-USP, Brazil); de F. Oliveira, Lucas; G. Sanchez, Danilo (Ribeirao Preto, Brazil)

In transgenic *Drosophila* the sciarid *BhC4-1* gene expression constitutes a late response to the increase of ecdysone titers that triggers metamorphosis. Previous studies, which employed a loss of function approach, have shown that *BhC4-1-lacZ* expression in the salivary gland is severely reduced in the absence of E74A, a transcription factor directly induced by ecdysone. To verify if the overexpression of E74A is sufficient to induce *BhC4-1-lacZ* expression in the salivary gland we have crossed the *P[hs-E74A]* line, which expresses E74A after heat shock treatment, with transgenic lines containing different fragments of the *BhC4-1* promoter region. Early third instar larvae were selected from the progeny and heat shocked for 30 minutes at 35 C, followed by recovery at 25 C during 6 hours. In *BhC4-1-lacZ* transgenic lines reporter gene expression in the salivary glands is initially detected in 0h prepupae. The overexpression of E74A induced *BhC4-1-lacZ* expression in the salivary gland of third instar larvae in transgenic lines from both the (-186/+40) and (-186/-58) series, indicating that E74A overexpression is sufficient to induce gene expression in the salivary gland. The results also suggest that E74A interacts with the (-186/-58) fragment, which corresponds to the salivary gland enhancer, previously mapped in the *BhC4-1* promoter region. *BhC4-1* expression was not induced in larvae from the (-3314/+40) series, suggesting that the (-3314/-187) fragment contains sequences that repress E74A activity in the *BhC4-1* promoter. We are currently using a one-hybrid approach to investigate if the interaction between E74A and the *BhC4-1* salivary gland enhancer occurs in a direct or indirect manner. Financial support: PRP-USP, CNPq, FAPESP.

Program/Abstract # 158

Quantitative and simultaneous determination of the transcriptional dynamics of two promoters at single cell level by bioluminescent reporters

Fuentes-Jiménez, Daniel (Instituto de Biotecnología, Mexico); Ohmiya, Yoshihiro (Biomedical Research Institute, Japan); Covarrubias, Luis; Wood, Christopher D. (Instituto de Biotecnología, Mexico)

Gene expression involves stochastic mechanisms that generate noise on the phenotype. Variation in gene expression is observed between individuals of the same cell population or even inside the same cell, in the case of interallelic variation. Stochastic natural variation and experimental observational error are also factors that must be taken into account in the study of phenomena at the single cell level - details generally masked when the phenotype is observed in cell-populations as whole. As a method to report gene expression, bioluminescent assays are gaining ground because of their sensibility, robustness and broad linearity. Insect luciferases have been used in single and dual-spectra assays on cell populations, and firefly luciferase has been used in single-cell assays in studies related to noise in gene expression. However, the intracellular behaviour and characteristics of diverse luciferase proteins are neither well characterised nor optimised for registering transcriptional dynamics over differing time scales (minutes to weeks). We are stablishing a dual-spectra bioluminescence assay for studying gene expression in single cells, with titratable reporter protein half-lifes, starting with click-beetle luciferases CBG99 and CBR, with green and red light emission respectively. To test their dynamic response we expressed them with the promoters of cell-cycle genes fos and cyclin A2. We compare the intensity and half-life of these and two other luciferases, ELuc and Luc2. To improve their dynamic response we are exploiting a titratable constituively-active-ubiquitin concatemer system fused to luciferase N-terminus. We thank the help provided by DGAPA (IN223810) and CONACYT-SEP (132478).

Program/Abstract # 159

Characterization of murine KLF10 5'-flanking region

Lee, Woon Kyu; Kim, Dong Hwa; Jang, Hae Jung; Jung, Jae Hun (Incheon, Korea,)

Krüppel-like factors (KLFs) are highly related zinc-finger proteins that are important components of the eukaryotic cellular transcriptional machinery and has been known as having an important role in cell proliferation, apoptosis, differentiation, and

neoplastic transformation. KLF10 firstly identified as an early induced gene in human fetal osteoblast cells (hFOB) after TGFβ stimulation and mimics TGFβ activity in many cell types. Even though it's possible functionality including tumor suppressor, the basal transcriptional regulation mechanisms of this gene is still unknown. Here, we cloned 2.1kb 5'flanking region of KLF10 and characterized it based on the basal level of transcription. The luciferase reporter assay showed that the promoter of mTIEG1 gene at positions from -101 to +68 played an important role in the basal promoter activity of mTIEG1 gene. This region (-101/+68) contained the transcriptional factor binding motif, one JunB and two Sp1 sites. Mutations at the JunB binding site, but not at the two Sp1 caused a dramatic decrease in the luciferase as well as fluorescence report activity. An electrophoretic mobility and super shift assays revealed that JunB protein specifically bound to this promote region. Taken together, our results suggest that the JunB but not Sp1 at mTIEG promoter may function as a positive regulatory factor for the basal transcriptional activity of mTIEG1 gene.

Program/Abstract # 160

Identification of Ryk target genes in regulating *Xenopus* gastrulation

Shin, Eun-Young; Park, Edmond Changkyun; Kim, Gun-Hwa (Korea Basic Science Institute, Korea)

Ryk is a member of atypical receptor tyrosine kinase family that consists of an extracellular WIF (Wnt inhibitory factor) domain, an intracellular atypical kinase domain, and a PDZ binding motif at C-terminus. Ryk has previously been shown to regulate canonical Wnt/β-catenin signaling by directly binding to Wnt ligands and Dishevelled. A recent study showed that Ryk also regulates noncanonical Wnt pathway in convergent extension (CE) movements by promoting Wnt11-mediated endocytosis. However, downstream of Ryk signaling during *Xenopus* embryogenesis is less well characterized. In this study, we report possible target genes of Ryk signaling that may regulate *Xenopus* gastulation. To identify target genes of Ryk signaling pathway, microarray analysis was performed with (1) uninjected, (2) Ryk mRNA overexpressed and (3) Ryk MO injected dorsal marginal zone tissues on Affimatrix GeneChip *Xenopus laevis* Genome 2.0 Array. We found 245 genes were upregulated in Ryk overexpressed tissues and downregulated in Ryk depleted tissues at 1.5-fold or above. GO annotation analysis showed that these genes are mainly associated with cell adhesion and movements. It strongly suggests that these genes may contribute to the regulation of *Xenopus* gastrulation.

Program/Abstract # 161

Identification of cellular and molecular mechanisms regulated by Sonic Hedgehog/Gli2 signaling during cerebellum development

Wojcinski, Alexandre; Joyner, Alexandra (Sloan-Kettering Institute, USA)

The cerebellum (Cb) is a foliated posterior brain structure involved in coordination of motor movements, motor learning, balance, and cognition. During postnatal development, the Cb undergoes rapid growth driven by proliferation of granule cell precursors (GCPs) in the external granule cell layer (EGL). GCPs then differentiate into granule cell (GC) neurons and migrate to the inner granule cell layer (IGL). Although it is known that Sonic Hedgehog (Shh) signaling through Gli2 is required to generate a sufficient number of cerebellar GCPs and formation of the lobules of the cerebellum (Corrales, *Development* , 2006), the molecular mechanisms involved in these processes are still poorly understood. In order to gain a more detailed understanding of how HH regulates production of GCPs, we analyzed the effect of altering HH signaling on GCP behaviors. Surprisingly, our results showed that inactivation of *Gli2* in a large proportion of GCPs at an early stage using an *Atoh1-Cre* driver is not sufficient to dramatically change the shape and the size of adult cerebellum. A more detailed analysis at different time points revealed that WT cells compensate for an early loss of mutant GCPs and then over proliferate as part of a compensation mechanism. In addition, we found that different areas of the cerebellum (vermis vs hemispheres) respond differently to a decrease or increase of HH signaling. We are testing which cells can be the source of cells that replenish the EGL in *Gli2* mutants by sensing the loss of GCPs and compensating by moving into the EGL and becoming GCPs that highly proliferate. Our results suggest there are feed back loops to ensure the normal proportion of each cell type is produced during cerebellum Development.

Program/Abstract # 162

The retina influences Wnt signaling and growth in the optic tectum

Rouse, Hannah (University College London, UK), Cerveny, Kara (Reed College, USA); Wilson, Steve (London, UK)

In the zebrafish visual system, the retina and optic tectum grow in register throughout life. Both regions add new neurons from proliferation zones at their margins with newly differentiated retinal ganglion cells projecting to, and making new synapses in the tectum. In situations where an eye is surgically removed, the contralateral side of the tectum is reduced in size. However, the mechanisms by which the retina affects growth in the optic tectum and/or survival of tectal cells are currently unclear. We have used unilateral enucleation to assess if and how growth in the optic tectum is affected when retinal input is abrogated. To determine the effect on the tectum, we developed a method to extract RNA from fixed half brains for use in both qPCR and for microarray analysis. We find that when one eye is removed early in development, larval zebrafish show both decreased expression of Wnt pathway genes axin2 and lef1 and a decreased level of neurogenesis in the uninnervated optic tectum. These results show that retinal input has a significant impact on proliferation in the optic tectum and on tissue-specific regulation of the Wnt pathway signaling in the tectal proliferative zone.

Program/Abstract # 163

Size regulation of dorsal root ganglia occurs in axolotls with an undersupply or an oversupply of neural crest

Zarzosa, Ana Lucia; Grassme, Kathrin; Taniguchi, Yuka; Tanaka, Elly; Epperlein, Hans-Henning (TU Dresden, Germany)

The attainment of the final size of tissues and organs is a remarkable example of regulative development, however, it is poorly understood. Our work provides evidence for a size regulation mechanism in developing axolotls when the Neural Crest (NC) is experimentally altered. In larvae (stage 41; 1 cm) that had received half (1 neural fold) or double the normal NC complement (4 neural folds) Dorsal Root Ganglia (DRG) were correspondingly smaller or larger. Strikingly, at a juvenile stage (5 cm), DRG in both cases had regulated their sizes to those in controls. To gain insight into size regulation processes we performed further grafting experiments and analyzed DRG size at intermediate timepoints. At 1.5 cm animal length, DRG from 1 neural fold larvae are still smaller (~30%) than control DRG, but attain full compensation at 2 cm. Preliminary data suggests there might be a difference in the number and proportion of proliferative and neurogenic, but not gliogenic cell divisions in this group in order to achieve compensation. In 4 neural fold animals of 2 cm length DRG are still bigger and show a significant increase in apoptosis relative to controls. It is possible that in animals with the double amount of NC, regulation would be coordinated by interactions with the target tissue. In this group, limb development seems to occur faster, while body length increases slower in comparison to controls. This data suggests an interaction between local and systemic signals to regulate the size of tissues in a proportional way. We conclude that overall size control of DRG in animals with an initial undersupply or oversupply of NC seems to be achieved at different rates and through different mechanisms for each case.

Program/Abstract # 164

Withdrawn

Program/Abstract # 165

JAIBA, a class II HD-ZIP transcription factor involved in the regulation of meristematic activity and important for correct gynoecium and fruit development in Arabidopsis

Zuñiga, Victor; Marsch Martinez, Nayelli; de Folter, Stefan (LANGEBIO, CINVESTAV-IPN, Mexico)

A novel transcription factor involved in fruit development in Arabidopsis was identified during a screen based on a fruit development expression profile study. This gene belongs to the class II HD-ZIP family. The JAIBA (JAB) transcription factor is necessary for proper male and female organ development and, together with CRABS CLAW, acts in the floral meristem determination process and in gynoecium marginal tissue development. Furthermore, the JAB gene has three splicing variants which encode for three proteins that could be functional. These JAB proteins interact with transcription factors involved in processes like meristematic activity, floral meristem determinacy and fruit development. Interestingly, these splicing variants have different promoter regions that confer differential expression patterns.

Program/Abstract # 166

Withdrawn

Program/Abstract # 167

A transfer RNAs (tRNAs) post-transcriptional modification and cell growth in Drosophila

Rojas, Diego; Glavic, Alvaro (University of Chile, Santiago, Chile)

tRNAs are present in all living organisms and are a fundamental part of the translation machinery. They are chemically-modified in specific conserved positions. Threonylcarbamoyl-adenosine (t^6A) is a universally conserved modification occurring at position 37 in tRNAs that pair A-starting codons, as the initiator tRNA ($tRNA_i^{Met}$). In yeast it is synthetized by Sua5 and Kae1 and its presence is required for proper codon recognition and translation accuracy, thus mutant for sua5 and kae1 present a slow-growth phenotype. It has been shown that $tRNA_i^{Met}$ is a limiting factor for growth in mammals and *Drosophila*. Therefore we wondered if this modification could be limiting for growth as well. Using the Gal4/UAS system we manipulated the levels of kae1 ubiquitously or in a tissue-specific fashion. Its ubiquitous knockdown caused pupae lethality and also apoptosis in wing imaginal cells when silenced locally. On the other hand, ubiquitous overexpression of kae1 generated larger animals constituted by larger cells. This enlargement can be partially prevented by reducing Kae1 or the methionine-tRNA synthetase, showing that growth effects are caused by the increase in Kae1 and related with the synthesis of the methionine-charged tRNAs. Additionally, silencing of Kae1 in the fat body caused smaller animals, however its overexpression did not generate larger animals, suggesting that the overgrowth produced by ubiquitous overexpression is not caused by the systemic hormonal growth control mechanism that involve the insulin-like peptides, but instead by a cell-autonomous phenomenon. Now we are studying the relationship between tRNAs, t6A and TOR kinase activity. Funding: FONDECYT 1100366, FONDAP 15090007, CONICYT AT 24121519

Program/Abstract # 168
Characteristic patterns of the incorporation of BrdU during DNA replication in different cells of the seminiferous epithelium of the rat
Ortiz, Rosario; Muñoz, Israel; Echeverría, Olga; Vazquez-Nin, Gerardo (Universidad Nacional Autonoma de Mexico, Mexico)
In the seminiferous epithelium we study the morphologic characteristics of DNA replication in rat spermatogonia and spermatocytes in preleptotene by means of immunodetection of the incorporation of bromodeoxiuridin (BrdU) during 30 min until 24 hours. The results show three patterns of labeling independently of their age. One characterized by foci, localized primarily to the periphery of the nucleus of spermatogonias type A and B, a second pattern in which the label is distributed in all the nuclear volume of preleptotene spermatocytes and finally, a pattern present in spermatocytes in leptotene, in which the label is restricted to only a region located anywhere the nucleus. These incorporation patterns can be used to identify the different cells types of the germinal line in phase S, which otherwise is particularly difficult during puberty. This work was supported by the grant : PAPIIT IN203308 UNAM

Program/Abstract # 169
Immunodetection of SYCP1 and SYCP3 during the first spermatogenic wave Wistar rat
Valenzuela, Yunuen; Ortiz, Rosario; Echeverría, Olga; Vazquez-Nin, Gerardo (Universidad Nacional Autonoma de Mexico, Mexico)
The primary spermatocytes have an exclusive structure called synaptonemal complex (SC), which is essential for the synapsis of homologous chromosomes. The SYCP1 (Synaptonemal Complex Protein 1) and SYCP3 (Synaptonemal Complex Protein 3) are involved in the assembly of the SC. It has been shown that the absence of any of the proteins constituting the SC causes alterations that lead to cell death. The aim of the present work is to characterize the distribution of SYCP1 and SYCP3 by means of immunodetections at light microscope level in prepubertal Winstar rats. In 13 days old rats labeling for SYCP3 is present in cells in preleptotene and leptotene stages. In 16 days old rats increases the number of cells positive to SYCP3 and appear cells positive to SYCP1, indicating the presence of zygotenes and early pachytenes. In 27 days old rats late pachytene and early spermatids are also present. In 16 and 27 days old rats some primary spermatocytes has an atypical distribution of SYCP3 labeling in the nuclear periphery. This distribution of the SYCP3 labeling may indicate alterations in the SC assembly that could lead to apoptosis of primary spermatocytes. This work was supported by the grant : PAPIIT IN203308 UNAM

Program/Abstract # 170
Cell death in atretic granulosa cells
Escobar, Maria; Vazquez-Nin, Gerardo; Casasa, Sofia; Garcia, Gethsemany; Echeverría, Olga (Universidad Nacional Autonoma de Mexico, Mexico)
Follicular atresia is the process by which most of the follicles existing in the ovary at birth are lost. Apoptosis cell death is involved in the follicular atresia in mammals, especially in granulosa cells. However we have observed that in all stages of atresia, numerous granulosa cells have characteristics different to the classical apoptosis. The aim of the present work is to characterize the process of cell death of granulosa cells during the atresia of adult rats. We have employed a detailed ultrastructural analysis, correlated with TUNEL assay as well as active caspase-3, LC3 and Beclin-1 immunolocalizations. We also evaluated the mRNA of Caspase-3 and LC3 by RT-PCR, as well as active caspase-3 and LC3 by Western Blot analyses. DNA fragmentation was demonstrated by means of electrophoreses in agarose gel. Our ultrastructural, immunohistochemical and molecular results demonstrate that granulosa cells death involves molecular mechanisms belonging to apoptosis and autophagy cell death, and in lesser degree cells are eliminated by both types of cell death simultaneously. This work was supported by the grant of CONACYT 180526.

Program/Abstract # 171
TACC3 is a crucial protein for bovine oocyte meiosis
Mahdipour, Mahdi; Leitoguinho, Ana Rita; Zacarias, Ricardo; Luteijn, Maartje; Van Tol, Helena; Ketting, Rene; Roelen, Bernard (Utrecht, Netherlands)
Transforming acidic coiled– coiled (TACC) proteins belong to a cluster of proteins associated with various cancers. Mammals express three TACC proteins: TACC1, TACC2 and TACC3 coded by three genes. TACC3 deficient mice displayed embryonic lethality, reduced cell number and mitotic defects which suggest an important role for this protein during early cell division. Although d epletion of TACC3 during meiotic oocyte maturation in mice resulted in inhibition of polar body extrusion and arrested meiosis I, its function during mammalian meiosis remains relatively unknown. In this project, with the help of quantitative RT-PCR we perceived that TACC3 transcript was expressed during bovine oocyte maturation and early embryo development until the 8 cell stage but decreased to non-detectable levels at the morula and blastocyst stages. Whole mount immunofluorescence on oocytes revealed that

TACC3 was localized around the DNA at metaphase I and II stages, concentrated tightly to the spindles, surrounding the chromosomes. Levels of TACC3 expression reduced after fertilization and TACC3 was undetectable after pronuclei formation. In addition, TACC3 expression was monitored after inhibition of Aurora-A kinase which is a protein proposed to phosphorylate TACC3 for its proper recruitment on the microtubules. After Aurora-A kinase inhibition, formation of meiotic spindle and chromosome alignment became impaired, polar body extrusion of oocytes was reduced and as progression of meiosis was distorted, oocytes exhibited reduced developmental competence. It is concluded that TACC3 has a direct function in oocyte meiosis and proper spindle assembly.

Program/Abstract # 172

Morphologic and molecular markers indicate developmental maturation-competence of mouse and human oocytes

Levi, Mattan (Tel Aviv University, Israel); Shulman, Adrian; Ghetler, Yehudith (IVF Unit, Meir Medical Center, Israel); Shalgi, Ruth (Tel Aviv University, Israel)

Background: Developmental maturation competence of mouse oocytes is correlated with central germinal vesicle (GV) and regulated by microtubules (MTs) and the presence of a chromatin ring. Fyn kinase is localized at the spindle and cortex of mouse oocytes and regulates oocyte maturation and MTs stabilization. The aim of the current study was to examine whether the position of the GV in mouse and human oocytes correlates with molecular and morphologic parameters as well as with maturation-competence. Methods: The spatial localization of GV and nucleolus, presence of a chromatin ring, localization of Fyn, MTs density and oocyte maturation were assessed in 153 human oocytes, 418 oocytes of young mice (2 months old) and 146 oocytes of old mice (12 months old) by confocal laser scanning microscope or differential interference contrast optics. Results: GV location was peripheral and age-independent in most of the human oocytes but age-dependent in mice oocytes; it was central in most of the young-mice oocytes and peripheral in most of the old-mice oocytes. Central GV, whether in human or mouse oocytes, correlated with central nucleolus, absence of Fyn at the GV, dense MTs network and high maturation competence; whereas peripheral GV correlated with peripheral nucleolus, presence of Fyn at the GV, flimsy MTs network and low maturation competence. No correlation was observed between GV position and presence of chromatin ring. Conclusion: Our results suggest that the central location of GV within the oocytes, absence of Fyn at the GV and presence of thick filamentous MTs at the ooplasm, can serve as predictors of successful developmental maturation and provide new insights for clinical in vitro maturation treatment.

Program/Abstract # 173

Oct4 is a useful marker for understanding PGC migration in Monodelphis domestica

Wright, Amelia; Cruz, Yolanda (Oberlin College, USA)

No satisfactory method or reliable marker currently exists for detecting primordial germ cells (PGCs) in marsupial embryos. Oct4, a cellular pluripotency marker, has been used extensively in mouse studies to track PGC migration during embryonic development. Oct4 is expressed in mouse tissues throughout prenatal development, but is restricted to the inner cell mass (ICM) at the blastocyst stage and to the epiblast after implantation. PGCs are specified from the epiblast at E7.0 and remain pluripotent through adulthood. We tested the hypothesis that Oct4 could be used as a PGC marker in the lab opossum, *Monodelphis domestica*, to track PGC migration in marsupial embryos. *M. domestica* is an excellent marsupial model for studying PGC migration due to its short gestation period and accessible embryos that lie free in the uterine cavity until day 13 of a 15-day gestation. Avidin-biotin-enhanced immunohistochemical analysis was used to detect Oct4 in opossum embryos of equivalent developmental stages to mouse E7.0 through E9.0. Our results indicate that Oct4-positive cells are detectable in day-10 through day-12 opossum embryos, in precisely the regions where PGCs would be located in developmentally equivalent stages of mouse. Thus, Oct4 appears to be a useful marker for tracking the migration of marsupial PGCs during embryogenesis. More importantly, our findings are consistent with the emerging role of Oct4 in maintenance of cellular pluripotency in the mammalian germ line.

Program/Abstract # 174

Oct60 is involved in the PGC formation as a germplasm component

Morichika, Keisuke; Shimada, Keigo (Rikkyo University, Japan); Kubo, Hideo (Tokyo Metropolitan Institute of Medical Science, Japan); Kinoshita, Tsutomu (Rikkyo University, Japan)

Primordial germ cells (PGCs) are germline cells that are specified from early embryogenesis. In mammal, Oct3/4 is expressed ubiquitously after fertilization. The expression of Oct3/4 is restricted in the inner cell mass at blastocyst stage, and thereafter detected only in PGCs. It is known that Oct3/4 conditional KO mouse showed depletion of germ cells. Oct3/4 belongs to transcriptional factor of POU family class V. In *Xenopus*, three POU class V genes, Oct60, Oct25 and Oct91 are expressed in different manner. Oct60 is maternally expressed in oocyte, but their role in PGC formation remains unknown. In this study, we examined a role of Oct60 in PGC formation. In order to examine the gene expression of PGC, synthesized RNAs of Venus-DEADSouth 3'UTR were injected into vegetal cortex of one-cell stage embryo, and GFP-positive PGCs were isolated form dissociated cells of endodermal region. RT-PCR analysis showed that among three POU-V genes, Oct60 is specifically expressed in PGC. Immunocytochemical examination demonstrated that Oct60 protein is localized in germ plasm and PGCs after gastrulation. To clarify the role of Oct60 in PGC formation, we injected mRNA of Oct60 into vegetal cortex of one-cell-stage embryo. Overexpression of Oct60 caused the up-regulation of PGC marker gene DEADEnd1 and the down-regulation of pan-endodermal marker Sox17α at gastrula stage. In addition,

truncated form of Oct60 disturbed PGC migration within the endoderm toward the genital ridge at tailbud stage. These results suggest that Oct60 plays the important role in PGC formation.

Program/Abstract # 175

Paracrine signalling as intraovarian regulator during *Xenopus laevis* oogenesis

Serrano, Maria de los Angeles; Luque, Melchor Emilio; Sánchez, Sara Serafina (INSIBIO - CONICET, Argentina)

Oogenesis in vertebrates has been well characterized as the result of a harmonic regulation between different signaling systems such as endocrine and juxtacrine . Throughout the last decade, paracrine factors like the bone morphogenetic proteins (BMPs) have been identified as fundamental for the correct progression of oogenesis. In this sense, BMPs have become integrated into the whole oogenesis process as intraovarian regulators. Not much is known about paracrine regulation during oogenesis in oviparous vertebrates, where the process has the additional complexity of the delicately controlled vitellogenesis. This is characterized by hepatic production of the glycoprotein vitellogenin (VTG) that is transported via the bloodstream to the ovary where it is incorporated by oocytes. It is known that in the amphibian ovary the three different stages previtellogenesis, vitellogenesis and maturation are regulated by endocrine and juxtacrine, mediated by gap junction, signaling. In the present study, we examine the participation of BMP factors during *Xenopus laevis* previtellogenesis and vitellogenesis. To analyse the participation of paracrine signaling, we first determined the gene expression of the different members of the BMP family through oogenesis. To determine a functional role of the paracrine factors and a possible interaction with endocrine and juxtacrine signaling, we performed different assays with the BMP pathway inhibitor LDN-193189. The results showed that BMPs are implicated in *Xenopus laevis* oogenesis, a complex process coordinated by a cross-talk between the molecules of different signaling pathways.

Program/Abstract # 176

Effects of glyphosate on structure and SF-1 expression in ovaries of zebrafish Danio rerio

Ammar, Dib; Armiliato, Neide; Nazari, Evelise; Nezzi, Luciane; Straliotto, Marcos; Müller, Yara (Universidade Federal de Santa Catarina, Florianópolis, Brazil)

Glyphosate is non-selective herbicide and highly soluble in water, and when applied in terrestrial systems, it percolates and infiltrates into the soil, eventually reaching the aquatic community and consequently affecting non-target organisms. Aiming to better understand the toxicity of glyphosate to female germ cells of the zebrafish Danio rerio , this study evaluated the structure, ultrastructure and expression of steroidogenic factor 1 (SF-1) in ovaries exposed to 65 µg/L of glyphosate [N-(phosphonomethyl) glycine] for 15 days. No morphological changes were recognized in the ovaries of exposed and non-exposed females. However, a significant increase in the diameter of previtellogenic and vitellogenic oocytes was observed after exposure to glyphosate. When we analyzed the ovarian ultrastructure, we observed, in the cortical regions of the oocytes, the presence of concentric membranes, appearing as myelin-like structures, associated with the external membranes of mitochondria, and also with the yolk granules. Also, a more significant immunolocalization of SF-1 was found in cortical yolk granules of *D.rerio* , after exposure to 65 µg/L of glyphosate. The subtle noxious effects of glyphosate on oocytes demonstrated here are a serious concern for fish reproduction and must be taken into account. These results contribute to the understanding of glyphosate toxicity to non-target organisms, showing subcellular and molecular impairments that affect the reproduction of female fish.

Program/Abstract # 177

Dynamic expression patterns of RaVasa during oogenesis and early embryonic development in *Rhynchosciara americana*

Rezende-Teixeira, Paula; Machado-Santelli, Glaucia (Universidade de São Paulo, Brazil)

The Dipteran *Rhynchosciara americana,* a native Brazilian insect, provides an interesting opportunity to study a different model of insect oogenesis and embryogenesis. The ovarian development in *R.americana* presents unique characteristics: the synchronic development of the ovarian follicles and only one giant nurse cell connected with the oocyte through ring canal. The last mitotic division of undifferentiated germ cells occurs early in its larval life. Each oogonium gives rise to two cells, with different fates: the oocyte and the nurse cell.

The initial analysis of sequences of a cDNA library constructed with poli A+ RNA of ovary from different ages of *Rhynchosciara americana* larvae showed messages related to different molecular functions and biological process. In the present work we isolated a *Rhynchosciara* homolog of vasa (*RaVasa*) and examined the spatial and temporal expression of *RaVasa* mRNA during the gonad and embryonic development. *RaVasa* mRNAs accumulated in ovarian follicles of *Rhynchosciara americana* in young larva ovary and adult fly ovary suggesting that *RaVasa* is maternally contributed to the *Rhynchosciara americana* eggs. Furthermore, the *RaVasa* protein is localized to the posterior pole of the egg and then incorporated into pole cells and remained expressed in germ cells throughout developmental stages. The molecular structure showed the presence of conserved domains and the *RaVasa* gene encodes an ATP-dependent RNA helicase of the DEAD box protein family. This protein is specifically expressed in the germ cell lineage and is required for multiple processes in the development and maintenance of primordial germ cells.

Program/Abstract # 178

A Transcriptome Analysis of Animal and Vegetal Half-Embryos of the Penaeid Shrimp *Marsupenaeus japonicas*

Hertzler, Philip Lamar (Central Michigan Univ, USA); Trewin, Carolyn; McWilliam, Sean; Sellars, Melony (CSIRO, Australia)

Penaeid shrimp represent an important global food resource, yet little is known of their basic developmental biology. To find genes involved in early development, *M. japonicus* embryos were manually separated at the 2-cell stage and allowed to develop to the time when controls had reached the limb bud stage. Animal and vegetal half-embryos were pooled; total RNA was isolated, converted to cDNA, and amplified for Ion Torrent sequencing. This resulted in 472,512 reads from vegetal half-embryos and 487,511 reads from animal half-embryos which were assembled as contigs and computationally subtracted from each other, producing 41,567 vegetal and 46,781 animal contigs. The transcriptome libraries were BLAST'd for selected developmental toolkit genes. Genes found included the sex determination genes *sex lethal* and *transformer-2* (both transcriptomes); potential germ line genes *ddx3x* (vegetal transcriptome only), *ddx17*, *mex-3* (animal transcriptome), *argonaute 1*, *ddx5*, *pumilio*, *SmB* , and *staufen* (both); mesoderm transcription factors *brachyury*, *twist* (vegetal), *mef2* , and *snail1* (both); the axial or segmentation genes *deformed*, *engrailed*, *hunchback*, *wingless/wnt-8* (vegetal), *kruppel*, *orthodencticle* (animal), *β-catenin*, *distal-less* , *giant* , and *hairy* (both). Other genes found included *par-6*, *trithorax*, *myosin*, *troponin C1* and *I* (vegetal), *cyclins A*, *B*, *I*, *polycomb* (animal), *gsk3*, *notch2*, *par-1* , and *retinoblastoma-1* (both). Contigs in each transcriptome were also ranked by number of repeats, indicating increasing probability that the contig was unique to one transcriptome and not the other. The sex determination, germ line, and mesoderm genes were examined more closely for developmental expression by qPCR. The results will be reported.

Program/Abstract # 179

Comprehensive screening of sexualization-induced genes in planarian

Matsumoto, Midori; Ueda, Kento; Takagi, Souta (Keio Univesity, Japan); Yoshitake, Kazutoshi; Gojyobori, Takashi (National Institute of Genetics, Japan)

The reproductive mode is classified into asexual reproduction and sexual reproduction, which have merit and demerit respectively. And there are some species, which change reproductive mode in order to produce offspring suitable for the environment. The planarian *Dugesia ryukyuensis* reproduces both asexually and sexually, and can switch from one mode of reproduction to the other. We have established the experimental method, which can change asexual reproduction to sexual reproduction in *D. ryukyuensis*, by feeding with the putative sexualizing substance contained in *Bdellocephala brunnea*. The sexualization process is divided into five distinct stages by histological changes and it has a point-of-no-return after which the worms could spontaneously develop sexual organs without feeding further with *B. brunnea*. In this study, we tried to identify the sexualization-induced genes by RNAseq, Real Time-PCR and RNA interference (RNAi). By this comprehensive screening, we selected five candidate genes for sexualization-induced genes. They increased or decreased the expression levels in the early stage of the sexualization. One candidate gene (C.3604) of them was annotated to *Schimidtea mediterranea* prohormone convertase 2 (PC2). By RNAi during sexualization, the knockdown of C.3604 did not develop sexual organs like ovary, testis, genital pore and copulatory apparatus. Therefore, it has been suggested that C.3604 has crucial role for producing the sexualizing substances and inducing sexualization.

Program/Abstract # 180

Meiotic chromosome behavior in the triploid planarian: Function of rad51 homolog in gametogenesis

Chinone, Ayako; Matsumoto, Midori (Keio Univesity, Japan)

It is generally assumed that triploid organisms reproduce only asexually, because they tend to produce aneuploid gametes due to problems of chromosomal pairing and segregation during meiosis. However, triploid individuals of *Dugesia ryukyuensis* can reproduce bisexually. They develop hermaphrodite sexual organs by artificial feeding of sexually matured planarians, produce functional gametes and begin reproducing by copulation. The previous report showed that meiosis occurs in both male and female germline. In this study, we observed the meiotic chromosomes in both germlines in the triploid planarian. The male germ-line cells are likely to eliminate haploid set of chromosomes before onset of meiosis, and then haploid sperms are exclusively produced. On the other hand, the female germ-line cells appear to stay triploid until meiotic prophase I, and then both haploid and diploid eggs are produced. To investigate the detailed processes and mechanisms of meiosis in both germline, we focused on *rad51* gene, which is known to assist in repair of DNA double strand breaks and be involved in pairing of homologous chromosomes during meiotic prophase I. We cloned a *rad51* ortholog (*Dr-rad51*) in *D. ryukyuensis* and found that *Dr-rad51* was expressed in oocytes and spermatocytes of triploid sexualized planarian. During the sexualizing process, *Dr-rad51 (RNAi)* worms failed to develop ovaries and delayed to develop testes but somatic sexual organs were unaffected. In addition, it appears that pairing of chromosomes was failed in oocytes of *Dr-rad51 (RNAi)* while sperms are produced normally. These results suggest that recombination regulated by Rad51 is necessary for at least oogenesis.

Program/Abstract # 181

Claudins are essential regulators of morphogenesis

Ryan, Aimee K. (McGill University / RI-MUHC, Canada), Baumholtz, Amanda; Collins, Michelle; Simard, Annie; Khairallah, Halim (McGill University, Montreal, Canada); El Andalousi, Jasmine; Gupta, Indra (RI-MUHC, Montreal, Canada)

Morphogenesis is a highly orchestrated series of events that transforms groups of cells into complex 3-dimensional tissues or organs. There are still large gaps in our understanding of how changes in behaviours of individual cells are coordinated with cellular rearrangements occurring at the level of the tissue. Tight junctions are appropriately positioned to coordinate these behaviours. We

are dissecting the roles of the claudin family of integral tight junction proteins in embryogenesis. Claudins are essential for tight junction formation. Claudin's extracellular loops determine the barrier properties of the tight junction and their C-terminal cytoplasmic tails interact with the tight junction cytoplasmic plaque to bridge the tight junction to the actin cytoskeleton. We have shown that the 17 chick claudins exhibit unique and overlapping expression patterns in the chick embryo. Using gain- and loss-of-function approaches we discovered that members of the claudin family are essential for morphogenetic milestones. Specifically, we found that Cldn-10 is required for asymmetric morphogenesis of the heart tube in chick embryos and that this function is dependent on specific amino acids that are required for its interaction with the actin cytoskeleton. In addition, using the C-terminal domain of the *C. perfringens* enterotoxin to simultaneously remove multiple claudins from tight junctions, we found that claudins are required for neural tube closure, somitogenesis and nephric duct formation. Our preliminary data suggest that claudins are required for apical cell constriction, interactions with the actin cytoskeleton, and proliferation during these morphogenetic events.

Program/Abstract # 182

Asymmetric division of luminal cells drives normal and ErbB2 induced epithelial stratification

Huebner, Robert J. (Johns Hopkins School of Medicine, USA), Lechler, Terry (Durham, NC, USA); Ewald, Andrew J. (Johns Hopkins Medical School Baltimore, USA)

The elongation of mammary ducts during branching morphogenesis is accomplished by terminal end buds (TEBs). TEBs are transient, stratified epithelial structures that arise from, and resolve back to, simple mammary ducts. We sought to identify the cellular mechanism of epithelial stratification and developed 3D organotypic culture and imaging techniques to visualize stratification in real-time. We report that stratification initiates from the asymmetric division of apically located luminal epithelial cells. Each asymmetric division yields one apically-located, polarized daughter cell and an unpolarized daughter located between the apical and basal cell layers. Stratification was not a clonal process, as we observed multiple asymmetric divisions within the same tissue. Internal daughter cells lacked apico-basal polarity and most intercellular junctions. These internal cells were proliferative and collectively migrated to accomplish branching morphogenesis. Importantly, mammary tumors undergo epithelial stratification during hyperplasia formation. We next determined the cellular mechanism of stratification following expression of the oncogene ErbB2. Oncogenic stratification also initiated through asymmetric division of apically located luminal epithelial cells, demonstrating that this cellular mechanism of stratification is conserved in development and disease. However, normal tissue restores a polarized, bilayered architecture at the end of branching morphogenesis while ErbB2 expression resulted in complete loss of polarity and tissue overgrowth. We are currently testing the relative contribution of downstream signaling components in the MAPK and Rac pathways to proliferation, migration, and polarity.

Program/Abstract # 183

Sox9 regulates branching morphogenesis during lung development

Rockich, Briana; Nagy, Melinda; Hyrcaj, Steven; Baker, Nicholas; Wellik, Deneen; Spence, Jason (University of Michigan, USA)

During lung development the highly complex, stereotyped pattern of branches is established by a process called branching morphogenesis. Disruptions in branching morphogenesis can lead to reduced lung function, respiratory distress and death. Many signaling pathways and transcription factors play a role in orchestrating this incredibly complex process. Here we show that the transcription factor Sox9 is an important regulator of branching morphogenesis and the conditional deletion of Sox9 from the lung results in severe developmental defects. In humans heterozygous mutations in Sox9 cause a severe disorder campomelic dysplasia (CD), a severe congenital disorder affecting many systems and tissues including the respiratory system. Many babies born with CD die in the neonatal period due to respiratory distress. The lung defects associated with these mutations are poorly described and understood. Here we show that when Sox9 is conditionally deleted from the lung epithelium, the lungs are hypoplastic and branching morphogenesis is perturbed. Cystic structures form at the branching tips, and are unable to undergo the normal series of bifurcations characteristic of this process. We found that the epithelium of the perturbed branching tips has disrupted cell morphology and does not establish a proper basement membrane. Through linage tracing we found that Sox9 marks the progenitor population of the branching tips; however, Sox9 does not seem to be required for the maintenance of the progenitor population. Taken together, our results suggest that Sox9 is likely to directly regulate branching morphogenesis by controlling cell shape and basement membrane deposition in the branching tips of the developing lung.

Program/Abstract # 184

Wnt9b regulates directed cell movement during kidney tubule diamter establishment

Carroll, Thomas J.; Pan, Xinchao; Schnell, Ulrike; Karner, Courtney (UT Southwestern Med Ctr, USA)

The epithelial tubule is a ubiquitous structure in the metazoan body plan. It is efficiently used as a mechanism to transport solids, liquids and gasses. Optimal function of tubular organs requires that the tubules establish and maintain the proper diameter. Somewhat surprisingly, we still understand relatively little of how this is accomplished. We found that Wnt9b, signaling through a non-canonical, Rho/Jnk pathway, is necessary to establish and maintain tubule diameter in the mouse kidney. Wnt9b mutants develop tubules with greatly expanded diameters, reminiscent of a human disease known as polycystic kidney disease. Although mutant tubules have a significantly increased number of cells making up their cross-sectional diameter, Wnt9b does not affect the number of cells in the tubule. Instead, Wnt9b functions by establishing planar cell polarity of cells within the epithelium. We hypothesize that this polarity is necessary for directed cell movements (similar to convergent extension) that establish the diameter and contribute to the length of the

tubule. Using live imaging of cultured kidneys, we are now characterizing cell movements during diameter establishment and the role of Wnt signaling in these processes.

Program/Abstract # 185

A Novel Non-cholinergic role for Acetylcholinesterase in Gut Morphogenesis

Pickett, Melissa Anne; Nascone-Yoder, Nanette (North Carolina State University, USA)

Acetylcholinesterase (AChE) is a serine protease that hydrolyzes the neurotransmitter, acetylcholine, at cholinergic synapses. In addition to this classical activity, AChE also has non-catalytic functions, particularly during neurite development in the brain. Since the gut develops in close association with the cholinergic enteric nervous system, we hypothesized that AChE may also have classical and/or non-classical functions in gut development. Exposure of *Xenopus laevis* tadpoles to three different chemical AChE inhibitors caused severe gut elongation defects. However, exposure to an acetylcholine agonist, which mimics the increased acetylcholine signaling caused by inhibition of AChE catalytic activity, did not produce gut abnormalities. Additionally, the gut defects elicited by AChE inhibitors could not be rescued by antagonistically blocking acetylcholine receptors. Together, these results suggest that AChE has important non-catalytic roles in gut morphogenesis. AChE shares homology with a family of cell-matrix adhesion molecules and, during neurite outgrowth, the non-catalytic role of AChE is thought to involve its binding to the matrix protein, laminin. Consistent with this mechanism, guts developing in the presence of AChE inhibitors exhibit disorganized neuronal outgrowth and disrupted laminin architecture, accompanied by disorganization of the adjacent smooth muscle. Increased apoptosis in the apposed gut endoderm, the rearrangement of which is necessary to generate gut length, is also elicited by AChE inhibition. These data provide evidence of a previously unrecognized non-classical role of AChE in gut development and increase our understanding of the potential etiology of congenital human gut defects.

Program/Abstract # 186

C-Jun N-terminal Kinase (JNK) maintains tissue integrity during cell rearrangement in the gut

Dush, Mike; Nascone-Yoder, Nanette (North Carolina State University, USA)

Tissue elongation is a fundamental morphogenetic process that generates the proper anatomical topology of the body plan and vital organs. In many elongating structures, tissue lengthening is driven by cell rearrangement, but the mechanisms that modulate intercellular adhesion to allow individual cells to change position without compromising overall structural integrity are not well understood. Although both Rho family GTPases and c-Jun N-terminal Kinase (JNK) are required for tissue elongation, the precise cellular role of JNK in this context has remained elusive. Here, we illuminate the role of JNK activity in the rearrangement of endoderm cells that underlies the elongation of the *Xenopus* gut tube. Whereas Rho kinase is necessary for cell intercalation and adhesive remodeling, JNK is required to maintain cell-cell adhesion and establish parallel microtubule arrays; without JNK activity, the reorganizing endoderm dissociates. Depleting polymerized microtubules phenocopies this effect, consistent with a model in which JNK regulates microtubule architecture to preserve adhesive contacts between rearranging gut cells. Thus, in contrast to Rho kinase, which generates actomyosin-based tension and cell movement, JNK signaling is required to establish microtubule stability and maintain tissue cohesion; both factors are required to achieve proper cell rearrangement and gut extension. This model of gut elongation has implications not only for the etiology of digestive tract defects, but sheds new light on the means by which intra- and intercellular forces are balanced to promote topological change, while preserving structural integrity, in numerous morphogenetic contexts.

Program/Abstract # 187

Integration of L-R Pitx2 transcription and Wnt signaling provides a mechanism for asymmetric gut morphogenesis

Kurpios, Natasza A.; Welsh, Ian; Afonso-Parra, Catalina; Gludish, David; Thomsen, Michael (Cornell University, USA); Bai, Yan; Martin, James (Baylor, USA)

A critical aspect of gut morphogenesis is initiation of a leftward tilt. Failure to do so leads to gut malrotation and catastrophic volvulus. The direction of tilt is specified by asymmetric cell behaviors within the dorsal mesentery (DM), which suspends the gut tube, and is downstream of Pitx2 , the key transcription factor responsible for the transfer of left-right (L-R) information from early gastrulation to morphogenesis. Although Pitx2 is a master regulator of L-R organ development, its cellular targets that drive asymmetric morphogenesis are not known. Using laser microdissection and targeted gene misexpression in the chicken DM, we show that Pitx2 -specific effectors mediate noncanonical Wnt signaling to activate Daam2, a formin protein and itself a Pitx2 target, linking actin dynamics to adherens junctions, to ultimately generating asymmetric cell behaviors. Our work highlights how integration of two conserved cascades may be the ultimate force through which Pitx2 sculpts L-R organs.

Program/Abstract # 188

Polarized collective cell movements drive antero-posterior folding to form the avian hindgut

Nerurkar, Nandan; Tabin, Cliff (Harvard Medical School, USA)

At the end of gastrulation, the endoderm forms the ventral surface of the developing embryo. Subsequently, through a series of poorly understood events, the initially flat endoderm is transformed into the gut tube, a cylindrical structure that gives rise to the epithelial lining of the respiratory and gastrointestinal tracts. This process has been largely assumed to proceed through a lateral-to-medial folding of the endoderm, mirroring primary neurulation in the ectoderm. However, this is directly contradicted by fate mapping

studies in chick and mouse demonstrating that cells near the extreme anterior and posterior poles of the initially flat endoderm translocate by nearly half the embryo's length to arrive at their final position in midgut. In the present work, hindgut formation was observed directly by multi-photon live imaging in the chick embryo. We identified a rapid and collective, polarized movement of endoderm cells into and through the caudal intestinal portal. These cell movements were intrinsic to the endoderm, and essential for hindgut formation. We conclude that the collective posterior flux of endoderm cells along the ventral midline drive antero-posterior folding to form the hindgut, whereby continued addition of cells to the forming hindgut causes a progressive folding into the elongating tail bud. Finally, we identified FGF signaling as required for these polarized cell movements, and ultimately for involution of the endoderm to form the hindgut. Work is ongoing to identify the physical forces that drive this process, and how FGF signaling regulates such forces during early organogenesis of the gut.

Program/Abstract # 189

Gut morphogenesis involves changes in cell shape that require ZFP568 and Hand1

Ulmer, Barbel Maria; Garcia-Garcia, Maria J. (Cornell University, USA)

During early mouse embryogenesis, the elongation and subsequent closure of the definitive endoderm gives rise to the primitive gut tube. Although these events are essential for the formation of the digestive and respiratory organs, relatively little is known about cellular and genetic events mediating the closure of the gut tube. We found that the KRAB-domain zinc-finger protein *ZFP568* and the bHLH transcriptional activator *Hand1* are required for proper gut morphogenesis. In both *Zfp568*^{chato} and *Hand1* mutants the definitive endoderm failed to form a proper foregut and hindgut pocket, did not elongate and did not zip up to close the gut tube. ISH with markers of early gut morphogenesis, such as *Shh*, *Nepn* and *hex1*, revealed that proper regional specification of the gut endoderm takes place in Zfp568^{chato} and *Hand1* mutants, but that these populations fail to translocate to form the foregut and hindgut pockets. We thus hypothesize, that ZFP568 and HAND1 are essential for cell movement and cell shape rather than cell differentiation in the definitive endoderm. Interestingly, we found that the formation of the foregut and hindgut pockets in wild type embryos correlates with changes in the shape of endoderm cells from a squamous to a cuboidal morphology. In contrast, endoderm cells remained squamous in *Zfp568* and *Hand1* embryos. By studying *Zfp568* and *Hand1* mutants, we are currently determining the contribution of these cell shape changes to gut morphogenesis and the underlying molecular mechanisms that control the folding of gut epithelia in vertebrate organisms.

Program/Abstract # 190

ADAMTS9 is a highly conserved protease crucial for gastrulation, left-right symmetry, neurulation, craniofacial development and intrauterine growth

Nandadasa, Sumeda; Nelson, Courtney; Somerville, Robert; Apte, Suneel (Cleveland Clinic Lerner Research Institute, USA)

Little is known about extracellular matrix (ECM) remodeling during gastrulation. We investigated the role of a secreted, but cell-surface bound metalloprotease, ADAMTS9 (A disintegrin and metalloproteinase with thrombospondin motifs 9), known to cleave ECM proteoglycans, using an allelic series. In mice, a unique cup-shaped structure known as the egg cylinder is formed prior to gastrulation, comprising two epithelial cell layers, the ectoderm and the visceral endoderm. The basement membrane between them is remodeled to accommodate the newly formed mesoderm. *Adamts9* was expressed at the onset of gastrulation (E 6.5) in distal ectoderm and the visceral endoderm cells, in the vicinity of the future node. During gastrulation, *Adamts9* expressing cells delaminated from the ectoderm to colonize the newly forming mesoderm. We found that *Adamts9* null embryos $^{(-/-)}$, die around E.7.0 without undergoing gastrulation and showed a disorganized ECM. We used a membrane-targeted, hypomorphic gene trap allele (*Adamts9*^{gt}), and tissue specific Cre recombinase drivers, Sox-2 cre, TTR-cre, and Brachyury (T)-cre, in combination with a floxed *Adamts9* allele (*Adamts9*^{Fl}) to investigate the functions of *Adamts9* in gastrulation and the post-gastrula embryo. Indeed, each of these mutants extended survival past gastrulation, suggesting a crucial role for membrane-bound ADAMTS9 during gastrulation, and distally acting secreted protease in late development. We show that ADAMTS9 is crucial for embryo turning, heart tube looping, neurulation, left-right symmetry, cranio-facial development, melanoblast survival, and for establishing a functional fetal circulatory system derived from the extra embryonic visceral endoderm.

Program/Abstract # 191

chem, a E3 ubiquitin ligase, is required for cell polarity and dorsal closure in *Drosophila melanogaster*.

Zamudio-Arroyo, José Manuel; Riesgo-Escovar, Juan R. (UNAM, Mexico)

chem encodes a putative E3 ubiquitin ligase. Isolated mutations in *chem* are embryonic lethal and have defective dorsal closure. Embryonic dorsal closure occurs towards the end of embryogenesis and is a process whereby the lateral epithelial cells change shape, stretching in a dorsal ward direction to close the embryo in its dorsal aspect. When this process does not occur properly, the resulting embryos die with a dorsal cuticular hole, a mutant condition known as 'dorsal open'. We isolated five new *chem* mutant alleles by chemical mutagenesis. The analysis of mutant cuticles revealed that besides dorsal closure defects, *chem* mutants have other defects as well: Early cellularization, germ band retraction, and head involution defects. *chem* dorsal closure phenotypes are very similar to the mutant *yurt* alleles phenotypes. *yurt* encodes a homolog of vertebrate cytoskeletal protein band 4.1. Genetic interactions between *yurt* and *chem* point to *chem* acting as a negative regulator of *yurt*. Immunolocalization of proteins involved in cell polarity like *crumbs*, β-catenin (*armadillo* in *Drosophila*), and another protein band 4.1 homolog in flies, *coracle*, in homozygous *chem* mutant embryos,

show that cell polarity is disrupted. This suggests that *chem* also participates as a negative regulator of proteins important for cell polarity during development of the *Drosophila* embryo.

Program/Abstract # 192

A moving zone of actomyosin contractility drives epidermal zippering and neural tube closure in ascidian embryos

Hashimoto, Hidehiko; Robin, François; Sherrard, Kristin; Munro, Edwin (University of Chicago, USA)

Neural tube closure is a key morphogenetic event in chordate development, but its underlying mechanisms remain poorly understood. We are investigating the cytomechanical basis for neural tube closure in ascidians - basal chordates that form a simple neural tube with < 100 cells. Ascidian neurulation occurs by neuroectoderm (Ne) folding, followed by unidirectional "zippering" in which the neural folds and adjacent epidermis (Epi) meet at the midline, then undergo junctional exchange (Ne/Epi -> Ne/Ne + Epi/Epi) in a posterior-anterior progression to form a simple tube beneath a epidermal sheet. Combining time-lapse fluorescence microscopy and immuno-staining of fixed embryos, we show that active non-muscle myosin II is highly enriched within a localized contractile zone (CZ) just ahead of the moving zipper where individual Ne/Epi junctions undergo rapid shortening. Laser ablation experiments suggest that junctional tension is highest in the CZ, and lower along newly formed Epi/Epi junctions just behind the zipper. Chemical inhibiton of Rho kinase abolishes both myosin enrichment and the increased tension in the CZ, and prevents Ne/Epi junction shortening and zipper progression. Kinetic analysis reveals that newly met epidermal cells remain transiently associated with the zipper, become highly elongated as the zipper moves anteriorly, and then release from the zipper and relax towards more isodiametric shapes. These data and computer simulations support a model in which localized actomyosin contractility ahead of the zipper provides the driving force for zipper progression, while junctional release and cell shape relaxation behind the zipper creates an essential force asymmetry to drive unidirectional zipper progression.

Program/Abstract # 193

The Claudin Family of Tight Junction Proteins Plays a Role in the Morphogenetic Movements that Drive Neural Tube Closure in Chick

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

Neural tube closure is dependent on the differentiation of ectoderm into neural and non-neural progenitors and the coordinated morphogenetic movements of these populations of cells. Our lab has shown that members of the claudin family of tight junction proteins are differentially expressed in the ectoderm prior to its differentiation into neural and non-neural progenitors and that these expression patterns are maintained throughout neurulation. To test my hypothesis that claudins are important for differentiation and for coordinating the morphogenetic movements of the ectoderm, I used the C-terminal domain of Clostridium perfringens enterotoxin (C-CPE) to remove a subset of claudins from the ectoderm of chick embryos during neurulation. GST-treated control embryos developed normally while GST-C-CPE-treated embryos had open neural tube defects (NTDs) that were classified according to their location along the anterior-posterior axis and their similarity to human defects: 7% of the embryos had an open NTD at the anterior end (anencephaly), 56% open at the posterior end (spina bifida) and 37% completely open (craniorachischisis). Whole mount *in situ* hybridization analysis of GST and C-CPE-treated embryos showed that expression of genes in the ectoderm (*Sox2* and *AP2*), genes that demarcate the boundary between neural and non-neural ectoderm (*Pax7*), and genes differentially expressed along the anterior-posterior neural ectoderm (*Pax6* and *Otx2*) were normally expressed indicating that differentiation and anterior-posterior patterning of the ectoderm occurred normally. These data suggest that claudins directly affect the morphogenetic movements required for neural tube closure but not initial differentiation of cells in neural and non-neural ectoderm.

Program/Abstract # 194

Control of apical constriction by dynamic calcium signaling during *Xenopus* neural tube closure

Suzuki, Makoto, (National Institute for Basic Biology, Japan); Hara, Yusuke; Sato, Masanao; Nagai, Takeharu (The Institute of Scientific and Industrial Research, Japan); Campbell, Robert (University of Alberta, Canada); Ueno, Naoto (National Institute for Basic Biology, Japan)

During early development of the central nervous system (CNS), progenitor cells undergo a typical shape change, called apical constriction, which makes the neural plate to bend mediolaterally to form the tubular structure. Actomyosin networks and their regulators drive apical constriction, yet how dynamically it is controlled in time and space is not fully understood. In this study, we investigated the possible role of calcium ion (Ca^{2+}) signaling using GFP/RFP-based Ca^{2+} indicators G-GECO/R-GECO. We confirmed that inhibition of Ca^{2+} channels delayed *Xenopus* neural tube closure, suggesting that Ca^{2+} signaling plays an important role(s) in apical constriction. From the long-term time-lapse imaging, we found that dynamic intracellular Ca^{2+} fluctuations occurred throughout the neural plate at single-cell to whole-tissue levels. Spatio-temporal patterns of the Ca^{2+} fluctuations appeared to be differentially regulated by the Ca^{2+} channel activity and the extracellular ATP, and the intracellular Ca^{2+} increase temporally preceded the repeated acceleration of the closing movements. Interestingly, the Ca^{2+} increase also temporally correlated with apical constriction, and the manipulation of cytoplasmic Ca^{2+} by caged IP_3 caused cell shape change similar to apical constriction. These data suggest that intracellular Ca^{2+} is a positive regulator of apical constriction. Therefore, this dynamic Ca^{2+}-dependent mechanism might act as a compensatory system for Rho/ROCK-dependent apical constriction, and enable *Xenopus* embryos to ensure the primitive CNS formation against environmental perturbations.

Program/Abstract # 195

Coordination of mitosis and morphogenesis: Role of a prolonged G2 phase during chordate neural tube closure

Ogura, Yosuke (University of Tsukuba, Japan); Sakaue-Sawano, Asako (Brain Science Institute, RIKEN, Japan); Nakagawa, Masashi (University of Hyogo, Japan); Satoh, Nori (Okinawa Institute of Science and Technology Promotion Corporation, Japan); Sasakura, Yasunori (University of Tsukuba, Japan)

Chordates undergo a characteristic morphogenetic process during neurulation to form a dorsal hollow neural tube. Neurulation begins with the formation of the neural plate and ends when the left epidermis and right epidermis overlying the neural tube fuse to close the neural fold. During these processes, mitosis and the various morphogenetic movements need to be coordinated. In this study, we investigated the epidermal cell cycle in *Ciona intestinalis* embryos in vivo using a fluorescent ubiquitination-based cell cycle indicator (Fucci). Epidermal cells of *Ciona* undergo 11 divisions as the embryos progress from fertilization to the tadpole larval stage. We detected a long G2 phase between the tenth and eleventh cell divisions, during which fusion of the left and right epidermis occurred. Characteristic cell shape change and actin filament regulation were observed during the G2 phase. CDC25 is probably a key regulator of the cell cycle progression of epidermal cells. Artificially shortening this G2 phase by overexpressing CDC25 caused precocious cell division before or during neural tube closure, thereby disrupting the characteristic morphogenetic movement. Delaying the precocious cell division by prolonging the S phase with aphidicolin ameliorated the effects of CDC25. These results suggest that the long interphase during the eleventh epidermal cell cycle is required for neurulation.

Program/Abstract # 196

A Proteomics Approach to Investigate Developmental Disturbances in Forebrain Formation of LRP2 Deficient Mice Using Mass Spectrometry

Paul, Fabian; Popp, Oliver; Dittmar, Gunnar; Hammes, Annette (Max Delbrueck Center for Molecular Medicine, Germany)

Recently, our lab revealed that the LDL receptor-related protein 2 (LRP2) is an essential component of the sonic hedgehog (SHH) machinery to orchestrate forebrain development (Christ et al., 2012). Mice deficient for LRP2 exhibit a broad range of severe forebrain malformations including forms of holoprosencephaly. However, little is known about the detailed dynamics and the role of LRP2 within the major signaling pathways. To this end, we want to further dissect potential modulatory effects of LRP2 on a proteomic level. Using state of the art mass spectrometry (MS) we will quantify proteins involved in formation of the central nervous system (CNS) expressed at crucial developmental stages (E8.5 to E9.5) in mouse embryos. This will enable us to detect differences in the CNS proteome of LRP2 null mice versus wild type controls. In collaboration with our MS core facility we tested chemical labeling (dimethyl label) and fathomed the protein amount that is sufficient to robustly quantify most of the CNS proteome at specific embryonic stages. The first MS runs revealed that for embryonic stage E8.5 the tissue (neural folds) of six embryos per group is adequate to achieve robust protein identification and quantification results. For E9.5, tissue from two embryos is sufficient. Overall, we were able to quantify over 3000 proteins at E8.5 and over 5000 proteins for embryonic stage E9.5 respectively. The first MS dataset with the LRP2 mutant embryos was already obtained and the analysis is currently ongoing. This will help us to further dissect the role of LRP2 in CNS formation. With the MS approach we also want to establish a workflow, which could serve as a blueprint for large-scale mutant analysis on the proteome level.

Program/Abstract # 197

SCO-spondin from embryonic cerebrospinal fluid is required for neurogenesis during early brain development

Vera, America; Stanic, Karen; Montecinos, Hernán; Caprile, Teresa (Universidad de Concepcion, Chile)

The central nervous system (CNS) develops from the neural tube, a hollow structure filled with embryonic cerebrospinal fluid (eCSF) and surrounded by neuroepithelial cells. Several lines of evidence suggest that the eCSF contains diffusible factors regulating the survival, proliferation and differentiation of the neuroepithelium, although these factors are only beginning to be uncovered. One possible candidate as eCSF morphogenetic molecule is SCO-spondin, a large glycoprotein whose secretion by the diencephalic roof plate starts at early developmental stages. In vitro, SCO-spondin promotes neuronal survival and differentiation, but its in vivo function still remains to be elucidated. Here we performed in vivo loss of function experiments for SCO-spondin during early brain development by injecting and electroporating a specific shRNA expression vector into the neural tube of chick embryos. We show that SCO-spondin knock down induces an increase in neuroepithelial cells proliferation concomitantly with a decrease in cellular differentiation toward neuronal lineages, leading to hyperplasia in both the diencephalon and the mesencephalon. Additionally, at the level of the posterior commissure, SCO-spondin is required for axon attraction and fasciculation. We further corroborated the long-range function of this protein in vitro, showing that the addition of inhibitory antibodies against SCO-spondin causes a reduction of neurodifferentiation and an increase of mitosis in mesencephalic explants cultured in eCSF. Our results suggest that SCO-spondin is a crucial eCSF diffusible factor regulating the balance between proliferation and differentiation of the brain neuroepithelial cells.

Program/Abstract # 198

Ift88 has an extraciliary role during neural convergent extension

McFarland, Rebecca J.; Brewster, Rachel (University of Maryland-Baltimore, USA)

Neural convergent extension (NCE), an early stage of neurulation, narrows and elongates the neural ectoderm prior to the formation of the neural tube. In zebrafish, NCE is driven by polarized cell migration directed towards the midline. Several studies have implicated ciliary genes in NCE, as they appear to interact with members of the planar cell polarity (PCP) signaling pathway, a key player in this

process. However, a clear mechanistic link between cilia and PCP signaling is currently lacking. In addition there is no direct evidence that ciliary genes mediate the cell behaviors that drive NCE. We investigate here the role of ciliary protein Ift88 in NCE in the zebrafish embryo. We demonstrate that in Ift88 morpholino-depleted embryos (in which maternal and zygotic mRNA is depleted) cells exhibit randomized protrusive activity and aberrant shape. In contrast, cells in which PCP signaling is disrupted appear to maintain medial-lateral polarization and elongate. Since the behaviors of ift88-depleted cells are similar to those observed when microtubules (MTs) are destabilized during NCE, we investigated a role for IFT88 in regulating the organization/stability of the MT network. Preliminary data reveal that MTs seem shortened and disorganized in Ift88-depleted embryos. As Ift88 is known to localize to centrosomes in addition to cilia, we propose that this protein nucleates/anchors cytoplasmic MTs. Together these findings point to a novel, extraciliary and PCP-independent role for ift88 in regulating MTs during NCE.

Program/Abstract # 199

Dysphagia and disrupted cranial nerve development in a mouse model of DiGeorge/22q11.2 Deletion Syndrome

Maynard, Thomas M.; Karpinski, Beverly; Fralish, Matthew (George Washington University, USA); Nuwayhid, Samer; Zohn, Irene (Children's National Med Center, USA); Wang, Xin; Mendelowitz, David; Moody, Sally (George Washington Univ, USA); LaMantia, Anthony (The George Washington Institute for Neuroscience, USA)

Dysphagia—difficulties with feeding, swallowing and nutrition—is common in infants and children with DiGeorge/22q11.2 Deletion Syndrome (22q11DS). We asked whether there are dysphagia-related developmental phenotypes in the *LgDel* 22q11DS mouse model. *LgDel* pups show evidence of feeding difficulties, including decreased weight gain and feeding-related, aspiration-based respiratory infections. Furthermore, activation of brainstem swallowing circuits elicits exaggerated synaptic responses in hypoglossal motorneurons from *LgDel* animals. A key developmental anomaly prefigures these phenotypes: disrupted cranial nerve (CN) differentiation. Sensory ganglion formation and axon growth is compromised in the trigeminal (V), glossopharyngeal (IX), and vagus (X)—which innervate targets essential for feeding, swallowing and digestion. The CN V disruption appears to result from altered patterning of the embryonic hindbrain, mediated by retinoic acid (RA); genetic modification of RA signaling in *LgDel* embryos rescues the CN V phenotype. In contrast, CN IX and X ganglia anomalies reflect primarily diminished dosage of the 22q11DS candidate gene *Tbx1*. Thus, 22q11 deletion—including, but not limited to *Tbx1*—results in altered development of CN V, IX and X. In parallel, 22q11DS-mediated alterations in RA signaling further alter CN V differentiation. These disruptions likely contribute to dysphagia in infants and young children with 22q11DS.

Program/Abstract # 200

PRDM12, histone methyltransferase factor is required for the regionalization of the trigeminal placode in *Xenopus leavis*

Matsukawa, Shinya; Michiue, Tatsuo (The University of Tokyo, Japan)

PRDM proteins are characterized by an N-terminal PR domain that is related to SET methyltransferase domain, and multiple zinc fingers that mediate sequence-specific DNA binding domain. It is known that PRDM proteins either act as direct histone methlytransferases or recruit histone-modifying enzymes to target promoters. Most of PRDM genes have been implicated in disease and developmental differentiation, but their functions in embryonic development is largely unknown. Here, we report the function of PRDM12 in *Xenopus* embryo. PRDM12 was expressed in two stripes of the neural plate and in the trigeminal placode region from early neurula stage and plays an important role for trigeminal placode formation. Our RT-PCR and WISH analysis showed that PRDM12 is a downstream effector of Pax3 but not overlap with FoxD3 known as early neural crest marker. Overexpression of PRDM12 suppressed several neural crest marker genes and these function was dependent on the PR domain and zinc finger domain by using deletion constructs of these domain. On the other hand, the inhibition of PRDM12 by antisense morpholino oligo (MO) caused the expansion of FoxD3 to the trigeminal placode region. Moreover, in the embryo injected with PRDM12 MO, several placode marker genes appeared slightly irregular in tailbud stage. Our findings suggest that PRDM12 restrict the neural crest region and regionalize the trigeminal placode region in *Xenopus* embryo.

Program/Abstract # 201

Morphogenesis of the vertebrate eye: cellular and molecular mechanisms

Norris, Anneliese; Streit, Andrea (King's College London, UK)

The vertebrate eye has dual origin: the lens arises from the surface ectoderm, while the retina, retinal pigment epithelium and optic nerve originates from the central nervous system. The coordination of cell fate and morphogenetic processes is crucial to form a functional eye. While cell fate decisions are a well studied, little is known about the cellular mechanisms that control eye formation in amniotes, and how both relate to each other. Here, we characterise the cellular events such as cell shapes from open neural plate stages to optic vesicle formation, along with proliferation and orientated cell division, and examine the contribution of each to the development of the eye. Using confocal microscopy and live imaging we provide the first detailed analysis of these processes in higher vertebrate. The main mechanisms that accompany epithelial morphogenesis are under the control of the non-canonical planar cell polarity (PCP) Wnt pathway. We have examined members of the PCP pathway, as well as Notch pathway components and we find that these are expressed in a restricted pattern from open neural plate stages to optic vesicle stages. We are currently investigating their role and how they interact. Ultimately this will allow us to establish the molecular mechanisms that control vertebrate eye morphogenesis and link them to cell fate allocation.

Program/Abstract # 202

Serotonin 2B receptor signaling is required for ocular morphogenesis in *Xenopus*

Ori, Michela; Marras, Giulia; Testa, Giovanna; De Lucchini, Stefania; Nardi, Irma (University of Pisa, Scuola Normale Superiore, Italy)

Recent work from our laboratory focused on the role of the serotonin 5-HT2B receptor in *Xenopus* craniofacial and ocular morphogenesis. *5-HT2B* gene is, in fact, expressed in the cranial neural crest cells (NCCs) which contribute to visceral arches and periocular mesenchyme (POM), a key signaling center required for eye morphogenesis including the choroid fissure closure. We demonstrated that pharmacologic and genetic alterations in 5-HT2B signaling cause ocular dysgenesis, characterized by small and dorsalized eyes, disorganized extraocular muscles, a shorter optic nerve and a failure of the choroid fissure closure or coloboma. To gain insight into the molecular mechanisms of 5-HT2B signaling in eye morphogenesis, we analyzed the gene expression profile of a number of key genes involved in POM development by in situ hybridization and qPCR in *5-HT2B* morphants. POM specific genes such as *Pitx2* and *Foxc2*, known to be regulated by the retinoic acid (RA), were upregulated and showed altered expression patterns. *Twist*, a marker of NCCs derived POM cells, revealed an accumulation of NCCs around the eye and near the ocular fissure suggesting a possible alteration in NCCs migration during the optic fissure closure and anterior eye segment formation. The *Vax2* gene, a marker of ventral retina did not change its expression domain. Interestingly, the expression of *Raldh3*, a RA generating enzyme, was upregulated in *5-HT2B* morphants resulting in an expanded expression domain in the ventral retina. On the whole these data support the notion that 5-HT2B signaling has a key role in the molecular networks of extrinsic factors governing ocular morphogenesis and suggest a possible interaction between 5-HT and RA signaling during POM development.

Program/Abstract # 203

Jitterbug(jbug)/Filamin is a Hindsight (Hnt) transcriptional target required for axon targeting and tendon cell adaptation to mechanical stress during Drosophila development.

Olguin, Patricio; Molina, Claudia; López, Estefanía; Sierralta, Jimena (Universidad de Chile, Chile); Oliva, Carlos (KU Leuven, Belgium)

How neurites grow directionally and find their specific layer in the brain to establish specific synaptic contacts remains poorly understood. The *Drosophila* visual system is an excellent model to study this general problem since it shares a similar organization to vertebrate visual system and its circuits are genetically hardwired and well characterized. This features along with the vast knowledge of signaling pathways and powerful genetics make it possible to explore molecular mechanisms at the cellular and subcellular level in the whole organism. Many factors play essential roles during axon growth and layer selection at the growth cone, as chemoatractant molecules and its receptors, cell adhesion molecules and cytoskeleton regulators. We have found that loss of function (LOF) of *jitterbug(jbug)/Filamin* a gene that encodes an acting binding protein that links the cytoskeleton with membrane receptors and participate of tendon adaptation mechanism to mechanical stress, results in layer targeting defects of photoreceptor (R) axons. These phenotypes are similar to those associated to the LOF of protein tyrosine phosphatase 69D (PTP69D) which is expressed both in tendon and R cells. Jbug/Filamin is expressed in photoreceptors under the control of the transcriptional regulator Hindsight and localizes at the membrane from apical to the axonal terminal. We propose that *jbug/Filamin* and PTP69D are components of a mechanotransduction mechanism that works in tendon and photoreceptor cells during its interaction with other tissues during development. Funded by FONDECYT Nº1120253 and Biomedical Neuroscience Institute, BNI, ICM.

Program/Abstract # 204

Molecular Characterization of Craniofacial Tendons in Zebrafish

Chen, Jessica W.; Tabin, Clifford J. (Harvard Medical School, USA); Galloway, Jenna L. (Center for Regenerative Medicine, Harvard Stem Cell Institute, Massachusetts General Hospital, USA)

Tendons enable movement by transmitting the force generated by the muscles to the bones. Not only is the integrity and strength of the attachment important, but the precise location of the connections between muscle, tendon and bone within the body must be strictly regulated for efficient motion. Due to an essential role of tendons in the mechanics of body movement, cases of tendon injury and degeneration are significant clinical issues. To date, there are a limited number of zebrafish studies analyzing tendon and ligament tissues and no reported expression of *scleraxis* (*scx*), the most robust mammalian marker of tendons and ligaments. To characterize the tendon tissue in the zebrafish, we have analyzed the expression of several robust mammalian markers of tendons and ligaments, including *scx*, *collagen 1a2* and *tenomodulin* (*tnmd)*, in the craniofacial musculoskeletal connective tissue regions. We find that *scx* is a robust marker of tendon progenitors in zebrafish, but is not required for the formation of differentiated craniofacial tendons as demonstrated by morpholino-mediated knockdown. Co-expression studies with muscle and cartilage markers demonstrate the presence of *xirp2a* at myotendinous junctions, and *scx* and *tnmd* at sites of muscle attachment to cartilage. Also, we show that the formation craniofacial tendon populations in zebrafish parallels what has been observed in higher vertebrates. Zebrafish craniofacial tendon progenitors are neural crest-derived, and can form in the absence of either muscle or cartilage. However, muscle is required for the maintenance of craniofacial tendon cell fate. We aim to expand our understanding of tendon formation and discover new potential therapeutic targets in tendon regeneration.

Program/Abstract # 205

Analysis of craniofacial defects in Six1/Eya1-associated Branchio-Oto-Renal Syndrome

Zhang, Haoran; Wong, Elaine Yee-man; Tsang, Sze Lan (The Univeristy of Hong Kong, China); Xu, Pin-Xian (Mount Sinai School of Medicine, USA); Sham, Mai Har (The Univeristy of Hong Kong, China)

Branchio-Oto-Renal (BOR) syndrome patients exhibit craniofacial and renal anomalies as well as deafness. BOR syndrome is caused by mutations in *Six1* or *Eya1*, both of which regulate cell proliferation and differentiation. The molecular mechanism underlying the craniofacial and branchial arch (BA) defects in BOR syndrome is unclear. We have found that Hoxb3 is up-regulated in the second branchial arch (BA2) of *Six1*$^{-/-}$ mutants. Moreover, Hoxb3 over-expression in transgenic mice leads to BA abnormalities which are similar to the BA defects in *Six1*$^{-/-}$ or *Eya1*$^{-/-}$ mutants, suggesting a regulatory relationship among *Six1*, *Eya1* and *Hoxb3* genes. The aim of this study is to investigate the molecular mechanism underlying abnormal BA development in BOR syndrome using *Six1* and *Eya1* mutant mice. Two potential Six1 binding sites were identified on the *Hoxb3* gene. *In vitro* and *in vivo* Chromatin IP assays showed that Six1 could directly bind to one of the sites specifically. Furthermore, using a chick in ovo luciferase assay we showed that Six1 could suppress gene expression through one of the specific binding sites. On the other hand, in *Six1*$^{-/-}$ mutants, we found that the Notch ligand Jag1 was up-regulated in BA2. Similarly, in *Hoxb3* transgenic mice, ectopic expression of Jag1 could be also detected in BA2. To investigate the activation of Notch signaling pathway, we found that Notch intracellular domain (NICD), a direct indicator of Notch pathway activation, was up-regulated in BAs of *Six1*$^{-/-}$*; Eya1*$^{-/-}$ double mutants. Our results indicate that *Hoxb3* and Notch signaling pathway are involved in mediating the craniofacial defects of Six1/Eya1-associated Branchio-Oto-Renal Syndrome.

Program/Abstract # 206

A family of FOX genes determines precise spatial patterns of growth and differentiation within facial bone and cartilage precursors

Balczerski, Bartosz; Louie, Kristin; Crump, Gage D. (Univ of Southern California-LA, USA)

FOX genes encode a large family of winged helix/forkhead transcription factors that have been shown to play multiple roles during development. While mutations in a number of FOX genes are known to cause craniofacial defects, how members of this large family coordinate development of the craniofacial skeleton remains unclear. In situ analyses in mice have led to a proposal that FOX genes form a complex expression code, much like the Dlx or Hox genes, that pattern the craniofacial primordia, yet this model remains to be tested at the functional level. In this study, we find that the homologous FOX genes of zebrafish (foxc1a , *foxc1b* , *foxd1* , *foxd2* , *foxf1* and *foxl1*) are also expressed in distinct patterns within the neural-crest-derived pharyngeal arches that are the precursors to the facial skeleton. By manipulating major signaling pathways, we show that these distinct expression patterns result from differential sensitivity of FOX enhancers to Hh, Fgf, Notch, Bmp and Edn signaling. This suggests that FOX genes act as integrators of multiple signaling cascades in the cranial preskeletal mesenchyme. Next, we use morpholino knock-down and conditional transgenic misexpression approaches to show that FOX genes have very specific requirements in controlling bone differentiation and cartilage growth in distinct regions of the developing face. In particular, we find that FOX genes act in region-specific manners to prevent the differentiation of dermal bone and increase the proliferation of cartilage precursors. In summary, our evidence in zebrafish supports the existence of a FOX code that shapes the future facial skeleton by precisely regulating the balance between the self-renewal and differentiation of skeletal progenitors.

Program/Abstract # 207

Alx-related frontonasal dysplasia: developmental mechanisms and evolutionary implications

Takahashi, Tokiharu; Mills, Peter; Dee, Chris (University of Manchester, UK)

The Alx gene family comprises three homeobox transcription factors in vertebrates, namely *Alx1*, *Alx3*, and *Alx4*. Interestingly, mutations in all the three human genes have been identified in three related craniofacial disorders, which have been recently established as Alx-related frontonasal dysplasia (FND). It encompasses a spectrum of severities but main characteristic features include ocular hypertelorism, malformations of the nose and forehead, and clefting of the facial midline. Most notably, loss of *Alx1* has severe orofacial clefting and extreme microphthalmia. In contrast, mutations of *Alx3* or *Alx4* cause milder forms of FND. Whilst *Alx1*, *Alx3* and *Alx4* are all known to be expressed in the facial mesenchyme, little is known about the function of these proteins during development.

Here, we report the establishment of zebrafish models of Alx-related FND. Morpholino knock-down of zebrafish *alx1* expression causes a profound craniofacial phenotype including loss of the facial cartilages and defective ocular development. In contrast, suppression of *alx3* produces no obvious phenotype. We demonstrate for the first time that Alx1 plays a crucial role in regulating the migration of cranial neural crest cells into the frontonasal primordia. Abnormal neural crest migration is coincident with aberrant expression of *foxd3* and *sox10*, two key genes of neural crest development. This novel function is specific to Alx1, and likely explains the marked clinical severity of *Alx1* mutation within the spectrum of Alx-related FND. *Alx1*, *Alx3* and *Alx4* have been originated by whole genome duplications at early vertebrate evolution, and we also discuss a possible link between Alx-related FND and the molecular evolution of Alx gene family.

Program/Abstract # 208

Normalized Shape and Location of Perturbed Craniofacial Structures in the *Xenopus* Tadpole Reveal an Innate Ability to Achieve Correct Morphology

Vandenberg, Laura Vandenberg; Adams, Dany; Levin, Michael (Tufts University, USA)

Embryonic development can often adjust its morphogenetic processes to counteract external perturbation. The existence of self-monitoring responses during pattern formation is of considerable importance to the biomedicine of birth defects, but has not been quantitatively addressed. To understand the computational capabilities of biological tissues in a molecularly-tractable model system, we induced craniofacial defects in *Xenopus* embryos, then tracked tadpoles with craniofacial deformities and used geometric morphometric techniques to characterize changes in the shape and position of the craniofacial structures. Canonical variate analysis revealed that the shapes and relative positions of perturbed jaws and branchial arches were corrected during the first few months of tadpole development. Analysis of the relative movements of the anterior-most structures indicates that misplaced structures move along the anterior-posterior and left-right axes in ways that are significantly different from their normal movements. Our data suggest a model in which craniofacial structures utilize a measuring mechanism to assess and adjust their location relative to other local organs. Understanding the correction mechanisms at work in this system could lead to the better understanding of the adaptive decision-making capabilities of living tissues and suggest new approaches to correct birth defects in humans.

Program/Abstract # 209

Morphogenetic Mechanisms Regulated by Non-Canonical Signaling in the Face

Geetha-Loganathan, Poongodi Geetha-Log (Life Sciences Institute, Canada); Nimmagadda, Suresh; Fu, Katherine; Richman, Joy (University of British Columbia, Canada)

WNTs that activate JNK-planar cell polarity pathways regulate convergent extension. The role of PCP pathways in later organogenesis is not as well studied, but recent work has shown that Wnt5a mediated PCP signaling is central to limb morphogenesis. In the period after neural crest cell migration has ceased, the prominences surrounding the primitive mouth elongate in the craniocaudal axis while becoming narrower in the perpendicular or medio-lateral axis. Initially we asked whether the necessary context to respond to putative non-canonical WNTs was present in the face. Indeed WNT11, a putative non-canonical WNT expressed in the avian face strongly activated a reporter for JNK activity. Furthermore, reporter activity depend on the DEP domain of Dishevelled, suggesting that PCP signaling was involved. In contrast, there was no activation of the canonical WNT reporter, SuperTopflash. We next targeted RCAS::*WNT11* retrovirus to the maxillary prominence (mp) in vivo. The majority of embryos developed notches in the upper beak and also caused an earlier shortening and widening of the mp connsistent with a defect in CE. These morphology changes were correlated with decreased expression of several human clefting genes. The data suggested that cell organization was disrupted by global expression of WNT11. Tracking labelled maxillary cells in the presence of Wnt11 or Wnt3a expressing cells implanted shows that the host cells were attracted to the source of Wnt11 and became greatly elongated whereas cells exposed to Wnt3a remained rounded and did not migrate. Taken together, the data suggest that the normal role of WNT11 is to control morphogenetic cell movements and to promote fusion of the lip via PCP signaling.

Program/Abstract # 210

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

Le Pabic, Pierre; Ng, Carrie; Schilling, Thomas (University of California-Irvine, USA)

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 Frizzled (Fz) signaling systems. While a requirement for components of the Fz 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- or Dachsous-orthologues in zebrafish results in craniofacial skeletal defects similar to those previously reported in Sox9a mutants, including defects in prechondrocyte stacking and polarity – two PCP-regulated behaviors in other contexts such as gastrulation - as well as a failure of chondrocytes to differentiate. Our chimaeric analysis demonstrates that Fat is both necessary and sufficient to coordinate polarity and differentiation of cartilage in a non-cell autonomous manner. Lastly, we find that Fat regulates expression of the cartilage differentiation gene Sox9a via the transcriptional co-repressor Atrophin2a. 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 tissue polarity.

Program/Abstract # 211

Two novel mouse models of craniofacial dysmorphology

Miller, Kerry Ann (Murdoch Childrens Research Institute, Australia); Tan, Tiong (Victorian Clinical Genetics Services, Australia); Welfare, Megan; Farlie, Peter (Murdoch Childrens Research Institute, Australia)

Approximately one third of all congenital abnormalities involve the craniofacial structures, where they are frequently associated with other clinical characteristics such as defects in the limbs and/or other organ systems. Thus delineating the molecular control of normal development in any individual structure will impact on our understanding of craniofacial dysmorphologies. Our current knowledge of the developmental processes governing anomalous development of the craniofacial complex is poor due to the deficits in our understanding of normal development of these structures. We have identified two novel ENU mouse models of two distinct human craniofacial dysmorphologies. Mutant *snoopy* embryos display a unilateral facial hypoplasia phenotype that involves the mandible, mid-face and ears. These characteristics are very similar in appearance to the human condition Goldenhar syndrome. The developmental origins of Goldenhar syndrome are not well documented and no genetic lesion has yet been associated with this

condition. *Kanyon* embryos have a mid-facial cleft, ocular anomolies (micropthalmia) and a variable mid-brain exencephaly, phenotypes that mimic human fronotonasal dysplasia. The facial cleft often varies in severity, from a disastrous lesion completely disrupting the face to a discrete cleft lip and palate phenotype. Thus, *kanyon* in its mildest form may also be a model for cleft lip and palate. Characterisation of these ENU mouse mutant strains will highlight the fundamental mechanisms responsible for normal development of the craniofacial structures. This data will facilitate the identification of underlying mutations in correlating human conditions.

Program/Abstract # 212

Fgf signaling in the control of craniofacial and tracheal gland development

May, Alison; Tucker, Abigail S. (King's College London, UK)

The submucosal glands (SMGs) of the respiratory system are specialized structures essential for maintaining human airway homeostasis. The significance of these glands is highlighted by their involvement in serious respiratory diseases such as cystic fibrosis, asthma and chronic bronchitis where both their phenotype and function are severely altered. Uncovering the normal developmental journey of SMGs of the conductive airways is essential to elucidate their role in these disorders, however, very little is known about their development and differentiation. To start to understand the molecular mechanisms involved we have investigated the development of both nasal and tracheal SMGs in the *Fgf10* mutant mouse. *Fgf10* is expressed in the mesenchyme around the developing SMGs, and in heterozygous mice the tracheal glands are reduced in number at a very early age, with an altered A/P distribution of the glands postnatally, a deficit that is not recovered in adults. This change in distribution is not due to a change in the tracheal cartilage rings and indicates that *Fgf10* is required for the first stages of SMG bud initiation and branching morphogenesis. In the nasal glands, some but not all glands were lost in the homozygous mutant, indicating that not all glands require *Fgf10* for initiation. Some of the SMGs present in the *Fgf10* homozygote were missing in the *Fgfr2b* mutant, suggesting compensation by another *Fgf* ligand. We aim to uncover the expression patterns of a number of *Fgf*s during early gland morphogenesis and to study the functional consequence of the reduction of SMGs in *Fgf10* heterozygotes by assessing the ability of these mice to respond to respiratory challenges, compared to wildtype littermates.

Program/Abstract # 213

Foxi3 is an essential regulator of tooth development

Jussila, Maria; Shirokova, Vera; Aalto, Anne; Sanz Navarro, Maria (University of Helsinki, Finland); Ohyama, Takahiro; Groves, Andrew (Baylor College of Medicine, USA); Mikkola, Marja; Thesleff, Irma (Institute of Biotechnology, University of Helsinki, Finland)

Transcription factor Foxi3 has been identified as the causative gene for the phenotype of the hairless dog breeds. These dogs have missing and misshapen teeth in addition to the hair phenotype. The function of Foxi3 in tooth development has not been studied previously. We show that *Foxi3* is expressed in the dental epithelium throughout tooth development, as well as in the epithelial stem cell niche of mouse incisors. We have studied the role of Foxi3 in tooth development by analyzing the phenotype of a conditional *Foxi3* knock-out mouse line (*Foxi3* cKO). To investigate downstream targets of Foxi3, we have performed a microarray analysis on teeth of *Foxi3* cKO and wild type embryos. To study the upstream regulation of *Foxi3*, we have analyzed *Foxi3* expression in different mutant mouse lines. In addition, we have used different proteins to induce *Foxi3* expression in embryonic skin and tooth, and analyzed the results with qRT-PCR. *Foxi3* expression is upregulated in K14-Eda mice overexpressing *Ectodysplasin* (*Eda*) and downregulated in *Eda*-deficient Tabby mice. In line with this *Foxi3* expression is induced in embryonic Tabby skin treated with Eda protein. In addition, *Foxi3* expression is induced in wild type skin and teeth treated with Activin A protein. We are currently analyzing the phenotype of the *Foxi3* cKOs and the microarray data. Our results show that Foxi3 is a new epithelial regulator of tooth development and that *Foxi3* lies downstream of Eda signaling. The phenotype of the hairless dogs resembles symptoms of ectodermal dysplasia, which is cause by mutations in the Eda pathway. Our data suggests the ectodermal dysplasia can be partly caused by reduced *Foxi3* expression.

Program/Abstract # 214

The Cadherin23, Harmonin, Myosin7aa, and Ift88 Usher syndrome protein complex assembles at the ER and is required for Usher protein trafficking

Blanco-Sanchez, Bernardo Blanco-San; Clement, Aurelie; Fierro Jr., Javier; Washbourne, Phillip; Westerfield, Monte (University of Oregon, USA)

In vertebrates, the scaffold and motor proteins, Harmonin and Myosin7a (Myo7a), interact physically with the cytoplasmic tail of the transmembrane protein Cadherin23 (Cdh23). These molecular interactions result in the formation of a macromolecular complex that is required for hearing, balance, and vision. In humans, mutations that disrupt the function any one of these or 8 other proteins result in Usher Syndrome, the most common cause of deafblindness. Little is known, however, about where, how, and which particular Usher proteins assemble together at the cellular level. We analyzed potential binding of the Cadherin23, Harmonin, and Myosin7aa Usher proteins, and whether Ift88 is required for their trafficking and localization in zebrafish. We used confocal microscopy of mechanosensory hair cells in conjunction with an in vivo whole-mount protein-protein proximity assay. Our data suggest that these four proteins are required not only for structural integrity of the mechanoreceptor, but also for its morphogenesis. We also found all four proteins in close proximity, consistent with them forming a complex. Analysis of mutants and morpholino-injected animals

revealed that the complex is preassembled at the level of the ER. Surprisingly, disrupting assembly of the complex affects not only the development of the intermediate compartment, a tubule like compartment formed by fusion of vesicles budded from the ER, but also hampers the trafficking of other Usher proteins. Thus, our results suggest that assembly of the complex is necessary for orchestrating the correct trafficking of Usher complexes through the secretory pathway to their final destinations.

Program/Abstract # 215

Interaction of Grxcr1 with the Usher protein complex in inner ear mechanosensory hair cells

Clément, Aurélie; Blanco-Sanchez, Bernardo (University of Oregon, USA); Panlilio, Jennifer (University of Miami, USA); Westerfield, Monte (University of Oregon, USA)

Usher syndrome is the leading cause of hereditary deafblindness in humans. To date, 11 genes have been identified as causative of Usher syndrome. The encoded proteins form complexes that are essential for development of the mechanosensory receptors of inner ear hair cells, but the detailed mechanisms of their functions are only incompletely understood. Glutaredoxin domain-containing cysteine-rich protein 1 (Grxcr1), not previously associated with Usher syndrome, is another protein known to be involved in hair cell mechanoreceptor development through its effect on the actin filaments that compose the hair bundle. We studied the role of Grxcr1 using morpholino oligonucleotides to affect the function of Grxcr1 in zebrafish and assayed whether Grxcr1 interacts genetically with Usher proteins. We observed that, consistent with the phenotype in mice mutant for *grxcr 1*, zebrafish larva deficient for Grxcr1 function displayed vestibular areflexia associated with dysmorphic mechanoreceptors. Although genetic interactions between Grxcr1 and the Usher type I proteins were not observed, we found that levels of the Usher type II proteins Dfnb31a and Dfnb31b were reduced when Grxcr1 was depleted. In addition, Espin, which like Dfnb31 plays a role in actin filament dynamics, was also diminished in Grxcr1 depleted animals. Together, our data suggest that in addition to its role in mechanoreceptor development, Grxcr1 may interact with the Dfnb31 Usher scaffold proteins.

Program/Abstract # 216

Expression of Wnt pathway genes coincides with processes of middle ear formation in chickens

Sienknecht, Ulrike J. (University Oldenburg, Germany); Fekete, Donna M. (Purdue University, USA)

Tympanic middle ears of vertebrates amplify and transmit air-borne sound to the inner ear. Middle ear components include the tympanic membrane, 1-3 ossicles and the oval window perforation of the periotic capsule. These structures, and the middle ear cavity itself, arise through the orchestration of tissues from diverse embryological origins, including the surface ectoderm, mesenchymal cells of both neural crest and mesodermal lineages, and the pharyngeal endoderm. The search for secreted signaling families that mediate the coordination of these morphogenetic events is ongoing. In situ hybridization of tissue sections through the embryonic chicken middle ear was conducted to evaluate how spatiotemporal maps of Wnt-related gene expression overlap with induction and chondrogenesis of the columella and otic capsule, as well as middle ear cavitation. Wnt11 labels migrating neural crest cells and is later transcribed at the tympanic membrane. In the tympanic mesenchyme, transcripts for Wnt11 superimpose with those of Fz1 and Fz7 receptors and the Wnt antagonist Dkk1. During middle ear cavitation Fz1 is present in the mesenchyme that finally becomes cleared off to give room for the surrounding otic cartilage which in turn transcribes Fz9. Early, the Wnt antagonist SFRP2 is prominently expressed in the condensing mesenchyme of the forming columella prior to chondrogenesis. Later, SFRP2 disappears from the columella and columellar chondrocytes express another Wnt anatgonist Frzb1, and also Fz9. In a complementary pattern to Frzb1, SFRP2 labels the columella perichondrium; it is also present in the surrounding mesenchyme. These patterns suggest a multistep involvement of the Wnt pathway during middle ear formation.

Program/Abstract # 217

Causes of Otitis Media in a New Mouse Model

Fuchs, Jennifer (King's College London, UK); Linden, Jennifer (Ear Institute, UCL, UK); Tucker, Abigail S. (King's College London, UK)

Otitis media (OM), the inflammation of the middle ear, is the most common disease and cause for surgery in infants. Chronic OM can be accompanied by excessive effusion (OME), often leading to conductive hearing loss. Though the pathogenesis of OM is multifactorial, ranging from infection to defects in ear morphology, there is evidence for genetic factors predisposing individuals to the disease. In this study we report that heterozygous Df1 (Df1/+)-knock out mice modeling DiGeorge syndrome (DGS) have a significant mono- or bilateral hearing impairment and a very high incidence of OME, similar to that observed in DGS patients. The severity of OME (thickness of mucosa, infiltration of inflammatory cells) was observed to correlate directly with the level of the hearing loss, making these mice an excellent novel model for studying the genetics behind the disease. MicroCT analysis of Df1/+ mice revealed that auditory bullae are smaller in these mice and may impact on correct function of the middle ear such as clearance and cavitation. Since *Tbx1* is the main candidate gene for the DGS phenotype, we have investigated its expression in developing tissues associated with the ear. *Tbx1* is expressed in the mesodermal core of the pharyngeal arches, which give rise to the muscles controlling the Eustachian tube (ET). Immunostaining against the skeletal muscle marker 12/101 revealed a decrease in myofiber in postnatal animals. Adult animals, however, showed a more diverse muscle phenotype. Defects in the size and condition of the ET muscles are likely to impair ET opening and closing and lead to defects in aeration of the middle ear, which are likely to increase susceptibility to OME. We aim to next investigate OME in *Tbx1* mutant mice.

Program/Abstract # 218

Lfng regulates the synchronized oscillation of the mouse segmentation clock via trans-repression of Notch signalling

Okubo, Yusuke, (National Institute of Health Sciences, Japan); Sugawara, Takeshi; Abe-Koduka, Natsumi (National Institute of Genetics, Mishima, Japan); Kanno, Jun (National Institute of Health Sciences, Japan); Kimura, Akatsuki; Saga, Yumiko (National Institute of Genetics, Japan)

The metameric features of vertebrates are based on the structure of the somites, which are sequentially produced (one by one) as a segmented cell mass from the anterior end of the presomitic mesoderm. The timing of this periodicity is controlled by the oscillation of gene expression, so called segmentation clock. In mice, the core component of the segmentation clock is the negative feedback loop that regulates Hes7 expression and incorporates another clock gene Lunatic fringe (Lfng), the product of which in turn represses Notch activation and generates Notch signal activity oscillations. In addition, a synchronization mechanism is required to form a sharp somite boundary. Although the intracellular mechanisms that underlie the activities of these oscillators are now well understood, the regulation of the intercellular coupling among clock cells that enable synchronization is largely unknown in mice. Notch signalling is required for the induction of several genes including clock genes, thus it has been difficult to analyze synchronization mechanisms independent of gene expression regulation. To overcome this difficulty, we used both experimental and theoretical approaches. Here we show, using chimeric embryos composed of wild-type cells and Delta like 1 (Dll1)-null cells, that Dll1-mediated Notch signalling is responsible for the synchronization mechanism. By analyzing Lfng chimeric embryos and Notch signal reporter assays using a co-culture system, we further find that Lfng represses Notch activity in neighboring cells by modulating Dll1 function. Finally, numerical simulations confirm that the repressive effect of Lfng against Notch activities in neighboring cells can sufficiently explain the synchronization in vivo. Collectively, we provide a new model in which Lfng has a crucial role in intercellular coupling of the segmentation clock through a trans-repression mechanism.

Program/Abstract # 219

Roles for Hoxa-5 in regulating chick cervical vertebral morphology

Mansfield, Jennifer; Chen, Jessica; Zahid, Soombal; Shilts, Meghan; Habbsa, Samima; Aronowitz, Danielle; Rokins, Karimah; Weaver, Sara (Barnard College, Columbia University, USA)

The vertebrate axial skeleton and its associated muscles and connective tissue develop from somites. Although somites form in the same way along the body axis, each vertebral segment develops with a unique morphology appropriate to its position. Hox transcription factors specify segmental identities prior to somite segmentation, but also continue to be expressed in segmented somites. Here, we examined the role of *Hoxa-5* in chick cervical somites using gain and partial loss-of-function approaches. We show that after somite segmentation, *Hoxa-5* is expressed in a sub-domain of lateral sclerotome, and that this restricted expression pattern is influenced by signals that pattern the somite medial-lateral axis (Shh and Fgf-8). *Hoxa-5* knockdown after segmentation specifically affects the morphology of ventral-lateral vertebral cartilage, which is derived from the *Hoxa-5* expression domain. We hypothesize that one role for chick *Hoxa-5* in patterning cervical segments is to locally influence precursors of the ventral-lateral vertebral cartilage, which develop differential morphologies across the cervical-thoracic transition.

Program/Abstract # 220

A Novel Mechanism Underlies Growth Plate Cartilage Column Formation

Romereim, Sarah M. (Northwestern University, USA)

Growth plate cartilage, the driving and shaping force of bone development, achieves directional growth by stacking proliferative zone chondrocytes into clonal columns to form a specific tissue architecture. The way in which these columns are formed is poorly understood and has been widely hypothesized to be similar to the migratory process of convergent extension. A novel application of time lapse confocal microscopy of the growth plate shows that recently divided cells do not migrate to take their place in the column. Instead, daughter cells rotate around the division interface. This process is regulated externally by signaling molecules and depends on cell adhesion. The discovery of this cell behavior not only provides insight into the mechanism of bone elongation but also offers a fresh perspective on directed growth in other systems.

Program/Abstract # 221

Opposing tensile forces and migratory behaviour drive tissue convergence during zebrafish laterality organ development

Pulgar, Eduardo; Santibañez, Felipe; Härtel, Steffen; Concha, Miguel (ICBM - BNI, University of Chile, Chile)

Changes in cell shape and tissue organisation involves the orchestrated integration of polarising cues from interdependent biomechanical processes. Cells interact with their environment by direct cell-cell and cell-matrix contact and through sensing diffusible factors such as migratory signals. Cellular responses to these cues enable cells to orient their polarity and develop a stereotyped supra-cellular organisation. How mechanical and chemical cues interact to control this phenomenon remains unclear. In our lab we are studying this issue during early development of the zebrafish laterality organ, known as the Kupffer's vesicle (KV). KV progenitor cells, the dorsal forerunner cells (DFCs), originate from ingression of dorsal marginal cells of the surface epithelium (EVL) at the onset of epiboly, a mechanism dependent by Nodal. Time-lapse microscopy showed that as epiboly progresses DFCs becomes increasingly elongated while converge to the midline. Detailed analysis of this early event revealed the presence of two main morphogenetic forces that display a biased spatial distribution along the animal-vegetal axis: (i) a vegetally-directed pulling force dependent on the attachment between DFCs apical membranes and the EVL-yolk cell, and (ii) a protrusive activity directed to the

animal pole depending on signals released by the deep embryonic blastoderm. Cell manipulation and computational simulations support an instructive role of these opposing cues in the control of early events of KV morphogenesis. We provide novel insights into the role of mechanochemical control of progenitor cells, during the coordination of vertebrate organogenesis. Grant sponsors: FONDECYT (1120558 and 1120579), the Scientific Millennium Initiative (P09-015-F)

Program/Abstract # 222

Cell cycle synchrony is lost before midblastula transition in zebrafish embryos.

Mendieta Serrano, Mario; Schnabel, Denhi; Lomelí, Hilda; Salas-Vidal, Enrique (Instituto de Biotecnología, Universidad Nacional Autónoma de México, Mexico)

In zebrafish, classical studies indicate that after fertilization rapid and synchronous cleavages occur until midblastula transition (MBT), when the cell cycle lengthens, cell division becomes asynchronous, cells start showing motile features and zygotic transcription begins (Kane and Kimmel, 1993). More recent studies demonstrated that cell divisions start to drift out of synchrony at the 4- to 8-cell stage transition, a drift that increases in subsequent cell cycles (Olivier et al., 2010). The aim of the present study was to further characterize cell cycle synchrony during the cleavage– and early blastula-period in zebrafish. By immunolocalization of the well-established mitosis marker, histone H3 phosphorylated at serine 10, we found evidence of mitotic asynchrony among blastomeres at the 2- to 4-cell stages transition. In order to obtain further evidence of the synchrony shift, we visualized the nuclear dynamics in living embryos during the first two cell cycles by injection of the DNA stain SYTOX Green. We found differences in mitosis progression among blastomeres in most of the 2- and 4-cell stage embryos analysed. Interestingly, from the 16-cell to 512-cell stage we demonstrate that nuclei number and mitotic indexes differ from those predicted by the classical synchronization model and the difference increased as development advanced. In addition we observed a novel pattern of mitotic clusters that coincided in time with the mitotic pseudo “waves” described to occur before the midblastula transition. Altogether, our findings indicate that early development is less synchronic than previously reported and that synchrony is not a requirement for proper development in zebrafish. Supported by IX201110 and IN205612.

Program/Abstract # 223

Loss of Dchs1b and Dchs2 leads to early developmental and cytoskeleton defects in the zebrafish embryo

Li, Nanbing (Jade) (Washington University, USA), Kim, Seok-hyung (Vanderbilt University, USA); Ma, Taylor; Helde, Kathryn; Moens, Cecilia (Fred Hutchinson Cancer Res Ctr, USA); Solnica-Krezel, Lilianna (Washington University, USA)

Dachsous (Dchs), an atypical cadherin with a large extracellular domain, has been shown to regulate planar cell polarity, tissue size and morphogenesis, and cell-cell adhesion in *Drosophila* and mammalian cell culture. Loss of Dchs1 function in mice leads to postnatal multi-organ defects and lethality. Using zebrafish as a model system, we characterize embryonic phenotypes of loss-of-function *dchs* mutants and begin to elucidate its function in vertebrates. In zebrafish, as in humans there are two *dchs* homologs (*dchs1* and *dchs2*), with duplication of *dchs1* (*dchs1a* and *dchs1b*). We have identified two nonsense mutations in *dchs1b* and one nonsense mutation in *dchs2*. All three mutations occur in the extracellular cadherin repeats domain that likely cause strong/complete loss of function. Whereas zygotic *dchs1b* and *dchs2* mutants show no obvious phenotype, maternal-zygotic (MZ) *dchs1b-/-* embryos present early defects in cytoplasmic segregation, cortical granule exocytosis, maternal mRNA translocation, and cell division. Both MZ*dchsb1-/-* and MZ*dchs2-/-* mutants show defects in epiboly as well as convergence and extension gastrulation movements. Moreover, MZ*dchs1b-/-* mutants present altered expression of the Spemann-Mangold gastrula organizer and mesodermal genes. Our studies indicate that these pleiotropic phenotypes may be caused by defects in the dynamics of the actin and microtubule cytoskeleton. We are using small molecules targeted to specific components of the cytoskeleton in order to identify the primary cytoskeletal defect. Additionally, we are using candidate gene approach to identify proteins that interact with Dchs. This work establishes a novel function for Dchs in early vertebrate embryogenesis.

Program/Abstract # 224

Cell and Tissue Interactions Organise Apico-basal Polarity During Lumen Formation in vivo

Ward, Laura, (King's College London, UK), Buckley, Clare; Clarke, Jon (King's College London, UK)

Much of our current knowledge of cell polarisation is based on cultured cellular aggregates polarising in a stable environment, which does not fully recapitulate the complex dynamics of embryonic development. To overcome this, our work uses live imaging of the transparent zebrafish embryo as a model in which to examine polarisation strategies during morphogenesis in vivo . Zebrafish neurulation involves the transformation of an initially solid primordium into an epithelial tube. We have recently shown that apical proteins localise to the point where a neural cell intersects the tissue midline, rather than at the cell’s anti-basal extremity. We are now investigating the mechanisms by which neural cells are able to sense the tissue architecture, assemble apical complexes at the midline and form a lumen at this point. During convergence, cells from each side of the neural rod interdigitate across the tissue midline and we have evidence to suggest that cadherin-based nascent adhesions are formed in the region where they meet. We have shown that this interdigitation is necessary for localisation of apical junctional proteins to the midline. We have additionally shown that basally located ECM components act to orientate apico-basal polarity, through examination of embryos lacking functional laminin. Strikingly, in the absence of a laminin-rich basal lamina, discrete regions of the neural tube show inverted polarity, with proteins normally present at the apical surface being mislocalised basally. In conclusion, we have shown that cellular interdigitation across the neural midline

works in concert with basal cues from the surrounding tissues for the correct spatial assembly of apical junctions and subsequent lumen formation.

Program/Abstract # 225

Developing a Staging Scheme for *Monodelphis domestica* embryos

Nellett, Kolleen, (Oberlin College, USA); Morrison, Jeremy (Greensburg, PA, USA); Cruz, Yolanda P. (Oberlin College Sci Ctr, USA)

The laboratory opossum, *Monodelphis domestica*, is the only marsupial maintained in laboratory colonies for biological studies that range from behavioral to genetic. The 2007 sequencing of its genome has enhanced the usefulness of this mammal in investigating the molecular basis of many developmental events routinely precluded by the extensive embryo-maternal tissue contact that occurs during implantation in eutherian mammals. Such events, especially organogenesis-related, occur at an exceedingly rapid pace during the last third of pregnancy—so much so that between days 10 and 11, for example, a litter of 14 opossum embryos could consist of individuals with somite numbers ranging from as few as 6 to as many as 28. We thus sought to devise a staging scheme based on the appearance of landmark anatomical structures (somites, optic and olfactory vesicles, etc.), rather than pregnancy days elapsed, to make meaningful comparisons with other vertebrate systems possible. Our results reveal the extent to which organogenetic events appear to be disordered, relative to those in mouse, chick or human embryo (for example, development of forelimbs, oral structures, and brain ventricles is precocious). These results will be useful for comparative studies involving organogenesis in these amniotes.

Program/Abstract # 226

PIAS-like protein Zimp7 participates in the Nodal signaling pathway during dorsal mesoderm development in zebrafish

Moreno, Roberto; Schnabel, Denhí; Salas, Enrique; Lomelí, Hilda (National Autonomous University of Mexico, Mexico)

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 human 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, the drosophila orthologue of the Zimp genes tonalli, 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 24 hpf. 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 dorsal mesoderm development. At 24 hpf, morpholino-mediated reduction of zygotic Zimp7 produced axial mesoderm defects -e.g. floor plate, precordal plate and notocord- alterations. These defects were accompanied by an up-regulation of nodal-related genes at gastrulation such as *squint, no tail* and *floating head*. Consistently, embryos over-expressing zimp7 RNA exhibited axial defects resembling those ones observed in squint mutants, like forebrain loss and cyclopia and down-regulation of nodal-related genes. Altogether our results indicate that Zimp7 might be interacting with the nodal-signaling pathway during mesoderm induction.

Program/Abstract # 227

Notochord vacuoles are lysosome-related organelles that function in embryonic axis elongation and spine morphogenesis

Ellis, Kathryn Leigh; Bagwell, Jennifer; Bagnat, Michel (Duke University, USA)

The notochord plays critical structural and signaling roles during vertebrate development. At the center of the vertebrate notochord is a large fluid-filled organelle, the notochord vacuole. While these highly conserved intracellular structures have been described for decades, little is known about the molecular mechanisms involved in their biogenesis and maintenance. Here we show that zebrafish notochord vacuoles are specialized post-Golgi structures and a new type of lysosome-related organelle. Through the use of dominant negatives, mutants, and pharmacological inhibitors we show that vacuole formation and maintenance requires late endosomal trafficking regulated by the vacuole-specific Rab32a and H^+-ATPase-dependent acidification. We establish that notochord vacuoles are required for body axis elongation during embryonic development and identify a novel role for notochord vacuoles in spine morphogenesis. Thus, the vertebrate notochord plays important structural roles beyond early development. We are currently using live imaging to further understand how the notochord acts as a hydrostatic scaffold during vertebrae formation. These experiments will provide insights into the cellular mechanisms behind congenital scoliosis.

Program/Abstract # 228

Cdx and Hox genes, and body axis extension of the mouse embryo

Neijts, Roel; Monteiro, Ana-Rita; van Rooijen, Carina; Deschamps, Jacqueline (Hubrecht Institute and UMC Utrecht, Netherlands)

Mouse Cdx and Hox genes are involved in regulating the posterior elongation of axial tissues that they will subsequently pattern. They work by maintaining active growth signaling in the embryonic growth zone. Cdx mutations cause axial truncation of varying severity depending on the identity and number of invalidated alleles. The Hox genes also participate in axial growth, in a way obeying spatio-temporal colinearity. While central Hox genes can replace Cdx genes in their axial growth promoting function, expression of posteriormost Hox genes must obligatorily remain silent until later embryonic stages. We will present data documenting the differential functional capacity of 3' to 5' Hox genes in modulating axial growth, exposing the relationship between Cdx and Hox genes.

Program/Abstract # 229

Withdrawn

Program/Abstract # 230

The Drosophila Z-disc protein Z(210) is an adult muscle isoform of Zasp52, which is required for normal myofibril organization in indirect flight muscles

Chechenova, Maria B.; Bryantsev, Anton; Cripps, Richard (The University of New Mexico, USA)

The Z-disc is a critical anchoring point for thin filaments as they slide during muscle contraction, therefore identifying components of the Z-disc is critical for fully comprehending how myofibrils assemble and function. In the adult Drosophila musculature, the fibrillar indirect flight muscles (IFMs) accumulate several high-molecular weight Z-disc proteins, the identities of which have to date been unknown. Here we use mass spectrometry and gene specific knockdown studies to identify one of these proteins, previously known as Z(210), as an isoform of the Z-disc protein Zasp52. The Zasp52 primary transcript is extensively alternatively spliced, and we describe its IFM-specific isoform. This isoform is detected in adult flies only, and not found in larvae. Finally, we demonstrate that Zasp52 in the fibrillar muscles is required for proper localization of another structural component of Z-discs, alpha-actinin, and for normal sarcomere structure, but not sufficient for distribution of some other structural sarcomere proteins, such as MLP84B and Sls. These studies expand our knowledge of Zasp proteins and their functions in muscle. Given the role of Zasp proteins in mammalian muscle development and disease, our results have broader relevance to muscle biology.

Program/Abstract # 231

Whole or Hole? Development of a Functional Diaphragm

Merrell, Allyson; Kardon, Gabrielle (University of Utah, USA)

The diaphragm is functionally the most important skeletal muscle in mammals, as it is essential for respiration. Strikingly, defects in diaphragm development are common birth defects (1:3000 births) that cause congenital diaphragmatic hernias (CDH) and result in high neonatal mortality and long-term morbidity. Despite its functional importance and the frequency and severity of CDH, our understanding of the embryonic origins, cell-cell interactions, and genetic mechanisms underlying diaphragm development normally and during herniation is limited. Using mouse genetic reagents, we have visualized for the first time the morphogenesis of the diaphragm's muscle, muscle connective tissue, and central tendon by identifying and genetically labeling the developmental sources of these tissues. We find that the morphogenesis of muscle and its connective tissue is tightly linked spatially and temporally. In addition, because the connective tissue can develop normally in the absence of muscle, the connective tissue is likely to be the driver of diaphragm morphogenesis. Furthermore, demonstrating the critical role of the connective tissue, we show via conditional mutagenesis that genetic defects in the connective tissue (and not the muscle) are the cause, with 100% penetrance, of CDH. By genetically labeling and visualizing the mutant connective tissue fibroblasts, we show that connective tissue is present throughout the diaphragm, but myogenic cells are locally devoid in the herniated regions. The presence of mechanically weak amuscular regions juxtaposed to stronger, muscularized regions allows liver to herniate through the weaker regions. Thus we show that the muscle connective tissue is critical for normal diaphragm development and CDH.

Program/Abstract # 232

Rab11 plays an indispensable role in the differentiation and development of the adult muscles in *Drosophila*

Singh, Divya; Roy, Jagat Kumar (Banaras Hindu University, India)

Rab11, an evolutionary conserved, ubiquitously expressed subfamily of small monomeric GTPase has been known to regulate diverse cellular and developmental events, by regulating the exocytic and transcytotic events inside the cell. Our studies show that Rab11 regulates *Drosophila* adult myogenesis by controlling proliferation and differentiation of the Adult muscle precursors (AMPs). Blocking Rab11 in the AMPs, which fuse to form the Indirect Flight Muscles (IFMs) of the fly results in rendering the flies completely flightless and non-viable. The IFMs comprising of the differentially patterned dorsal longitudinal muscles and dorsal ventral muscle are affected to different extents. Abrogating normal Rab11 function or knocking down its function results in severely disrupted IFM structure. DLMs forming from larval templates are reduced in number along with a significant reduction in their fibre size. On the other hand, the de novo developing DVMs are frequently absent. The DLMs in Rab11 hypomorphs are highly reduced, showing as a small constricted mass in one half of the thorax. Furthermore, we found that, Rab11 function is essential for the growth of these muscles during later half of adult myogenesis, as on altering Rab11 in the IFMs results in degenerated muscles and broken fibres. Finally, we show that loss of Rab11 activity in the AMPs result in acquisition of migratory characteristic of myoblast as they show cellular protrusion at their polar ends accompanied with loss of cell-cell contacts. We surmise a functional requirement of Rab11 at early stages of muscle development and our data provide the first line of evidence of a trafficking protein playing an indispensable role in regulating the adult muscle development.

Program/Abstract # 233

The gene regulation in skeletal myogenesis in medaka, Oryzias latipes

Tani, Saori, (USA), Kusakabe, Rie; Inoue, Kunio (Kobe, Japan)

We have analyzed microRNA (miR) expression and function in medaka (Oryzias latipes), a small fresh water fish. miRs are non-coding RNA molecules of 22 nt long, which silence the target mRNAs by imperfect base-pairing with the 3'UTR. In mammals, miR-1, -206 and -133 are involved in myoblast proliferation and differentiation. We identified miR-1, miR-206 and miR-133 genes in the

medaka genome. These miRs are encoded as six different genes organized into three bi-gene clusters. RT-PCR and in situ hybridization (ISH) showed that these miRs are specifically transcribed as continuous precursor RNA molecules in the skeletal muscle during embryogenesis. ISH using LNA-modified oligoprobes showed that 22-nt mature miR molecules accumulate in the trunk skeletal muscle. miR-206 and miR-133 were also expressed in the precursor cells of the pectoral fin muscles, in which miR-1 was not detected. Moreover, inhibition of miR-206 function using antisense morpholino oligonucleotide disrupted skeletal muscle formation, especially in the pectoral fin muscle. These results suggest that medaka miR-206 might play important roles in the pectoral fin myogenesis. We are currently analyzing expression of muscle-related transcription factors, *MyoD*, *Myf5*, *Pax3*, *Pax7* and *Lbx*, which are potential molecular players in the pectoral fin myogenesis in medaka. Our study would provide insights into the gene regulation in skeletal muscle formation both at the transcriptional and posttranscriptional levels.

Program/Abstract # 234
GTPase control of blood vessel morphogenesis
Cleaver, Ondine B.; Koo, Yeon (UT Southwestern Medical Center, USA); Xu, Ke (Harvard University, USA); Davis, George (University of Missouri, USA)
Cardiovascular function depends on the formation of blood vessels by endothelial cells (ECs). However the cellular and molecular mechanisms that coordinate to drive this process are only beginning to be unraveled. We carried transcriptional screening of embryonic blood vessel endothelium and found enrichment of a family of EC-specific effectors of the GTPases Rho, Rac, Rap and Cdc42. We recently demonstrated the requirement for a novel GTPase-interacting protein called Rasip1, and its binding partner the RhoGAP, Arhgap29, for endothelial tubulogenesis. Rasip1 null mice display aberrant localization of junctional complexes, and loss of adhesion to extracellular matrix, resulting in failure of functional blood vessels. Depletion of either Rasip1 or Arhgap29 in cultured HUVECs caused increased RhoA/Rock/Myosin II activity, suggesting that Rasip1 and Arhgap29 function together to suppress RhoA-dependent internal contractility. In addition, both Cdc42 and Rac1 activity were dramatically downregulated in siRasip1/siArhgap29 treated cells. Here, we dissect the functional domains required for Rasip1 function and show its control of cell adhesion via regulation of endocytosis. Current studies are aimed at elucidating the mechanisms by which GTPases drive basic cellular behaviors that culminate in blood vessel formation.

Program/Abstract # 235
MED23, a subunit of the global transcription complex, Mediator is essential for vascular remodeling and regulation of WNT signaling during cranial ganglia formation
Bhatt, Shachi, (Stowers Institute for Medical Research, USA), Sandell, Lisa (Louisville, KY, USA); Youngwook, Ahn; Krumlauf, Robb; Trainor, Paul (Stowers Institute for Medical Research, USA)
A close physical relationship between nerves and blood vessels is crucial for proper neuro-vascular function. Thus, it is not surprising that nerves and blood vessels share molecular and cellular signals during development. Here we describe the mouse mutant, *snouty*, obtained from a forward genetics screen which exhibits defects in vascular remodeling and cranial sensory neuron formation. These neuro-vascular defects result in embryonic lethality. *snouty* carries a point mutation in *med23*, a ubiquitously expressed subunit of transcription co-factor, Mediator. Detailed analyses reveal that loss of *med23* disrupts multiple steps of cranial placode formation, which leads to defects in cranial sensory neurons and ganglia development. Interestingly, these placodal defects are associated with elevated levels of WNT signaling and genetic suppression of WNT signaling partially restores cranial ganglionic neuron differentiation. Vascular remodeling defects in *snouty* embryos are associated with elevated levels of *vegfa* and defects in endothelial cell-cell adhesion as observed by fewer tight junctions. *snouty*, thus represents a unique mouse model for investigating the link between global gene transcription and spatio-temporal signaling during embryogenesis. Our findings suggest a novel role for transcription co-factor MED23 in vascular and neural development and highlight a surprising link between the MED23 containing Mediator complex and WNT signaling in regulation of neuro-vascular development.

Program/Abstract # 236
Endoderm convergence controls myocardial migration
Lin, Fang; Ye, Ding (The University of Iowa, USA)
In vertebrates, heart formation requires the migration of bilateral myocardial precursors to the midline, where they form the primitive heart tube. The adjacent endoderm is critical for this migration, but the underlying mechanisms remain unclear. Myocardial migration in zebrafish requires signaling mediated by sphingosine-1-phosphate (S1P) and its cognate G protein-coupled receptor, S1pr2. Our recently published data revealed that S1pr2 signals through a $G\alpha_{13}$/RhoA-dependent pathway to control convergent movement of the endoderm, and that this in turn promotes myocardial migration. We have used transgenic lines in which endodermal and myocardial cells are labelled with distinct fluorescent proteins at early developmental stages to determine how endoderm convergence controls myocardial migration. Our analysis revealed complex and dynamic associations between the myocardial and the endoderm during their migration. The endoderm rapidly converged towards the midline through the 14 somite stage (14s), at which point this migration slowed to a minium. In contrast, the myocardial cells underwent three distinct steps of migration: 1) before 14s, they migrated toward the midline with the endoderm, from a position dorsal to the endoderm; 2) at 14s, they migrated to the ventral side of the endodermal layer (translocation); and 3) after 14s, they migrated toward the midline beneath the endoderm. Our results suggest myocardial cells migrated by two distinct modes: first by passive migration that depends on endoderm convergence, and then by active migration that

appears not to be directly regulated by endoderm convergence. Furthermore, defects in endoderm convergence induced by a deficiency for S1pr2/Gα_{13} signaling impaired both the passive and active modes of myocardial precursor migration. We are currently investigating the mechanisms by which S1pr2/Gα_{13} controls both endoderm convergence and myocardial migration. Our study is expected to establish a framework for the interplay between the endoderm and myocardial precursors during heart-tube formation.

Program/Abstract # 237

A role for Claudin-10 in left-right axis patterning

Collins, Michelle M.; Ryan, Aimee (McGill University, Canada)

Asymmetric organ positioning within the limited space of the body cavity is critical for normal physiological function. The origin of this asymmetry is initiated during gastrulation in an evolutionarily conserved molecular cascade. We have identified that a transmembrane tight junction component, *Claudin-10*, plays a role in directing asymmetric organ positioning in the chick. Claudins are integral components of tight junctions. Within the tight junction, claudins play a critical role in the regulation of the movement of ions and small molecular within the paracellular space. Additionally, claudins link the tight junction to the actin cytoskeleton via interactions within their cytoplasmic tails with adaptor and scaffolding proteins. Here, we report that *Claudin-10* mRNA and protein are asymmetrically expressed on the right side of Hensen's node, a critical site where bilateral symmetry is broken. 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 *Nodal, Lefty, Pitx2c,* and *cSnR* is randomized in manipulated embryos. Mutagenesis of charged residues in the domain determining ion permeability properties did not affect the function of Claudin-10 in asymmetric organogenesis. However, mutation of two sites in the cytoplasmic tail (PDZ-binding domain and a putative phosphorylation site at S218) abolished the ability of Claudin-10 to randomize the direction of heart looping in gain-of-function studies, suggesting that interactions with cytoplasmic proteins are critical for Claudin-10 function. We are currently exploring the mechanism by which Claudin-10 functions in patterning the left-right axis.

Program/Abstract # 238

Dynamic cell rearrangement driving early heart tube formation and looping

Saijoh, Yukio; Kidokoro, Hinako (University of Utah, USA); Tamura, Koji (Tohoku University, Japan); Okabe, Masataka (The Jikei University School of Medicine, Japan); Schoenwolf, Gary (University of Utah, USA)

The vertebrate heart forms by the fusion of the paired left and right fields of precardiac mesoderm, which are originally separated on the either side of the embryonic midline. The short and symmetric primitive heart, undergoes rapid elongation along the A-P axis as the fusion proceeds, transforming from a straight morphology into a C-shaped loop. The asymmetric looping usually orients the heart tube toward the right side of the embryo, and several laterality genes that are expressed exclusively on the left side of the heart field have been shown to control the directionality of the looping. However, the behaviors/properties of cells that are modified by the left-right signals to drive morphogenesis remain unknown. To address this, we first analyzed in detail morphological changes of the heart tube during C-looping and found that C-looping is accomplished by asymmetric tissue growth between the left and right heart rudiments. Using cell labeling with fluorescent dyes and time-lapse microscopy, we further investigated how precardiac tissues are arranged during heart tube formation and C-looping. Our observations suggested that heart precursor cells undergo dynamic rearrangement during these processes to transform the sheet-like structure of the precardiac mesoderm into a single elongated tube and to shape the primitive heart. Detailed analyses comparing movements of groups of labeled cells in the left and right heart primordia revealed that each primordium undergoes different patterns of rearrangement during heart morphogenesis. We will discuss differences in cell properties between left and right heart tissues that control differential tissue rearrangement and tissue growth to drive asymmetric looping.

Program/Abstract # 239

Importancia de microRNAS en la embryogénesis del tracto de salida ventricular derecho. Estudio en el embrión de pollo

Sanchez Gomez, Concepcion, (Hospital Infantil Federico Gomez, Mexico), Perez, Carmen (UNAM, Mexico)

El objetivo fue determinar el patrón de expresión de 13 miRs cardiacos en la región del corazón embrionario de pollo incluyendo al cono, tronco y saco aórtico durante su transformación en tractos de salida y troncos arteriales (St.24-36HH). Se seleccionó 13miRs cardiacos, se disectó la zona de interés y se obtuvo tejido adulto de tractos de salida ventrículares y troncos arteriales. Se extrajo el RNA total por el método de Trizol, se amplificó por RT-PCR. La expresión relativa se calculó con el método ΔCT y se empleo Anova una vía y una prueba post-hoc Tukey $p \leq 0.005$. En St.24HH, la mayoría de los miRs estaban subexpresados respecto a la expresión en estructuras maduras, excepto cuando se comparó con el tracto de salida ventricular derecho. En este caso, el tejido embrionario mostró la mayoría de los miRs sobreexpresados. Por búsqueda bioinformatica y en la literatura, se encontró que miRs 206, 23b, 24 y Let7 están relacionados con TGF- b , importante en la TEM necesaria para el desarrollo de las crestas conales. Estos miRs fueron evaluados de St.24-36HH y se investigo sus blancos. Al comparara tejido embrionario con tracto de salida ventricular derecho se halló que en St. 24HH cuando TEM ya no es importante para el desarrollo de crestas conales, miR206 y 23b están sobreexpresados, después desaparecen. El blanco de miR206 es la endotelina y de miR 23b la acido hialurónico sintetasa, ambos modifican la MEC. miR24 inhibe la síntesis y secreción de TGF- b , que promueve la regulación de TEM. Estos eventos en conjunto provocan descenso de TEM,

facilitan cambio en patrón morfogenetico del mesénquima de crestas conales hacia la proliferación y maduración del tejido. Let7 no participa.

Program/Abstract # 240
The Cellular Basis of Limb bud Initiation
Gros, Jerome, (Institut Pasteur, France), Tabin, Cliff (Harvard Medical School, USA)
Vertebrate limbs first emerge as small buds at specific locations along the trunk. While a fair amount is known about the molecular regulation of limb initiation and outgrowth, surprisingly, the cellular events underlying these processes have remained less clear. Here we show that the mesenchymal limb progenitors arise through localized Epithelial-to-Mesenchymal Transition (EMT) of the coelomic epithelium specifically within the presumptive limb fields, and that this process is the initiating event of limb bud formation. This EMT is absolutely necessary to initiate limb buds and is regulated at least in part by Tbx5 and Fgf10 (key players in the molecular circuitry known to genetically control limb initiation). The finding that limb buds initiate earlier than previously anticipated through an EMT event and not through differential proliferation rates, as previously proposed, represents a paradigm shift that changes the way one views the earliest events of limb formation and redefines the question of how the limb buds are placed at the proper axial location.

Program/Abstract # 241
Gene regulation that initiates Sonic hedgehog expression in the limb bud
Tamura, Koji, (Tohoku University, Japan), Matsubara, Haruka; Yokoyama, Hitoshi (Tohoku University, Japan)
Sonic hedgehog (*Shh*) is a key molecule that plays a central role in limb morphogenesis. *Shh* expression in the early limb bud begins at a restricted small area of the lateral mesoderm as a very small dot at the posterior margin of the bud. Genetic and embryological evidences in the mouse and chick have suggested that positive regulators, such as *dHAND*, *Hoxd11/d12* and *Tbx2/3*, and negative regulators, including Gli3R and *Alx4* , restrict the domain of *Shh* expression, determining the position of posterior-localized *Shh* in early limb bud. However, available data in published reports and our own detailed observations indicate that these genes are expressed in a region larger than the epression domain of *Shh*, suggesting that the posterior-restricted initiation of *Shh* expression cannot be completely explained by any combinations of expression of these genes. To overcome this incompleteness, we further focused on *Tbx2/3*, *Hoxd12* and *Fgf8* and examined the expression pattern of these genes in detail in sections. *Tbx2*, an upstream inducer of *Shh* , is expressed at the posterior (and anterior) periphery of the limb bud mesenchyme, and *Tbx2* expression is negatively regulated by *Fgf8*. *Hoxd12*, which can induce *Shh*, is expressed in posterior margin of the limb bud mesenchyme simultaneously *Shh*. FGFs are known to have a function in maintenance of *Shh* expression with a feedback loop. *Fgf8* is expressed in a special epithelial tissue, the apical ectodermal ridge (AER). We found that *Shh* expression begins at a small area within *Tbx2/3* and *Hoxd12* positive domain underneath the posterior margin of *Fgf8* expression. These results suggest that the initial position of the *Shh* expression domain corresponds to the *Tbx2/3* and *Hoxd12*-positive region underneath the *Fgf8*-positive ectoderm at the posterior edge of the AER.

Program/Abstract # 242
Expression and functional analysis of transcription factor AP-2ß in limb development
Seki, Ryohei (MRC National Institute for Medical Research, UK); Suzuki, Takayuki (Nagoya University, Japan); Yokoyama, Hitoshi; Tamura, Koji (Graduate School of Life Sciences, Japan)
Tetrapods have two pairs of limbs, which exhibit diverse morphology in digits. The length of a digit depends on the number and length of phalanges. To elucidate the mechanisms that regulate these two parameters, we focused on a human familial syndrome called Char syndrome causing digit malformation, including shortness and disappearance of the skeleton. It has been reported that the responsible gene is the transcription factor *AP-2β*. Thus, we hypothesized that in normal development, *AP-2β* regulates the length and number of skeletal elements in digits. We first investigated using chick embryos whether *AP-2β* carrying the same mutation as that in Char syndrome gave rise to skeletal malformation of digits. The resultant limbs showed shortness and disappearance of the skeleton. The expression analysis of *AP-2β* revealed a good correlation between the digit length and duration of *AP-2β* expression. Further investigations suggested that *AP-2β* functions downstream of FGF signals from the apical ectodermal ridge, an epithelial structure essential for limb outgrowth. Gain of function of *AP-2β*, however, resulted in no alteration in limb skeletal pattern. Co-overexpression of *AP-2β* with *AP-2α*, which is another member of the AP-2 family and is also expressed in the distal limb bud, resulted in limb skeletal malformations. To further elucidate the function of *AP-2β*, we examined alterations in cell proliferation, cell death and expression pattern of some key genes involved in limb outgrowth or morphogenesis when the dominant-negative *AP-2β* was introduced. Based on the results, we will discuss the mechanism of digit morphogenesis from the viewpoint of *AP-2β* function.

Program/Abstract # 243
Role of transcription factor EVI-1 in chondrogenesis
Cela, Petra; Balkova, Simona (Institute of Animal Physiology and Genetics, Czech Republic); Horakova, Dana; Buchtova, Marcela (University of Veterinary and Pharmaceutical Sciences, Czech Republic); Richman, Joy M. (Life Sciences Institute, University of British Columbia, Canada)
Ecotropical viral integration site 1 (*EVI-1*) is a transcription factor essential for vascularisation and cell proliferation during embryogenesis. In our previous experiment, we analysed its expression during chicken embryonic development. Since strong

expression was observed in the limb buds close to but not within the cartilage elements, we focused on the effect of *EVI-1* on chondrogenesis in stage 24 limb micromass cultures. Cells were transfected with an siRNA to target chicken *EVI-1* at the time of plating. After six days of cultivation, the level of chondrogenesis was evaluated in cultures by Alcian blue staining. Perinodular inhibition of chondrogenesis in cultures was observed. Downregulation of *EVI-1* after siRNA treatment was confirmed by qPCR. Since *EVI-1* was expressed adjacent to the apical ectodermal ridge and this structure is a source of FGFs, we wanted to analyse the influence of FGFR inhibitors on *EVI-1* gene expression. Local injection of FGF inhibitor PD161570 into the limb bud at stages 20–22, downregulated *EVI-1* expression was determined by qPCR. Next, we performed gain-of-function experiment, where FGF2 beads were implanted into the right wings at stage 20. We observed upregulation of *EVI-1* expression 16h after treatment. In summary, it was found that *EVI-1* levels need to be maintained in order for chondrogenesis to occur. In addition *EVI-1* may be a novel gene mediating the effects of FGF on chondrogenesis. This work was supported by GACR (grant 304/09/0725) to MB and CIHR grant to JMR.

Program/Abstract # 244

Effects of homocysteine on mesenchymal cells during limb development on chick embryos

Bourckhardt, Gilian; Kobus, Karoline; Cecchini, Manuela; Müller, Yara; Ammar, Dib; Nazari, Evelise (Universidade Federal de Santa Catarina (UFSC), Brazil)

Hyperhomocysteinemia is a metabolic condition resultant of folic acid dietary deficiency. This condition is related with the occurrence of congenital anomalies that include limb defects. High levels of homocysteine (Hcy) can induce DNA damage and cell cycle arrest due to non-remethylation of Hcy to methionine. The aim of this study was to investigate whether high levels of Hcy can affect the mesenchymal cell dynamics d uring limb development. Chick embryos were treated with 20 µmol D-L Hcy/50 µL saline at E2 and analyzed at E6. Control embryos were treated with 50 µL saline. To identify cells in proliferation and proteins involved in cell cycle we performed immunolocalization and flow cytometry analyses using antibodies anti-phosphohistone H3 (mitosis marker), anti-p53, anti-p21 and anti-PCNA. No significant differences on cell proliferation rate were observed between Hcy-treated and control embryos. Thus, we observed a downregulation of proliferating cell nuclear antigen (PCNA) and the p21 protein, both involved in the G1 phase of cell cycle progression. On the other hand, the Hcy induces in mesenchymal cells of the limbs, an upregulation in expression of p53 protein, which can be activated by DNA damage. Additionally, we observed an increase of apoptosis rates. Our results indicate that the Hcy-treatment changes the mesenchymal cell dynamics during limb development of the *G. domesticus*.

Program/Abstract # 245

Inhibition of Hedgehog Signaling is Necessary for ß-Catenin-Regulated Interzone Differentiation and Joing Morphogenesis

Rockel, Jason; Yu, Chunying; Whetstone, Heather (The Hospital for Sick Children, Canada); Craft, April (University Health Network, Canada); Reilly, Katherine; Alman, Benjamin (The Hospital for Sick Children, Canada)

The mechanisms responsible for articular chondrocyte (AC) development are incompletely elucidated. ACs derive from Gdf5-expressing interzone cells and differentiate through a distinct pathway compared growth plate chondrocytes (GPCs), which do not derive from interzone cells . Hedgehog (HH) signalling is active in chondrocytes, primarily in GPCs. In osteoarthritis, a degenerative disease of articular cartilage, the HH-regulated GPC developmental program is r ecapitulated in ACs. Thus inhibition of HH signalling may be necessary for normal interzone cell differentiation, joint morphogenesis, and the maintenance of ACs. Using transgenic mice and ex vivo cultured embryos, we found that inhibition of HH signalling maintained interzone populations cell autonomously but had no effect on joint or skeletal morphogenesis. In contrast, activation of HH signalling inhibited interzone cell differentiation and maintenance in a cell non-autonomous manner. Interestingly, transgenic mice that had activated HH signalling in interzone cells developed osteochondrodysplasias and morphological abnormalities including ectopic joint cartilage, reduced AC differentiation and undifferentiated cells within the joint space. HH signalling also reduced Wnt/β -catenin activity in interzone progeny. Constitutive activation of β-catenin rescued HH-induced knee joint abnormalities and partially rescued the osteochondrodysplasias. Treatment of hindlimb organ cultures with FGF18, a β-catenin target gene, also rescued HH-induced joint abnormalities. These data indicate that HH signalling needs to be downregulated in interzone cells for β-catenin-regulated AC differentiation and joint morphogenesis.

Program/Abstract # 246

Characterizing gene expression dynamics between Shox2 and Hox genes during limb development

Neufeld, Stanley John (University of Calgary, Canada), Scott, Alexandra; Wang, Fan (Durham, USA); Cobb, John (University of Calgary, Canada)

The proper development of the vertebrate limb relies on homeobox genes of both the *Hox* and *Shox* families of genes. In mice and humans, mutation of certain members of either gene family results in similar phenotypes, such as malformed or shortened limb segments. We have previously established that *Shox2* and *Hox* genes genetically interact in the mouse limb, supporting the view that these genes function together. To gain further insight, we are analyzing their relative expression dynamics through double mRNA FISH in whole embryos. This analysis reveals an intriguing pattern where *Shox2* expression prominently overlaps with proximal-acting *Hox* genes, and is complementary to the expression of distal-acting *Hox* genes. This dichotomy is established as early as E10.5, suggesting that these relative dynamics could be important for the proper development of the discrete segments of the limb. We are currently assessing the possibility of cross-regulatory control between *Hox* genes and *Shox2*, and also the possibility that these genes

coordinately regulate the expression of a downstream target gene. Overall, this analysis will provide insight into transcriptional control that operates during normal limb development, and aspects that fail during genetic disease.

Program/Abstract # 247

Interdigital mesoderm acts as a signaling center instructing digit joint formation

Huang, Bau-Lin (National Cancer Institute-Frederick, USA); Koyama, Eiki; Pacifici, Maurizio (Children's Hospital of Philadelphia, USA); Mackem, Susan (National Cancer Institute-Frederick, USA)

The number and position of joints is one of the morphologic hallmarks of digit identity. Interdigital mesoderm (IDM) has been implicated in regulating digit identity, but its exact role and whether it regulates digit joint formation are unclear. Digital joints are lost in the 5'HoxdDel (Hoxd11,12,13 -/-) mouse. Using Gdf5Cre lineage tracing analysis to mark presumptive joint progenitors (interzone, IZ) in 5'HoxdDel, Gdf5+ cells are absent from, but surround the IZ region, which consists of Sox9+ chondrogenic cells. Wnt/βcatenin signaling has been shown to play a key role in joint formation by repressing the chondrogenic program in the IZ. Our genetic analysis of a conditional 5' Hoxd allele reveals that " late " 5'Hoxd function is required for digit joint formation (to E12.5) and we tested whether 5 ' Hoxd genes act in the βcatenin pathway. Introducing a conditional, stabilized βcatenin allele (exlox3 βcatenin; βCatGOF) restores normal joint formation and joint markers in 5'HoxdDel digits. To determine tissue requirements for rescue by βCatGOF, either cartilage- (Sox9CreER) or interdigit-specific (OsrCre) Cre lines were used. Surprisingly, joint formation was restored in 5'HoxdDel digits only by OsrCre/βCatGOF but not Sox9CreER/βCatGOF. Genetic lineage tracing demonstrates that OsrCre/βCatGOF cells are found only in IDM and do not contribute to IZ. These results indicate a non-cell autonomous role for βcatenin acting in IDM to regulate digit joint formation, and reveal that IDM can act as a signaling center to instruct digital joint formation. BMP signaling has been proposed to regulate digit identity from the IDM and we are currently testing the role of Bmp and other potential targets regulated by 5'Hoxd and βcatenin.

Program/Abstract # 248

Interdigit BMP signaling is essential for programmed cell death and is implicated in digit formation

Kaltcheva, Maria M.; Pajni-Underwood, Sangeeta (National Cancer Institute-Frederick, USA); Harfe, Brian (University of Florida College of Medicine, USA); Lewandoski, Mark (National Cancer Institute-Frederick, USA)

Shaping of the embryonic limb involves many processes including growth, differentiation, and programmed cell death (PCD). These processes integrate complex information from multiple signaling cascades such as the BMP and FGF pathways. Our previous work has shown that BMP signaling regulates interdigit (ID) PCD indirectly by modulating the secretion of FGFs from the apical ectodermal ridge, which act as cell survival factors to the ID mesenchyme. However, this does not exclude a direct role for BMPs in PCD. We therefore genetically examined the direct role of BMPs as triggers of ID PCD. To reduce BMP signaling to the ID mesenchyme we inactivated the gene encoding receptor BMPR1A within the ID. This results in retention of ID tissue, syndactyly, in adult mice due to a decrease of ID PCD during embryogenesis. To test redundancy between BMPR1A and BMPR1B in PCD we inactivated ID *Bmpr1A* in a *Bmpr1B* null background. This compound mutant has a further decrease in PCD and a significant upregulation of *Gdf5* expression in the ID mesenchyme. GDF5 is a TGFβ family ligand that can bind BMP receptors but has not been previously implicated in regulating ID PCD. During our analysis we also discovered a potential role of the ID tissue in digit formation. *Bmpr1B* null digits are short with abnormal development of their phalanges. This defect is completely rescued in digit one when we inactivate *Bmpr1A* in the ID. To fully understand the role of ID BMP signaling on normal limb development we are inactivating ID *Bmp2, 4,* and *7.* Preliminary analysis of these mutants reveals a syndactylous phenotype, supporting a role of BMP signaling in ID PCD. This work will elucidate the role of ID BMP signaling in directly regulating PCD and digit formation.

Program/Abstract # 249

Tramtrack69 regulates epithelial tube expansion in the Drosophila ovary through Paxillin and the homeobox protein Mirror

Peters, Nathaniel C.; Berg, Celeste (University of Washington, USA)

Epithelial tubes are the infrastructure for multicellular organs and tissues. Faithful tube morphogenesis requires the precise orchestration of cell signaling, shape, polarity, migration, and adhesion. The *Drosophila* ovary provides a robust, accessible model for epithelial tube morphogenesis. The somatic epithelium that encases each developing egg chamber forms and then expands a pair of tubes during late oogenesis, and the lumens of these tubes mold the eggshell's two dorsal respiratory appendages (DAs). Thus, the DAs of the laid egg provide an external readout for the efficacy of DA-tube morphogenesis. The Tramtrack69 (TTK69) transcription factor is required for DA-tube expansion; the *twin peaks* (*ttk*^{twk}) mutation reduces TTK69 levels during late oogenesis and disrupts DA-tube expansion, resulting in stunted DAs. Microarray and in situ hybridization analyses comparing wild-type and *ttk*^{twk} ovaries implicate the focal-adhesion scaffold Paxillin and the homeobox-protein Mirror as TTK69 effectors of DA-tube expansion. Tissue-specific RNAi against *Paxillin* and *mirror* produces DA defects that are enhanced in *ttk*^{twk} heterozygotes, demonstrating function in DA-tube formation and genetic interactions with *ttk*^{twk}. Although Mirror patterns the DA tubes prior to morphogenesis, we show that Mirror regulates DA-tube expansion independently of patterning. Mirror positively influences *Paxillin* expression, and over-expression of *Paxillin* can partially suppress the *mirror* RNAi defect. Additionally, *shibire* (*Drosophila* Dynamin) and *lamina ancestor* regulate DA-tube expansion downstream of TTK69. Thus, we have identified TTK69 effectors required for tube expansion, several of which are novel regulators of epithelial tube morphogenesis.

Program/Abstract # 250

Embryonic and uterine changes during mouse embryo implantation observed using a clearing technique.

Baiza Gutman, Luis Arturo; Sánchez Santos, Alejandra; Gómez Jiménez, Jaime; Martínez Hernández, María Guadalupe (FES Iztacala, UNAM, Mexico)

Mouse embryo implantation involves the adhesion of blastocyst to uterine luminal epithelium and the invasion of endometrium by the trophoblast and includes uterine changes in order to support embryo development. The earliest macroscopic uterine response to embryo signals is an increased vascular permeability detected after high molecular weight dye injection. Our aim was to analyze changes during early differentiation of implantation zones by a clearing procedure and its temporal relation with the vascular response to implantation. Uteri of CD1 mice on 3 to 8 days of gestation where cleared using H_2O_2 and benzylic alcohol. The vascular response was detected by extravasation of tripan blue. When we first detected the vascular response on the afternoon of gestation day (GD) 4 (already at 18 h), a morphologically differentiated uterine zone of implantation (IZ) was not clearly defined, furthermore a progressive growing of antimesometrial endometrium in IZ was observed until the uterine lumen was occluded and the embryo was firmly adhered in the top of growing endometrium at 17 h on GD 5, these events were followed by the formation of egg cylinder stage immersed in a mass of decidual tissue (GD 6 to 8). Since early morning on GD 5 a clearly defined IZ and an antimesometrial small invagination in the center of the zone (implantation chamber) were observed. In conclusion, uterine vascular response to embryo signals is followed by deep morphological changes in the lumen and wall of uterus associated with the endometrial interaction with the developing concepti, which can be associated with uterine remodeling and the formation of placenta. Supported by PAPIT, DGAPA, UNAM, grant IN230611.

Program/Abstract # 251

Directional rearrangement of planar polarised cells underlies the elongation of *Drosophila* renal tubules.

Saxena, Aditya; Denholm, Barry (University of Cambridge, UK); VijayRaghavan, K (National Centre for Biological Sciences, India); Skaer, Helen (University of Cambridge, UK)

The Malpighian tubules (MpTs) are single-cell layered epithelial tubes that serve as the principal excretory organs in *Drosophila*. In a period lasting only 5 hours during embryonic development, the MpTs undergo a dramatic transformation in shape increasing in length four-fold whilst the number of cells surrounding the lumen reduces from 8-12 to just 2 cells. This change in tissue architecture occurs in the absence of cell proliferation or significant changes in cell shape. Using a combination of time-lapse imaging and cell tracking we find that directional cell intercalation underpins MpT elongation. Employing SLAM protein as a reporter, we show for the first time that cells in elongating MpTs are planar polarised and that EGF signaling is necessary for the establishment and/or maintenance of this polarity. Further, live imaging of the actomyosin cytoskeleton reveals that myosin II shows dynamic pulsatile behavior that is also planar polarised in MpT cells during the process of tissue elongation. We will discuss the molecular and cellular mechanisms by which EGF signaling regulates planar polarity and we suggest a model in which the direction of cell intercalation is biased by the generation of asymmetric tensions in tubule cells, resulting from the planar polarised distribution of myosin II.

Program/Abstract # 252

Towards a common model of symmetry breakage: 'early determinants' act in the context of cilia-driven leftward flow

Blum, Martin; Walentek, Peter; Tisler, Matthias (University of Hohenheim, Germany); Danlichik, Michael (Oregon Health & Science Univ, USA); Schweickert, Axel (University of Hohenheim, Germany)

In fish and mammalian neurula embryos midline epithelia harbor polarized monocilia which rotate in a clockwise manner to produce a leftward extracellular fluid flow. Flow induces asymmetric gene expression, resulting in asymmetric organ morphogenesis and placement. In frog, earlier asymmetries were described for serotonin and the ion pump ATP4. The 'ion-flux' model proposed that an ATP4-generated voltage gradient drives serotonin through gap junctional communication (GJC) to asymmetrically enrich on one side of the embryo, breaking symmetry upstream of flow. We show that GJC was needed later in development for the transfer of asymmetric cue(s) from the midline to the lateral plate mesoderm. Serotonin and ATP4 were symmetrically expressed and required for Wnt-mediated setup of leftward flow. The GRP of ATP4 morphants revealed fewer, shortened and misaligned cilia. ATP4 was essential for Wnt/ß-catenin regulated *Foxj1* induction and Wnt/PCP dependent cilia polarization. Serotonin was involved in the specification of the superficial mesoderm (SM). The SM represents an epithelium localized above the Spemann organizer, from which the GRP develops during gastrulation. Importantly, serotonin already accumulates in the epithelial superficial cell layer of the blastula before SM specification. We hypothesize that other "early" determinants are required for SM specification as well. A model will be presented which unites opposing hypotheses into a common, evolutionarily conserved mode of symmetry breakage.

Program/Abstract # 253

The Congenital Heart Disease gene, GALNT11, glycosylates Notch to orchestrate cilia type and left-right asymmetry

Yuan, Shiaulou; Boskovski, Marko T. (Yale, USA); Pedersen, Nis Borbye; Goth, Christoffer Knak (University of Copenhagen, Denmark); Makova, Svetlana (Yale, USA); Clausen, Henrik (University of Copenhagen, Denmark); Brueckner, Martina; Khokha, Mustafa K. (Yale, USA)

Heterotaxy (Htx) is a disorder of left-right (LR) body patterning, or laterality, that is associated with major congenital heart disease. The etiology and mechanism underlying most human Htx is poorly understood. In vertebrates, laterality is initiated at the embryonic left-right organizer (LRO), where motile cilia generate leftward flow that is detected by sensory cilia, which transduce flow into

downstream asymmetric signals. The mechanism that specifies these two cilia types remains unknown. We now show that the O-glycosylation enzyme GALNT11 is crucial to such determination. We previously identified GALNT11 in a patient with Htx, and now demonstrate, in *Xenopus*, that *galnt11* activates Notch signaling. By mass spectrometry, GALNT11 glycosylates NOTCH1 peptides with GalNAc, a previously undescribed form of Notch glycosylation. Surprisingly, this glycosylation can increase Notch1 peptide cleavage by ADAM17, suggesting a novel mechanism for Notch activation. We further developed a quantitative live imaging technique for *Xenopus* LRO cilia and show that knockdown of *galnt11* or *notch1* converted immotile LRO cilia into motile cilia and produced a laterality defect reminiscent of loss of the ciliary sensor Pkd2. Strikingly, paralyzing a subset of these converted motile cilia by knockdown of axonemal dynein *dnah9* rescued laterality, thereby suggesting that motility masks the sensory function of motile cilia. Together, our data demonstrates that Galnt11 modifies Notch, establishing an essential balance between motile and immotile cilia at the LRO to determine laterality and identifies a novel mechanism for human Htx.

Program/Abstract # 254

N-cadherin locks left-right asymmetry by ending the leftward movement of Hensen's node cells

Saude, Leonor; Mendes, Raquel V. (Instituto de Medicina Molecular, Portugal); Martins, Gabriel G. (Centro de Biologia Ambiental, Portugal)

The stereotypic left-right (LR) asymmetric distribution of internal organs is due to an asymmetric molecular cascade in the lateral plate mesoderm (LPM) that has its origin at the embryonic node. In chicken embryos, molecular asymmetries at Hensen's node are created by leftward cell movements that occur transiently. What terminates these movements, and moreover what is the impact of prolonging them on the LR asymmetry cascade, was entirely unknown. We show that leftward movements last longer when N-cadherin function is blocked and cease prematurely when N-cadherin is overexpressed on the right side of the node. The prolonged leftward movements lead to loss of asymmetric expression of *wnt3a*, *fgf8* and *nodal* at the node region. This originates an abnormal expression of the asymmetric genes *cer1* and *snai1* in the LPM, resulting in a mispositioned heart. We conclude that N-cadherin stops the leftward cell movements, and that this termination is an essential step in the establishment of LR asymmetry.

Program/Abstract # 255

Novel complementary asymmetric gene expression of linked genes at the Pitx2 locus establishes a role for chromatin regulation of L-R patterning

Welsh, Ian Christophe; Chen, Frances; Kurpios, Natasza (Cornell University, USA)

The transcription factor *Pitx2* is required for the asymmetric development of multiple organs including that of the dorsal mesentery (DM), a structure whose dynamic changes in cell behavior are critical for directing the chiral rotation of the midgut. To identify the downstream cellular effectors that mediate *Pitx2* influence on cell behavior, our lab performed a laser capture microdissection and microarray analysis of the left and right DM at the onset of gut rotation. Unexpectedly, we found that the most differentially expressed gene in the right DM is located immediately adjacent to *Pitx2* on chicken chromosome 4. Furthermore, additional genes from this locus, both proximal and distal to *Pitx2*, also exhibit mirrored right-specific DM expression. Significantly, we discovered that this novel complementary expression occurs early during L-R patterning at both the node and in the lateral plate mesoderm well prior to formation of the DM. These data suggest this phenomenon is a fundamental characteristic of regulatory mechanisms directing expression of *Pitx2*. To our knowledge, this is the first report of such binary expression of linked genes across the L-R axis. Evolutionarily, the organization and content of this locus, including the presence of a large (~600kb) gene desert proximally flanking *Pitx2*, is highly conserved. The gene desert harbors numerous conserved noncoding elements (CNEs) with established transcriptional or structural regulatory function. We propose a model where long range interactions amongst these CNEs differentially organizes chromatin at the locus in nuclei on the left or right to drive asymmetric expression of *Pitx2* or its neighbors during L-R organogenesis.

Program/Abstract # 256

RhoA GTPase Signaling During Development of the Left-Right Body Axis

Amack, Jeffrey D.; Wang, Guangliang (State University of NY Upstate Med Univ, USA)

Evidence from patients and animal models implicates Rho GTPase signaling pathways in establishing left-right (LR) asymmetry in vertebrate embryos, but the underlying mechanisms remain unclear. We are using the zebrafish embryo to identify and characterize the role(s) of RhoA signaling during development of LR asymmetry. The small GTPase RhoA is a molecular switch that can activate downstream effectors including Rho kinase (Rock) proteins to modulate cytoskeletal dynamics and control several cell behaviors. Previously, we found that the Rho kinase Rock2b mediates cell shape changes that establish an anteroposterior (AP) asymmetric distribution of motile cilia in Kupffer's vesicle, which is necessary for these cilia to generate asymmetric fluid flow and direct normal LR patterning. Partial depletion of RhoA protein levels altered AP asymmetry in Kupffer's vesicle, but also revealed defects in cilia formation. While cilia phenotypes were not observed in Rock2b deficient embryos, antisense depletion of another Rho kinase, Rock2a, or brief treatments with a Rho kinase inhibitor disrupted cilia and LR development. Videomicroscopy of beads injected into Kupffer's vesicle demonstrated that interfering with RhoA or Rock2a function disrupted asymmetric fluid flow. Finally, LR defects in RhoA deficient embryos were partially rescued by ectopic expression of constitutively active Rock proteins, indicating RhoA indeed signals through Rho kinases to control LR asymmetry. These results uncover new roles for RhoA signaling via different downstream effectors (Rock2a and Rock2b) that control multiple steps of LR development.

Program/Abstract # 257

Fritz Governs Ciliogenesis in *Xenopus laevis*.

Kim, Su Kyoung (Univ of Texas-Austin, USA); Park, Tae Joo (Ulsan Metropolitan City, Korea); Abitua, Phil B. (University of California-Berkeley, USA); Wallingford, John B. (Univ of Texas-Austin, USA)

Cilia are evolutionarily conserved microtubule-based organelles projecting from nearly all vertebrate cells, and ciliary defects result in a variety of human disorders known as ciliopathies. Recent studies have shown that several planar cell polarity (PCP) proteins are essential for cilia functions. Here, we focused on Fritz, known as a novel PCP effector protein in *Drosophila*, in multi-ciliated cells in the epidermis of *Xenopus laevis* embryos. To investigate the role of Fritz, using confocal and scanning electron microscopy, we discovered that Fritz localizes along the ciliary axonemes and that knockdown of Fritz causes severe reductions in axonemes length and number. Then, using pull-downs and mass-spectrometry, we identified the Chaperonin Containing T-complex polypeptide 1 (CCT) and septin as interacting partners of Fritz. CCT is the key chaperonin interacting with septins, and both have been implicated in ciliogenesis. Using tagged CCT subunit constructs, we found that the tagged CCTα and CCTε co-localize with Fritz along the ciliary axonemes of multi-ciliated cells. Knocking-down of Fritz results in the accumulation of CCT at the apical cytoplasm in multi-ciliated cells; however, we confirmed that Fritz does not affect the CCT holoenzyme assembly. Septins, another interacting partner of Fritz, are novel cytoskeletal elements. Using septin antibodies, we found that endogenous septins also localize along the axonemes and accumulate in the apical cytoplasm of multi-ciliated cells in Fritz morphants. Similar ciliary defects were observed in septins morphants. Our data reveal that Fritz is essential for ciliogenesis, and that CCT and septin may interact with Fritz to control ciliogenesis in *Xenopus* multi-ciliated cells.

Program/Abstract # 258

Molecular basis of principles of regeneration: distalization and intercalation

Agata, Kiyokazu, (Kyoto University, Japan

Regeneration is always conducted under the control of positional information. The distal portion of the body is formed immediately after wound healing around the cut surface (a step called 'distalization'), and interaction of the newly formed distal portion and the remaining proximal portion may next induce reorganization of positional information, and lost tissues are then intercalatively generated to restore the original structures ('intercalation'). We proposed that this is probably a general principal of regeneration from invertebrates to vertebrates (Agata et al., Dev. Growth Differ., 49, 73–78, 2007). Recently we found that the molecular basis underlying diatalization and intercalation is also very similar among different regeneration events, although the tissues acquiring distal characteristics and cells participating in the regeneration of lost tissues vary among different animals and different regeneration systems. Here we will compare the molecular mechanisms of planarian regeneration and newt limb regeneration and then discuss common aspects of regeneration.

Program/Abstract # 259

GXD: A Gene Expression Resource for Developmental Biologists

Smith, Constance M.; Finger, Jacqueline H.; Hayamizu, Terry F.; McCright, Ingeborg J.; Xu, Jingxia; Eppig, Janan T.; Kadin, James A.; Richardson, Joel E.; Ringwald, Martin (Jackson Lab, USA)

By integrating large amounts of mouse developmental expression information, and by making these data readily accessible and easily searchable, the Gene Expression Database (GXD) supports investigators in their quest to understand the molecular mechanisms of developmental and disease. GXD contains expression information from wild-type and mutant mice and integrates different types of expression data, including those derived from RNA in situ and immunohistochemistry experiments. Expression data from the literature is added to the database by the GXD staff. Data is also acquired directly from researchers, including groups doing large-scale expression studies. GXD currently contains 1.4 million expression results for nearly 13,700 genes. As well, GXD has nearly 250,000 images of expression data, allowing users to retrieve the primary data and interpret it themselves. By being an integral part of the larger Mouse Genome Informatics (MGI) resource, GXD combines its expression data with other genetic and disease-oriented data. Thus, GXD can provide tools that allow users to evaluate expression data in the larger context and search by a wide variety of parameters and in ways unavailable elsewhere. Recent interface enhancements include the capability to search for expression data for genes that are associated with specific phenotypes and/or human diseases. Data summaries have become more interactive and include export features that make it possible to download these data for further analyses. GXD is available through the MGI web site at www.informatics.jax.org/expression.shtml. GXD is supported by NIH grant HD062499.

Program/Abstract # 260

The Sanger Mouse Genetics Project: High Throughput Recessive Lethality and DMDD Screens

Galli, Antonella; Ramirez-Solis, Ramiro; Estabel, Jeanne; White, Jacqui; Tuck, Elizabeth; Jones, Catherine; Green, Angela; Hooks, Yvette; Souter, Luke; Ryder, Edward; Adams, David (Wellcome Trust Sanger Institute, UK); Mohun, Tim; Wilson, Robert (MRC, UK)

The Sanger Institute Mouse Genetics Project (MGP) is a major contributor in the worldwide effort to develop mouse models to understand human genetic diseases. Over the next five years, the MGP aims to generate, cryopreserve and perform primary phenotypic characterization of over 800 lines of mice . A standardised battery of primary phenotyping tests is performed on all lines without any prior assumptions about gene function. To date, 37% of the 699 lines studied by the MGP are classified as lethal or sub-viable at postnatal day 14 due to non-Mendelian homozygous viability rates. To explore potential defects during embryogenesis, the

viability and morphology of over 200 of these lines have been assessed during organogenesis at embryonic day 14.5 (E14.5). Any dysmorphology including growth retardation, oedema, craniofacial, skeletal and neural tube defects are recorded and annotated. This recessive lethality screen has now been extended thanks to a strategic award from the Welcome Trust involving the Deciphering Mechanisms of Developmental Disease (DMDD) consortium, an innovative and ambitious programme of research involving members of the UK developmental biology community. This programme includes additional phenotyping tests (encompassing embryonic days E9.5, E14.5 and E18.5) and will use a combination of comprehensive whole embryo 3D imaging, placental histopathology, transcriptomics and nervous system functionality assessment in order to identify abnormalities in embryo structure and development. All data will be made freely available, enabling individual researchers to identify lines relevant to their research and provide valuable insight into novel gene functions and new mouse models of human developmental disorders.

Program/Abstract # 261
MMAPPR: Mutation Mapping Analysis Pipeline for Pooled RNA-seq
Hill, Jonathon T.; Demarest, Bradley; Bisgrove, Brent; Gorsi, Bushra; Su, Yi-Chu; Yost, H. Joseph (University of Utah, USA)
Forward genetic screens in model organisms are vital for identifying novel genes essential for developmental or disease processes. One drawback of these screens is the labor-intensive and sometimes inconclusive process of mapping the causative mutation. In order to leverage high-throughput techniques to improve this mapping process, we have developed a Mutation Mapping Analysis Pipeline for Pooled RNA-seq (MMAPPR) that works *without* parental strain information or requiring a pre-existing SNP map of the organism, and adapts to differential recombination frequencies across the genome. MMAPPR accommodates the considerable amount of noise in RNA-seq datasets, calculates allelic frequency by Euclidean distance followed by Loess regression analysis, identifies the region where the mutation lies and generates a list of putative coding region mutations in the linked genomic segment. MMAPPR can exploit RNA-seq datasets from isolated tissues or whole organisms that are utilized for gene expression and transcriptome analysis in novel mutants. We tested MMAPPR on two known mutant lines in zebrafish, *nkx2.5* and *tbx1*, and used it to map two novel ENU-induced cardiovascular mutants, with mutations found in the *ctr9* and *cds2* genes. MMAPPR can be directly applied to other model organisms, such as *Drosophila* and *C. elegans*, that are amenable to both forward genetic screens and pooled RNA-seq experiments. Thus, MMAPPR is a rapid, cost-efficient, and highly automated pipeline, available to perform mutant mapping in any organism with a well-assembled genome.

Program/Abstract # 262
The Role of Long Noncoding RNAs in Regulating Chicken Limb Patterning
Schwartz, Matthew G., (Harvard Med School, USA), Ulitsky, Igor; Bartel, David P. (Whitehead Institute for Biomedical Research, MIT, Howard Hughes Medical Institute, USA); Tabin, Clifford J. (Harvard Med School, USA)
Recently, the known repertoire of functional RNAs has been expanded by the discovery that long noncoding RNAs (lncRNAs) influence development and differentiation by regulating gene expression by a diverse array of mechanisms. In addition, it has become clear that there is nearly pervasive transcription throughout the genome. While the number of protein-coding genes remains relatively static across evolution, higher organisms tend to have a larger noncoding portion of the genome—suggesting a potential role for lncRNAs in increasing organismal complexity. LncRNAs have been implicated in many developmental processes, but there remains limited *in vivo* evidence for the functionality of lncRNAs in development. The developing chicken limb bud is readily accessible and permissive to developmental manipulations *in ovo*, making it an excellent model for examining such roles. Using RNA-Seq and 3P-Seq, we identified lncRNAs differentially expressed across seven stages of chicken embryonic development as well as in forelimbs and hindlimbs at HH21/22 and HH25/26. Our analysis uncovered a subset of 7,197 candidate intergenic lncRNAs (lincRNAs), which are enriched at genomic loci nearby to developmental transcription factors and many of which are spliced and/or have multiple isoforms. Analysis of additional lncRNA candidates, including antisense and intronic lncRNAs, is currently ongoing. A whole-mount *in situ* hybridization screen of candidate lncRNAs enriched in the limbs verified many novel lncRNAs with developmentally interesting spatial and temporal expression patterns. Candidates are currently being functionally tested by overexpression and knockdown via RCAS virus infection and *in vivo* electroporation in the developing chicken limb.

Program/Abstract # 263
Genome-wide approaches reveal dynamic Foxh1-mediated gene regulation during mesendoderm specification in *Xenopus tropicalis*
Le, Rebekah Le; Chiu, William; Blitz, Ira; Cho, Ken (University of California-Irvine, USA)
The Nodal signaling pathway is necessary for vertebrate mesoderm and endoderm specification. The receptor-activated Smad2/4 complex functions together with the maternal transcription factor Foxh1 as key transcriptional activators of Nodal target genes. To gain a comprehensive understanding of the Nodal signaling gene regulatory network, we employed a high-throughput sequencing approach to investigate the role of Foxh1 during *Xenopus tropicalis* mesendoderm development. We performed mRNA-seq analysis to identify Nodal signaling and Foxh1 targets in the gastrula embryo, where Nodal signaling was abrogated using the pharmacological inhibitor SB-431542 and Foxh1 was knocked down using an antisense morpholino oligo. A comparison of regulated genes revealed a critical, but not exclusive, role for Foxh1 in the transcriptional mediation of Nodal signaling. We also utilized ChIP-seq to investigate *in vivo* Foxh1 binding patterns over the time course of mesendoderm development from blastula to early gastrula. Our current analysis uncovered Foxh1 binding to cis-regulatory regions of over 5,000 genes at the blastula stage, and nearly 2,000 at the early gastrula

stage. Among these, only 1,000 genes were Foxh1-bound at both stages, suggesting that Foxh1 has both common and unique regulatory roles at each stage. *De novo* motif analysis identified TF binding motifs with strong enrichment under the Foxh1 peaks, including Foxh1, Smad2/3, HEB, and others representing potential novel co-factors. Our current results illustrate the dynamics of Foxh1-mediated gene regulation during early embryogenesis, demonstrating the crucial need for a temporal understanding of the Nodal signaling gene regulatory network controlling mesendoderm specification.

Program/Abstract # 264

A whole genome approach to explore the gene regulatory network controlling germ layer patterning in the *Xenopus tropicalis* gastrula

Paraiso, Kitt; Blitz, Ira; Chiu, William; Cho, Ken W.Y. (University of California-Irvine, USA)

The vertebrate body plan is laid out during the blastula and gastrula stages. Differential expression of genes in specific regions of the embryo acts as markers and specifiers of future cell fates. The complex circuitry that governs the regulation of gene transcription can be usefully summarized with gene regulatory networks. In *Xenopus*, multiple published gene regulatory networks exist to explain gastrulation but most of the work done thus far involve probing interactions between a small subset of expressed genes. With the power of high-throughput sequencing, we have the opportunity to probe for network interactions on the genome-wide scale. Here, we explore for possible network interactions through correlations between gene expression in different embryonic regions and DNAse I hypersensitive regions gene promoter. First, we dissected the early gastrula embryo into five regions – the animal cap (ectoderm), dorsal marginal zone (dorsal mesoendoderm), ventral marginal zone (ventral mesoendoderm), lateral marginal zones (lateral mesoderm), and vegetal (endoderm). We performed RNA-seq on each region and compared their transcriptomes. Using DNAse-seq on early gastrula, we identified possible regulatory regions actively interacting with transcription factors. Given these information, cross-referenced to known transcription factor expression patterns and consensus binding sites, we can infer the regulation of gene transcription with regionally specific expression. We will present the most current findings.

Program/Abstract # 265

Coordinating neurogenesis: Roles of REST and Hoxb1 binding modules integrating neural fate determination

De Kumar, Bony; Parrish, Mark; Paulson, Ariel; Gottschalk, Aaron; Scott, Carrie; Conaway, Ron; Krumlauf, Robb (Stowers Institute for Medical Research, USA)

Hox genes encode a family of transcription factors that play key regulatory roles in determining the anterior–posterior properties of many tissues in developing embryos. An excellent example of this regulatory role is Hoxb1, which displays restricted expression in a segment (rhombomere 4) of the developing hindbrain. Loss of Hoxb1 results into a transformation of rhombomere (r) 4 to and r2 identity and leads to abnormal neuronal differentiation, defective axonal guidance, abnormal facial nerve development and partial neonatal lethality. However, very little is known about how Hox genes control the cellular processes and programs through downstream target genes to fulfill these roles in the CNS. We used programmed differentiation of mouse embryonic stem (ES) cells into neuro-ectoderm fates and mouse tissues in combination with ChIP-Seq technology to perform genome-wide analyses of Hoxb1 binding regions and identity potential target genes. In addition to known types of Hox binding motifs our analyses revealed enrichments for novel classes of sequences that integrate input from Hoxb1. These include sites for REST, SP1, Blimp-1, Pax-4, Gata6, Krox and Lmo2 binding motifs. Furthermore, unbiased motif sampling using MEME identified novel binding motifs with no previously defined binding properties. Regulatory assays in chicken embryos demonstrated that many of these sites functioned as neuronal enhancers. The REST sites are of particular interest because REST represents a repressor complex that actively blocks genes important in neuronal differentiation. REST activity is maintained in non-neuronal tissues to prevent neural fates and it must be down-regulated or eliminated in the CNS to properly coordinate neurogenesis. A significant number of Hoxb1 binding peaks have closely associated REST Motifs and ChIP-seq data indicate that the REST complex binds to these sites in undifferentiated ES cells. The close association or tethering of the REST and Hoxb1 binding sites provide a potential mechanism for coordinating cell differentiation programs in neurogenesis. Hoxb1 may remove the REST repressor complex and activate selected loci to regulate neurogenesis. Our analyses are uncovering novel interactions between Hox proteins and other factors that underlie their role as master regulators of patterning and morphogenesis.

Program/Abstract # 266

Genomic differences between spontaneously aborted fetuses and live-born patients with trisomy 21

Torres, Leda; de Robles, Ximena; Sanchez, Silvia; del Castillo, Victoria (Instituto Nacional de Pediatría, Mexico); Orozco, Lorena; Carnevale, Alessandra (Instituto Nacional de Medicina Genómica, Mexico); Grether, Patricia (Instituto Nacional de Pediatría, Mexico); Mayen, Dora Gilda (Hospital Angeles Lomas, Mexico); Frias, Sara (Instituto Nacional de Pediatría/Instituto de Investigaciones Biomédicas, UNAM, Mexico)

Aneuploidies have been identified in at least 5% of pregnancies and are the leading genetic cause of spontaneous abortions and birth defects, 1 of 150 abortions with aneuploidies has trisomy of chromosome 21 (T21). To date there is not known why a fetus with T21 continues to develop or is aborted. In order to found genomic differences between live births and abortions with T21 we studied 12 patients, 8 of them with congenital heart defects (CHD), 5 abortions with T21 and 10 healthy donors. gDNA was extracted from every individual. Affymetrix Genome Wide Human SNP 6.0 microarrays were performed; we identified common regions with loss or gain of genomic material employing the Chromosome Analysis Suite of Affymetrix; each region was analyzed using UCSC genome

browser. The genomic differences observed were the following: 2/10 healthy individuals showed copy number variants (CNV) consisted of gains in 3p12.3, 9q21.11 and 17q21.31, and loss in 4q13.2. In T21 patients with CHD we observed a gain in 9q21.11, loss of heterozygocity (LOH) in 10p12.31 and 19q13.2. In T21 patients without CHD we observed LOH in 4q13.3, 6p22.1, 8q22.22, 15 q13.3, 16q22.1 and loss in 4q13.3 and 14q11.2. In T21 abortions we detected LOH in the regions 4p15.1, 10p12.31 and 20q11.21. The most evident difference among the groups is that live births either T21 or controls, had some regions with CNV that are absent in T21 abortions, this CNV could balance the extra chromosome 21 in live births, while in abortions its absence could compromise the normal development. Our study contributes to find possible reasons for trisomy 21 embryo survival.

Program/Abstract # 267

Microarray analysis of the embryonic skull vault.

Barrell, William; Healy, Christopher (Craniofacial Development and Stem Cell Biology, UK); Ota, Masato (Section of Molecular Craniofacial Embryology, Japan); Ohazama, Atsushi (Craniofacial Development and Stem Cell Biology, UK); Dionne, Marc (Centre for the Cellular and Molecular Biology of Inflammation, UK); Liu, Karen (Craniofacial Development and Stem Cell Biology, UK)

The mammalian skull vault is formed from two distinct embryonic cell populations, with the frontal bones and intervening suture arising from neural crest cells whereas the parietal bones are mesodermally derived. The coronal suture, which separates the frontal and parietal bones, is of mixed origins. As the brain grows the skull must be able to accommodate the increasing size, therefore it is crucial for the timing of ossification and suture fusion to be tightly regulated. Previous reports on postnatal bones suggest that differential gene expression is correlated with the different embryonic origins. However, there have been few unbiased analyses focusing on embryonic expression patterns. In this study, we have performed microarray analyses on dissected frontal and parietal bones, interfrontal and coronal sutures at 15.5 and 18.5 days post coitum (DPC). We then performed pairwise analyses between tissues and time points, as well as hierarchical clustering of the differentially expressed genes. In addition, gene ontology (GO) process analysis is presented, revealing dynamic changes during skull vault formation. Finally, by comparing expression in frontal and parietal bones, we identified high levels of sclerostin domain-containing protein 1 (Sostdc1) in the frontal bone at 15.5DPC. Sostdc1 is a secreted protein reported to inhibit both the BMP and Wnt signaling pathways. Microcomputed tomography (mCT) and cephalometric measurements revealed that Sostdc1 knockout mice have altered skull shapes compared to wildtype controls. All together, our data provide a springboard for further analyses of the dynamic changes necessary for proper development of the embryonic skull vault.

Program/Abstract # 268

Interactions Between Organizer Genes and Early Neural Ectodermal

Klein, Steven L. (National Science Foundation, USA); Moody, Sally; Neilson, Karen (George Washington University, USA)

Neural induction involves Organizer inhibition of factors that repress neural genes: BMP, Wnt & Nodal. However, Organizer genes probably activate early neural ectodermal genes as well. We previously determined the transcriptional relationship between 4 early neural ectodermal genes that form a gene regulatory network controlling the progression of neural ectodermal precursors to neural stem/progenitor cells. We next studied the interactions between these neural genes, and the Organizer genes to provide a more-complete view of neural induction. We used an ectopic induction assay by expressing Organizer genes in *Xenopus* blastomere precursors of epidermis and assaying the expression of FoxD4L1, Sox11, Gmnn, & Zic2 by ISH at neural plate stages. We found that the factors that induced FoxD4L1/Sox11 & Gmnn/Zic2 were not identical. Induction of FoxD4L1 & Sox11 required blocking BMP & Wnt signaling, whereas Gmnn & Zic2 were induced by blocking only Nodal or BMP. FoxD4L1 & Sox11 were only induced by Siam & Twn, whereas Gmnn & Zic2 were induced by Siam & Twn, which partially required FoxD4L1 activity, as well as by Lim1, FoxA4, Otx2 & Pou2. FGF but not Nodal was required for the Siam-mediated induction of all 4 genes. These findings show that two independent pathways lead to neural gene expression. In one, Siam/Twn activate FoxD4L1 transcription, directly and indirectly via FGF signaling and in the absence of both BMP & Wnt signaling, and FoxD4L1 in turn activates Sox11, Gmnn & Zic2. In the second, Gmnn & Zic2 are activated by other Organizer factors, both directly and indirectly in the absence of BMP and/or Nodal. These studies provide greater detail of the molecular interactions that regulate the induction of neural ectoderm.

Program/Abstract # 269

To be or not to be - mutually exclusive neural and non-neural ectodermal competence territories are established at the neural plate border

Schlosser, Gerhard (National University of Ireland), Pieper, Mareike; Ahrens, Katja (Brain Research Institute, University of Bremen, Germany)

Cranial placodes give rise to many sensory organs and ganglia of the vertebrate head. All placodes are now known to arise from a common panplacodal primordium located around the anterior neural plate. It has been proposed that this primordium and the neural crest arise from a common precursor, the neural plate border region. However, using tissue grafting in *Xenopus* embryos we show that during gastrulation two mutually exclusive competence territories are established at the neural plate border. Whereas competence for induction of neural plate, neural plate border and neural crest markers is confined to neural ectoderm, competence for induction of panplacodal markers is confined to non-neural ectoderm. We also show that Dlx3 and GATA2 are required cell-autonomously for panplacodal and epidermal marker expression in the non-neural ectoderm, while ectopic expression of Dlx3 or GATA2 in the neural plate suppresses neural plate, border and crest markers. Overexpression of Dlx3 (but not GATA2) in the neural plate is sufficient to induce different non-neural markers in a signaling dependent manner, with epidermal markers being induced in the presence, and

panplacodal markers in the absence of BMP signaling. Taken together, this suggests a non-neural vs. neural origin of placodes and neural crest, respectively, strongly implicates Dlx3 in the regulation of non-neural competence, and shows that GATA2 contributes to non-neural competence but is not sufficient to promote it ectopically.

Program/Abstract # 270

Comparative analysis of the promoter sequences of the MADS-Box Gene APETALA3 from the homeotic flower Lacandonia schismatica and its sister taxon, Triuris brevistylis (Triuridaceae)

Rodriguez Mega, Emiliano (UNAM, Mexico); Piñeyro Nelson, Alma (UC Berkeley, USA); Garay Arroyo, Adriana (UNAM, Mexico); Álvarez-Buylla, Elena (UC Berkeley, USA)

The *Lacandonia schismatica* plant presents unusual "inside-out" hermaphrodite flowers where central stamens are surrounded by peripheral carpels. Based on the ABC model of floral organ determination, the simplest explanation for this phenotype is that B-function has been displaced towards the flower center. This hypothesis has been corroborated in recent works, where it has been shown that the spatio-temporal expression patterns of *LsAP3* is restricted to the apex of the flower meristem in all stages of floral axis development. Thus, during ontogeny, the concomitant expression of *LsAP3* and *LsPI* in the center of the floral meristem enables B-function to be exerted. Although the genetic-molecular data obtained up to date provide a sufficient explanation for the unique spatial inversion of stamens and carpels in *L. schismatica* flowers, the molecular bases for the atypical expression pattern of *LsAP3* are still unknown, but could be due to changes in the *cis*-regulatory sequences of this gene (putatively involving mutations in *cis*-regulatory motifs in the promoter sequences), or due to alterations in *trans*-regulatory factors. In order to further understand the *LsAP3* regulation in *L. schismatica*, we address the *cis*-regulatory changes that could have occurred in the promoter region of *LsAP3* and compare them with those occurring in the promoters of the two AP3 genes present in *Triuris brevistylis* (*TbAP3v1* and *TbAP3v2*), the dioecious sister taxon of *L. schismatica*. The two principal strategies used in this investigation will be the detection of conserved motifs through phylogenetic footprinting analyses of all three promoter sequences, as well as the generation of GUS reporter lines in *Arabidopsis thaliana* for each AP3 promoter. This work is supported by CONACyT (180098; 180380; 167705; 152649; 105678) and DGAPA, UNAM (IN204011-3; IN203113-3; IN226510-3; IB201212-2) grants.

Program/Abstract # 271

Investigating the role of MADS-box protein networks in the establishment of the floral meristem of Lacandonia schismatica and Triuris brevistylis

Herrera Martinez, Joel (UNAM, Mexico); Piñeyro-Nelson, Alma (UC Berkeley, USA); Garay-Arroyo, Adriana; Álvarez-Buylla, Elena (Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico)

The study of homeotic genes and their interactions in angiosperm model systems has enabled the unraveling of the Gene Regulatory Networks (GRN) that underlie floral organ specification. According to the ABC model of floral organ determination, three different classes of functions undertaken by particular homeotic genes determine the identity of floral organs; A class specifies sepals; A and B specify petals; B and C specify stamens and C specifies carpels. Extensions of this model have incorporated the function of the *SEPALLATA* (*SEP*) genes into the E class. *SEP* genes are expressed in all floral whorls and are necessary for ABC gene function. The ABCE genes are transcription factors of the MADS-box family (except the A function gene *AP2*), and have been shown to exert their function through the formation of whorl-specific protein tetramers. It has been proposed that changes in the protein interaction patterns among MADS-box transcription factors may lead to shifts in floral organ morphogenesis, favoring the evolution of novel floral arrangements as the one found in the genus *Lacandonia* (Triuridaceae), which harbors the only two known bisexual species with central stamens surrounded by carpels. The developmental and molecular genetic bases for this unique phenotype have started to be unraveled but it remains to be investigated if the protein-protein interactions that occur among endogenous floral MADS-box genes have evolved to have different interactions and/or affinities that directly impact floral morphogenesis in *Lacandonia*. In this work we tested the protein-protein interactions of endogenous B, C and E genes of *L. schismatica* and its sister taxon *Triuris brevistylis* in a Yeast two and three Hybrid System. This work is supported by CONACyT (180098; 180380; 167705; 152649; 105678) and DGAPA, UNAM (IN204011-3; IN203113-3; IN226510-3; IB201212-2) grants.

Program/Abstract # 272

The Floral Organ Cell Fate Determination Gene Regulatory Network: A Network-level Molecular Evolutionary Analysis Across 18 Angiosperm Genomes

Davila-Velderrain, Jose (Universidad Nacional Autonoma de Mexico, Mexico); Servin-Marquez, Andres (Universidad Autonoma de Nuevo Leon, Mexico); Alvarez-Buylla, Elena R. (Universidad Nacional Autonoma de Mexico, Mexico)

The floral organ specification gene regulatory network (FOS-GRN) of *Arabidopsis* has become a model GRN for Systems Biology approaches to developmental mechanisms. The FOS-GRN constitutes a robust developmental module which orchestrates the sub-differentiation of floral meristemic cells during early stages of flower development. Genetic and expression analyses in diverse species have suggested that some components of this FOS-GRN tend to share similar developmental functions in flower morphogenesis. However, we still do not know to what extent are the FOS-GRN components conserved during angiosperm evolution, or the constraints that the network structural and dynamical properties impose upon the molecular evolution of such components. To approach such questions, we performed a comparative genomics approach and evaluated the conservation of the network components in 18 flowering plant species that span angiosperm evolution and which have fully annotated genomes. We then conducted molecular

evolutionary analysis to study the patterns of sequence evolution of all the FOS-GRN genes during angiosperm evolution. We found that all the genes of the FOS-GRN are generally conserved, and we also found several gene duplications in certain FOS-GRN nodes. Our analyses suggest that strong functional constraints have been the main force driving the evolution of the genes in the FOS-GRN, although some nodes seem to have been more constrained than others. Overall, our results are consistent with a scenario where the functional (dynamical) independence of a developmental module and the strong interdependence of its components play a major role in constraining evolutionary rates at the molecular level of individual module components.

Program/Abstract # 273

Of Butterfly Wings and Hopeful Monsters: the loci of discrete evolution

Martin, Arnaud (Cornell Univ, USA); Papa, Riccardo (Univ of Puerto Rico-Rio Piedras, Puerto Rico); Orgogozo, Virginie (CNRS, France); McMillan, Owen (Smithsonian Tropical Research Inst, Puerto Rico); Reed, Robert (Cornell Univ, USA)

Is phenotypic evolution gradual and consisting of many mutations of small effects, or saltational and based on *Hopeful Monster* mutations resembling developmental biology experiments? Answering this question empirically requires the identification of the genes that drive variation in the wild. The wing patterns of *Heliconius* butterflies are involved in predator avoidance and are a model of choice for the study of developmental evolution due to their explosive diversity. We fine-mapped two genes responsible for pattern variation in this genus. Cis- regulatory variants of the transcription factor optix switch color pattern identities prior to pigmentation[1] , and cis- regulatory variants of the *WntA* ligand determine differences in pattern shape[2] . Both genes qualify as genetic hotspots of evolution since they have been repeatedly involved in driving adaptive phenotypic variation[3] . How widespread is this gene re-use phenomenon? In a compilation of 1000 alleles that cause phenotypic differences among animals, plants and yeasts[3] , more than 100 hotspot genes drove the repeated evolution of a wide range of traits. While *Hopeful Monsters* are uncommon, several natural alleles of large effect have been shown to result from the aggregation of multiple small-effect mutations at the same hotspot locus, thus reconciling micromutationist models with the empirical observation of large-effect variants. This synthesis suggests that evolution is repeatable, gradual at the mutational level, and that phenotypic saltation is due to an accumulation of mutations at single genes. [1]Reed RD et al. 2011 Science 333:1137-1141 [2]Martin A et al. 2012 PNAS 109:12632-12637 [3]Martin A, Orgogozo V. 2013 Evolution 10.1111/evo.12081

Program/Abstract # 274

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

Werner, Thomas (Michigan Technological University, USA); Shigeyuki, Koshikawa (U of Wisconsin-Madison, USA) Williams, Thomas (Univ of Dayton, USA); Bollepogu Raja, Komal Kumar (Michigan Technological Univ, USA); Carroll, Sean (Univ of Wisconsin-Madison, USA)

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 of convergent evolution. We optimized an in situ hybridization technique for the developing thorax and abdomen and show that the *yellow* and *tan* genes are expressed in identical patterns 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* and *tan* expression patterns.

Program/Abstract # 275

Insights into origin of new elements in the Dorso-Ventral patterning network in dipterans.

Hodar, Christian; Cambiazo, Veronica (INTA - Universidad de Chile - CRG , Chile)

Evolutionary changes of Dorso-Ventral patterning network (DVN) among Diptera, results in morphology changes of the embryonic ectoderm. Thus, *Drosophila* species differentiate a single extra-embryonic membrane, the amnioserosa (AS), in contrast to the amnion and serosa observed in lower dipterans. This transition might have involved the recruitment of new genes to the network. Some components of the DVN have been compared between *Drosophila* and other lower dipterans, which shared a common ancestor 150-200My ago but, since AS origin was estimated ~100My ago and *Drosophila* radiation occurs after that event, the question then arises as to if members of the DV network are novelties in *Drosophila* lineages or they appear concomitant with the amnioserosa origin. Using RNA sequencing of early embryos of *Musca domestica* (~100My of divergence with *D. melanogaster*) we searched orthologs of DVN to determinate whether their expression correlated with amnioserosa origin. We identified an ortholog for *D. melanogaster* CG6234 gen, which until now had been only found in Drosophilidae group and loss-of-function of this gene results in AS alterations. In situ hybridizations (ISH) in whole-mount embryos reveal a conserved temporal and spatial expression of CG6234 mRNA.

Additionally, using ISH and immunofluorescence we determine the expression pattern of zen and localization of phosphorylated Mad (pMad), main regulators of the dorsal ectoderm patterning in *Drosophila*. Our results suggest that the genetic network triggered by Mad and Zen in dorsal domains of *Drosophila* are conserved in other cyclorrhaphan flies and CG6234 might be originated prior *Drosophila* radiation as part of the network involved in amnioserosa formation. Fondecyt 3110129 & 1120254

Program/Abstract # 276

The arthropod segmentation clock and what it tells us about the origin and evolution of segmented body plans

Peel, Andrew (Univ of Leeds, UK); Sarrazin, Andres (Pontificia Universidad Catolica de Valparaiso, Chile); Averof, Michalis (Institut de Genomique Fonctionnelle de Lyon, France)

Many biological processes occur under the control of 'molecular clocks'. One such process is the rhythmical formation of mesodermal somites in an anterior-to-posterior progression along the primary body axis of vertebrate embryos. Somites are blocks of tissue that give rise to segmented structures; e.g. vertebrae and their associated muscle. In collaboration, Andrés Sarrazin, Michalis Averof and myself have recently provided rigorous proof that the body units (segments) of an arthropod, the beetle *Tribolium castaneum*, also form via the activities of a segmentation clock. We have shown that homologues of *Drosophila* pair-rule genes (*odd-skipped* and *even-skipped*) exhibit oscillatory expression, with a period of 95 minutes (at 30°C), within cells of the posterior growth zone during the formation of *Tribolium* abdominal segments. This finding suggests that vertebrate somites and arthropod segments form using similar developmental principles. Given the evolutionary distance separating vertebrates and arthropods this finding might also imply that a segmentation clock played an ancient and ancestral role in animal development. However, vertebrate segmentation clocks consist of a complex network of 40-100 oscillating genes, none related to the two known *Tribolium* oscillating genes. It is therefore too early to conclude that the arthropod and vertebrate segmentation clocks are evolutionarily related. I will report our recent work and discuss what it does, and does not, tell us about the origin and evolution of segmented body plans. More generally, I will discuss what recent studies on arthropod segmentation mechanisms have taught us about the evolution of developmental gene networks.

Program/Abstract # 277

Actin-based cytokinetic twist breaks Left-Right symmetry in C. elegans

Tiongson, Michael; Bao, Zhirong (Memorial-Sloan Kettering Cancer Center, USA)

In *C. elegans*, the establishment of the left-right body plan can be traced back to the third cleavage of embryogenesis. At the end of the 4-cell stage, sister cells ABa and ABp initiate division synchronously, with their spindles aligned to the LR axis. As their contractile rings ingress, ABa and ABp each engage in a whole cell twisting movement such that by the end of the division, the left side daughters are moved anterior relative to the right side daughters. At this point, the ABa and ABp daughters intercalate to solidify their cellular positions. As previous micromanipulations showed, this resulting positional bias is sufficient to specify the handedness of the animal. Using micromanipulation to force the right AB daughters more anterior to the left is enough to reverse all normal L\/R asymmetries of the worm. In order to generate a cellular twist, a molecular motor must be implemented to generate such a force. Observing that the twist seemed to be coupled to the cytokinesis ring contraction, we directed our investigation towards actomyosin. We found that 45% of adult worms homozygous for mutant alleles of *act-2* exhibitted situs inversus totalis (reversal of left-right body plan asymmetry), indicating near randomization of handedness choice. Using high-resolution time lapse fluorescent microscopy, we discovered that *act-2* homozygous embryos exhibited similar reversal rates during the 4-6 cell division. In addition, disrupting actin polymerization through *pfn-1* RNAi also resulted in near randomization of handedness at the 4-6 cell division (~40%). In these embryos, ABa and ABp cellular twist is absent and symmetry is not broken until the daughters intercalate randomly at the end of division. Finally, we have observed handed actin structures that correlate with the LR symmetry breaking event. We are currently using quantitative image analysis to investigate how disrupting actin polymerization affects these handed structures and LR symmetry breaking.

Program/Abstract # 278

Non-stochastic assignment of asymmetry in the vertebrate ancestral brain

Boutet, Agnès (Centre de Biochimie, France); Lagadec, Ronan; Laguerre, Laurent; Godart, Benoît; Mazan, Sylvie (CNRS UPMC, France)

L/R asymmetry exists in the vertebrate epithalamus but its evolutionary origin is largely unknown.
This subdivision of the dorsal diencephalon composed of the habenula and the pineal organ/parapineal nucleus displays stricking anatomical asymmetries in many species. In *actinopterygii*, an asymmetric Nodal (*cyc*) expression precedes the differential L/R epithalamus morphogenesis. However in zebrafish Nodal signalling is not controlling the habenular asymmetry *per se* but the migration of the parapineal organ toward the left habenular nucleus. From these results it has been proposed that Nodal signalling was not breaking symmetry in the brain but rather directing laterality. On the other hand, since the asymmetric nodal expression has not been reported in any other vertebrate taxon, it was thought that the asymmetry was stochastic at the basis of the vertebrate lineage. In this work we have characterized components of the Nodal pathway from a *chondrychthe* and an *agnatha* and found them all expressed in the embryonic diencephalon. In addition this expression was always reported on the left in both species. Clear molecular asymmetries were reported in the habenula of catsharks in spite of the absence of parapineal nucleus. Lampreys are not devoid of parapineal but the organogenesis of this structure starts clearly after habenular asymmetry set up suggesting that the differential morphogenesis of the habenula is not depending on a nodal-dependant migration toward the left of the parapineal as it is the case in

teleostei. Our data evidenced that the brain asymmetry program has originated in the dorsal diencephalon independently of parapineal migration and that the laterality imposed by Nodal is an ancestral vertebrate trait.

Program/Abstract # 279

Early, nonciliary role for microtubule proteins in left–right patterning is conserved across kingdoms

Lobikin, Maria (Tufts University, USA); Wang, Gang; Xu, Jingsong (Univ of Illinois College of Medicine, USA); Hsieh, Yi-Wen; Chuang, Chiou-Fen (Cincinnati Children's Hospital Research Foundation, USA); Lemire, Joan; Levin, Michael (Tufts University, USA)

Many types of embryos' bodyplans exhibit consistently oriented laterality of the heart, viscera, and brain. Errors of left–right patterning present an important class of human birth defects, and considerable controversy exists about the nature and evolutionary conservation of the molecular mechanisms that allow embryos to reliably orient the left–right axis. We have found that the same mutations in the cytoskeletal protein tubulin that alter asymmetry in plants also affect very early steps of left–right patterning in nematode and frog embryos, as well as the chirality of human cells in culture. Our unbiased proteomic analysis identified multiple maternal proteins that are consistently localized to the left and right of the *Xenopus* embryo during the first two cleavages. In the frog embryo, tubulin α and tubulin γ-associated proteins are required for the differential distribution of these maternal proteins to the left or right blastomere at the first cell division. Our data reveal a remarkably-wide molecular conservation of mechanisms initiating left–right asymmetry and characterize novel aspects of left-right patterning occurring within the cytoplasm of early blastomeres. The origin of laterality is intracellular, ancient, and highly conserved across kingdoms - a fundamental feature of the cytoskeleton that underlies chirality in cells and multicellular organisms.

Program/Abstract # 280

Evolution of Placode-Derived Neurons Assessed by Cell Type-Specific Transcriptional Profiling

Shimeld, Sebastian; Patthey, Cedric (University of Oxford, UK)

A major challenge in understanding the evolution of the vertebrate body plan is to model how gene usage evolved to produce the cranial placodes from which the paired sensory organs arise. Although our knowledge of placode development is growing, the function and evolution of the genetic regulatory networks underpinning the specification of differentiated cell types are not well known. In particular, we lack specific molecular markers for the placode-derived neurons. Combining dissection, FACS sorting and next-generation sequencing in chicken, we have developed a method for the establishment of cell type-specific transcription profiles in order to study the evolution and development of sensory neuronal cell types.

Program/Abstract # 281

SeaBase – A new tool to analyze RNAseq data and a big step on our way toward a Nematostella gene interaction network

Fischer, Antje (MBL,USA); Cosentino, Carlo (Università degli Studi Magna Graecia Catanzaro, Italy); Smith, Joel (MBL, USA)

The evolutionary origin of fundamental developmental programs such as axis specification and germ layer specification is still unsolved. Cnidarians, the sister group of Bilaterians, have a seemingly simpler body plan and form only two embryonic germ layers, a one-way gut and are traditionally viewed as radial symmetric. Many of the embryonic patterning genes in Bilaterians are present in Cnidarians with little known about their gene regulatory network (GRN). The sea anemone *Nematostella vectensis* is a particularly suitable cnidarian model system. We present our first steps towards resolving the GRN for early development in *Nematostella*. Using an Illumina HiSeq to perform quantitative RNA-seq, we established a high-density gene expression time course, from fertilization to gastrulation. We use SeaBase, a new multispecies web resource for sharing and analyzing RNAseq data, to visualize and compare absolute transcript levels for each gene. SeaBase is a powerful tool for differential gene expression analysis, between different developmental stages, perturbations and species. The comparison of gene expression levels will help to determine the statistical dependencies between all gene pairs, a measure of genetic interaction. The resulting testable network model will offer first insights into which genes are the most interconnected and thus provide the starting point for detailed network analyses. Comparing the *Nematostella* GRN to known interactions in other species will advance our understanding about evolutionary changes of the developmental GRN across metazoans. To initiate the comparison we are performing quantitative RNA-seq time courses in the snail *Crepidula fornicata* and the ctenophore *Mnemiopsis leidyi*.

Program/Abstract # 282

Evolutionary Origins of the Vertebrate "New Head"

Abitua, Philip; Wagner, Eileen; Levine, Mike (UC Berkeley, USA)

In their classic paper (Science 220, 268-273; 1983) Gans and Northcutt proposed that, "most of the morphological and functional differences between vertebrates and other chordates occur in the head…". Several distinctive features of the vertebrates arise from cranial placodes and a special form of neural crest, the ectomesenchyme, which produces a variety of mesodermal derivatives including connective, skeletal and muscular tissues. We are using the simple sea squirt, *Ciona intestinalis*, to investigate the evolution of these cell types since it is a close relative of the vertebrates. I will provide evidence that the *Ciona* tadpole contains a cell lineage that is homologous to neural crest, which can be reprogrammed into "ectomesenchyme" by the misexpression of *Twist*. Additional studies suggest that the anterior neural plate border of the Ciona embryo produces GnRH-expressing neurons, which we are studying to elucidate the origins of the chordate endocrine system.

Program/Abstract # 283

Your Inner Inner Fish: Analysis of Pharyngeal Segmentation in Vertebrates

Shone, Victoria; Graham, Anthony (MRC Centre for Developmental Neurobiology, UK)

Pharyngeal arches are bulges found on the lateral surface of the head of vertebrate embryos. They form a segmented series that the pharynx is built on, and a complex array of tissues and signals are involved in their formation. Arches become segmented when endodermal pharyngeal pouches develop and elongate along a dorsoventral axis, eventually fusing with the ectodermal pharyngeal clefts. We aim to determine if a Hox code present within the endoderm is responsible for regionalization of the pouches. We also aim to characterise the interaction between ectoderm and endoderm when they make contact, and to analyse the differences in pouch morphology within and across different vertebrate species. To analyse this interaction, ectoderm and endoderm were separately labelled using various methods including fluorescent immunohistochemistry, CCFSE labelling, and lineage tracing in mouse and zebrafish reporter lines. This work has revealed how these tissues interact at each of the pouches, generating the unique morphology of each pouch which is maintained as they mature. Another interesting feature within vertebrates is the varying number of pharyngeal arches: lamprey have 9, sharks have 7 and chicks have 5. To elucidate how these arches were lost during vertebrate evolution, we have used neuronal antibody labeling to determine where cranial nerve innervation has been lost and hence where the arches have been lost. These results will be discussed.

Program/Abstract # 284

In vivo evidence for a novel and direct role of Cdx proteins in trunk neural crest cell development

Sanchez, Oraly; Pilon, Nicolas (UQAM, Montreal, PQ, Canada)

Cdx genes (*Cdx1, Cdx2* and *Cdx4*) encode homeodomain transcription factors critical for development of the posterior chordate embryo with notable well-known key roles in anterior-posterior (AP) patterning and axial elongation. Cdx members are expressed in all three germ layers of the caudal embryo from e7.5 to e12.5. However, specific Cdx functions in the neurectoderm are still poorly understood because of functional redundancy and early embryonic lethality. Moreover, although important emergent roles for Cdx proteins in neural tube and neural crest formation have been described, it is still unknown whether these roles are tissue-autonomous or not (via the mesoderm). To circumvent functional redundancy and early embryonic lethality as well as to address a direct role for Cdx proteins in neural development, we have generated a conditional (Cre-dependent) pan-Cdx loss-of function mouse model expressing a previously described Cdx dominant negative fusion protein (EnRCdx1; Sanchez-Ferras et al., JBC 2012) under the control of the ROSA26 promoter. The efficacy of this approach has been validated by crossing our novel line, called $R26R^{EnRCdx1/+}$, with $T\text{-}Cre^{Tg/+}$ mice. In agreement with the key Cdx role in mesodermal AP patterning as well as the dominant nature of EnRCdx1, $R26R^{EnRCdx1/+}$::$T\text{-}Cre^{Tg/+}$ double transgenic mice display vertebral patterning defects as severe as those observed in Cdx compound mutants. Most interestingly, directing expression of EnRCdx1 specifically in the dorsal neural tube and pre-migratory neural crest cells using the *Pax3pro-Cre* line results in pigmentation defects that phenocopy those observed in $Pax3^{Sp/+}$ mutants. Taken together with other data describing a direct and crucial regulation of a *Pax3* neural crest enhancer by Cdx proteins, our work constitutes the first *in vivo* demonstration for a direct role of Cdx proteins in trunk neural crest cell development.

Program/Abstract # 285

Endothelin Signaling Balances Identity of Neural Crest Cells in the First Pharyngeal Arch

Tavares, Andre Luiz Pasqu; Clouthier, David (Univ. of Colorado at Denver, USA)

Endothelin-1 (Edn1)-induced signaling of the endothelin-A receptor (Ednra) is crucial for dorsal-ventral (D-V) patterning of cranial neural crest cells (CNCCs) within the mandibular pharyngeal arch. Targeted deletion of *Edn1* or *Ednra* in mice causes perinatal lethality due to severe craniofacial birth defects that include homeotic transformation of mandibular arch-derived structures into more maxillary-like structures, indicating a loss of NCC identity. CNCCs express *Ednra* whereas Edn1 is derived from the overlying ectoderm, core paraxial mesoderm and pouch endoderm. To define the pathways that establish a more dorsal/ ventral fate in the first pharyngeal arch, we created transgenic mice containing a silent Edn1 expression cassette (*CBA-Edn1*). When Edn1 was overexpressed in CNCCs (*CBA-Edn1;Wnt1-Cre*), the overexpression caused a homeotic transformation of maxillary structures into more mandibular-like structures. This transformation was preceded by a proximal expansion of distal genes (*Dlx3, Dlx5* and *Hand2*) and disruption of proximal genes (*Dlx2, Twist1, Pou3f3, Six1* and *Eya1*) in the first arch. We focused on Six1, as it plays roles in vertebrate development, with mutations in Six1 and/or its partner Eya1 found in patients with branchio-oto-renal syndrome, a developmental disorder that is characterized by hearing loss, branchial arch defects and renal anomalies. While *Six1*-null mouse embryos have facial defects, deletion of one allele of Ednra partially rescued the defect ($Six1^{-/-};Ednra^{+/-}$). Together, our results suggest that proximal first pharyngeal arch is competent to form either a mandible or a maxilla and that inductive/repressive signals that include Six1 and Edn1 are responsible for driving CNCCs into either fate.

Program/Abstract # 286

Craniofacial Ontogeny in Turtles: putative role of bone morphogenetic proteins in the lack of palatal shelves

Abramyan, John; Leung, Kelvin; Richman, Joy (University of British Columbia, Canada)

Turtles are an enigmatic group of vertebrates whose unique skull morphology is still at the forefront of scientific discussion. While turtles pass through a conserved stage of primary palate development found in all amniotes, they diverge during secondary palate

ontogeny. The typical condition for amniotes is to form outgrowths from the medial sides of the embryonic maxillary prominences called palatal shelves. In mammals, the shelves fuse in the midline and form a bony hard palate that completely separates the nasal and oral cavities. In birds and squamates, palatal shelves develop on the lateral sides of the oral roof but remain unfused, leaving a natural cleft. In our study, we conclusively excluded the presence of vestigial palatal shelves at any time during the ontogeny of the craniofacial complex in the turtle (*Emydura subglobosa*). Furthermore, through comparative analysis of avian and testudine craniofacial gene expression patterns, we have identified a distinct lack of mesenchymal Bone Morphogenetic Protein 2 (BMP2) expression in the maxillary prominences of *E. subglobosa*, a protein that is required for proliferation in the face. This lack of expression was in turn correlated with a lack of proliferation increase expected in the putative palatal shelf outgrowth region when compared to chicken embryos. In previous work we showed that when BMP signaling is blocked in the chicken embryo maxillary prominence, a complete loss of palatal shelves occurs. We propose that the absence of BMP expression in the maxillary prominences is associated with the lack of proliferation and this contributes to palatal shelf loss in turtles. JA is a recipient of an NIH F32 award, the work was funded by an NSERC operating grant to JMR.

Program/Abstract # 287

Expression timing of Gdf11 reveals positional diversity of the hindlimb in vertebrates

Suzuki, Takayuki; Matsubara, Yoshiyuki (Nagoya University, Japan); Hattori, Ayumi; Ogura, Toshihiko (Tohoku University, Japan); Se-Jin, Lee (Johns Hopkins Univ, USA); Kuroiwa, Atsushi (Nagoya University, Japan)

Previously, we have reported that *Tbx5/Tbx4* specified wing/leg identity in the chick, and they were necessary and sufficient for limb initiation. In this research, we were looking for upstream molecule of *Tbx5/Tbx4*. We found that GDF11 could control *Tbx4* and *Pitx1* expression in the chick lateral plate mesoderm (LPM). *Gdf11* knockout mice show homeotic transformation of rostral bone to caudal bone, including posterior shift of hindlimb position. We studied expression pattern of *Gdf11* in the chick embryos. *Gdf11* expression starts suddenly from 10 somite stage at presomitic mesoderm (PSM). After implantation of bead soaked with GDF11, ectopic *Pitx1* and Tbx4 expression were induced, compared to inhibition of *Tbx5* expression. Further, when we co-implanted FGF8 expressing cells with GDF11, only *Tbx4* was induced in extra limb bud. This extra limb had leg type structure morphologically. We also found that ALK4 receptor signaling is involved in GDF11 signaling at LPM by mouse whole embryo culture. We conclude that expression timing of *Gdf11* at PSM determines hindlimb position through *Pitx1* and *Tbx4* expression at LPM. In this conference, we will also show expression timing of *Gdf11* in several tetrapod species and discuss how leg field starts to develop in vertebrates.

Program/Abstract # 288

The participation of Wnt/Ca+ signaling and the Wnt antagonists DKK and SFRP in digit formation during limb development.

Farrera Hernandez, Alejandro ; Bustamante, Marcia; Flores-Hernández, Erick; Robles-Flores, Martha; Orozco-Hoyuela, Gabriel; Chimal-Monroy, Jesús (UNAM, Mexico)

During limb embryogenesis, mesenchymal cells condense to give rise to chondrogenic blastema that prefigures the skeletal elements. Limb chondrogenesis begins once Sox9 is expressed, promoting the formation of precartilage condensations and the subsequent differentiation. It is known that WNT/β-catenin induces proliferation in the distal zone of the limb and inhibits chondrogenesis. However, has been proposed that the non-canonical Wnt pathways oppose Wnt/β-catenin pathway. On the other hand, albeit Wnt5a promotes to Sox9 expression and chondrogenesis, *in vitro* promotes by stimulating calcium signaling by CAMKII and NFAT activation, its role in limb development is poor known. The aim of this work was if Wnt/Ca+ participate in the Digit Crescent (DC), for this purpose, we determine the presence of intracellular calcium with FURA 2AM. Results showed calcium staining near the DC. To see differences in Wnt signaling, we implanted presoaked beads with antagonists and put them in the tip of the digits, we used two types of Wnt antagonist; DKK1, a specific Wnt/β-catenin antagonist and SFRP1 a general Wnt antagonist. In both treatments we observed absence of phalanges by cartilage staining; also the treatment with the NFAT inhibitor 11R-VIVIT blocks the chondrogenesis at the distal zone of the limb, reducing the size of phalanges or with the complete loss of the cartilage. We observed differences of *Sox9* expression between both WNT antagonists; while Dkk1 increased *Sox9* expression in the first 12 hours, SFRP1 reduce *Sox9* expression in the first 8 hours. This data suggest that Wnt/Ca+ signaling may participate in DC chondrogenesis. Support: CONACyT grant 53484 and 168642, DGAPA-UNAM grant IN214511 and IN220808.

Program/Abstract # 289

The Origin of the Thumb Patterning System

Tanaka, Mikiko; Onimaru, Koh (Tokyo Institute of Technology, Japan)

Evolution of gene regulatory networks is regarded as a driving force of morphological evolution. Recent molecular studies for vertebrate fin-to-limb transformation suggests that autopod-related gene regulation predate the acquisition of autopod structures. However, the mechanism of how gene regulatory changes led to fin-to-limb transformations remains unknown. Here, we examined fin development of a dogfish *Scyliorhinus canicula*, and provide evidence suggesting that anterior-posterior patterning changes in fins could have triggered anatomical transformations such as digit acquisition and reduction of fin radials. Anterior radials of *S. canicula* fins expressed a gene set shared with the anterior elements of mouse limbs such as digit I, radius and deltoid process. Nevertheless, a homologous element of *Gli3* anterior limb enhancer from chondrichthyan drove reporter expression throughout the limb buds of chick embryos. Thus the anterior-posterior patterning system may have been modified through alteration of the *cis*-element of *Gli3*, resulting in transformation of anterior fin radials into the digit I, radius, and deltoid process of tetrapod limbs.

Program/Abstract # 290

Developmental genetics of evolved tooth gain in sticklebacks

Cleves, Phillip; Jimenez, Monica (UC Berkeley, USA); Nunez, Stephanie (Univ. of Michigan, USA); Schluter, Dolph (University of British Columbia, Canada); Kingsley, David (Stanford U. and HHMI, USA)

Teeth are a classic model for studying organogenesis and morphological evolution. Despite the incredible phenotypic diversification in dentition in vertebrates, our understanding of the molecular and developmental basis behind this variation is limited. A derived benthic freshwater stickleback population has evolved a two-fold gain in ventral pharyngeal tooth number compared to their ancestral marine counterparts. This evolved tooth gain provides an excellent system to study the molecular basis of evolved dental variation. To ask when during development evolved tooth gain appears, we generated lab-reared developmental time courses of a low-toothed marine population and this high-toothed freshwater population. Early in development, no differences in dental patterning are observed. However, at late larval stages, differences in tooth number, an increase in tooth plate area, and a decrease in tooth spacing arise. We identified genomic regions controlling these evolved patterning changes by mapping quantitative trait loci (QTL) controlling tooth number, area, and spacing in a marine by freshwater F2 cross. One large effect QTL controlling tooth number fine-maps to a genomic region containing an excellent candidate gene, *Bone morphogenetic protein 6 (Bmp6)*. Stickleback *Bmp6* is expressed in developing teeth, and no coding changes are found between the populations. However, by quantitatively comparing allele specific expression of *Bmp6*, we find *cis*-regulatory changes have elevated the relative expression level of the freshwater *Bmp6* allele at late, but not early, stages of development. Ongoing genetic and transgenic approaches will functionally test *Bmp6* as a candidate for underlying evolved tooth gain in sticklebacks.

Program/Abstract # 291

The Roles of Canonical Wnt Signaling in Developing Teeth of Polyphyodont Lizards

Holmes, Scott N.; Richman, Joy (University of British Columbia, Canada)

Though most dentate vertebrates replace their teeth at least once in the course of their lives, the process of tooth replacement is poorly understood. This is mainly because the major tooth development model is the mouse which only has one generation of teeth. Our previous work suggested that tooth renewal in geckos might involve dental epithelial stem cells and that these putative stem cells become transit amplifying cells when exposed to canonical Wnts. Here we further investigate this idea using adult leopard geckos (*Eublepharis macularius*). First we mapped areas of high canonical WNT signaling using antibodies to activated beta-catenin. Nuclear staining was principally found in the outer enamel epithelium and successional lamina. Ameloblasts were noticeably negative for beta catenin. Injections of 1M LiCl over a 1 week period caused a significant increase in proliferation in the successional lamina ($p<0.05$), outer enamel epithelium and cervical loop ($p<0.001$) as compared to the control side which was injected with NaCl. Areas not affected by LiCl included the dental lamina next to the terminal tooth, a region previously shown to contain label-retaining cells. One putative ligand that could be regulating proliferation in vivo is *Wnt7a* since expression is highest in the outer and inner enamel epithelium. Based on our work, we conclude that proliferation of defined regions of the dental epithelium is regulated by canonical Wnt signaling. Slower dividing regions of the dental lamina that contain putative stem cells may require signals in addition to Wnts to stimulate the formation of transit amplifying cells.

Program/Abstract # 292

Dlx2 overexpression disrupt the development of teeth in mouse

Dai, Jiewen (Shanghai Ninth People's Hospital, Shanghai Jiao Tong University, China); Wang, Xudong (Shanghai Ninth People's Hospital, Shanghai Jiao Tong University, Shanghai Key Laboratory of Stomatology, China); Shen, Guofang (Shanghai Ninth People's Hospital, Shanghai Jiao Tong University, China)

Objective: To explore the role of Dlx2 overexpression in the teeth development in mammal. Method: A transgenic mouse for specifical overexpression of Dlx2 in cranial neural crest cell was constructed, and the phonotype in this mouse was observed using gross observation, micro CT scan and histological examination. Result: Except for craniofacial deformities and thoracolumbar spinal kyphosis that was reported in our previous study, the mouse also exhibited abnormity in teeth, including cross-bite in incisor, shortened teeth root, and disrupted histological structure in dentin and cementum. Conclusion: Dlx2 overexpression would disrupt the development of teeth, when combined with previous report that Dlx2 null mutation also would cause abnormal development of teeth, it is clear that the expression level and the spatiotemporal expression patterns of Dlx2 might play crucial and subtle roles in regulating the development of teeth in mammal.

Program/Abstract # 293

Natural "experiments" and Sonic hedgehog in the evolution of odontogenesis

Grieco, Theresa (UC Berkeley, USA)

The high degree of conservation in gene networks for odontogenesis demonstrates that vertebrates have created highly adaptive and morphologically variable phenotypes from similar developmental underpinnings. There is consensus in the developmental literature that the beginning stages of tooth development are marked by a *Sonic hedgehog*-expressing odontogenic band stage which delimits the cells competent to form teeth. Depending on the taxon, this area of epithelium then gives rise to a *Sonic hedgehog*-expressing dental lamina or individual tooth placodes and then to tooth germs from those structures. A survey of odontogenesis literature reveals a few

examples whose *Shh* expression requires additional explanation: snakes and crocodylians. Emerging *in situ* hybridization data from the frog *Silurana (Xenopus) tropicalis* may increase the need to revisit the odontogenic band, as it provides the first tooth gene expression data for any amphibian taxon. I present a developmental series of *S. tropicalis* prometamorphic tadpoles (Nieuwkoop and Faber stages 55-58), the time period encompassing the odontogenesis of first-generation teeth. This series presents an important comparative datapoint in representing a vertebrate whose life history has been modified to include an extended, specialized, and toothless larval stage. I evaluate these comparative data from an evolutionary perspective, identifying phenotypes which indicate that developmental mechanisms for tooth competence, tooth initiation, and mouth formation may be decoupled in developmental time.

Program/Abstract # 294
Endless pigeons most colorful: genetics and development of feather pigment diversity among domestic rock pigeons
Domyan, Eric; Kronenberg, Zev; Guernsey, Michael; Vickrey, Anna; Cassidy, Pamela; Shapiro, Michael (University of Utah, USA)
Centuries of selective breeding have crafted tremendous phenotypic diversity among breeds of domestic rock pigeon (*Columba livia*), providing a rich model to investigate the process of phenotypic variation. To understand the genetic basis of this diversity, we recently sequenced and interrogated the genomes of 40 pigeons from breeds with distinct sets of derived traits. Here we present the preliminary identification of several mutations underlying color variation within and among pigeon breeds. Interestingly, one non-synonymous mutation associated with ash-red feather color is located in the *Tyrosinase-related protein 1* (*Tyrp1*) gene. This dominant mutation reduces the efficiency of signal peptide cleavage. In addition, we identify putative null alleles of this gene that act recessively to cause brown feather color; therefore, the dominant ash-red allele may represent a neomorphic allele. *Tyrp1* is thus a strong candidate for the *blue/ash-red/brown* locus of classical pigeon genetics. We also identify a mutant allele of *Slc45a2* that is associated with a reduction in feather pigmentation and is a strong candidate for the *dilute* locus of classical pigeon genetics. By determining the genotype of birds at these two loci, we are able to explain some of the most common feather colors among domestic pigeons.

Program/Abstract # 295
Developmental basis of phallus reduction during bird evolution
Herrera, Ana M; Shuster, Simone (University of Florida, USA); Perriton, Claire (University of Reading, UK); Cohn, Martin (Howard Hughes Medical Institute, USA)
One of the most puzzling events in evolution is the loss of the phallus in birds. All birds reproduce by internal fertilization, but only ~3% of birds have retained a phallus capable of intromission. Behavioral studies have implicated sexual selection as the evolutionary mechanism responsible for phallus reduction; however, the underlying developmental mechanism is unknown. We investigated external genital development in two sister clades, *Galliformes* (land fowl), which lack an intromittent phallus, and *Anseriformes* (waterfowl), which have well developed phalluses, as well as two out groups, *Paleognathae* (emus) and *Crocodilia* (alligators). Galliform embryos undergo cryptic development of a genital tubercle, but later this undergoes apoptosis and regresses. A derived pattern of *Bmp4* expression was identified in galliform genital tubercles. Functional experiments show that Bmp activity is necessary and sufficient for apoptosis and regression of the genital tubercle. Our results suggest that evolutionary acquisition of a novel *Bmp4* domain in the distal genital tubercle underlies loss of an intromittent phallus in galliforms.

Program/Abstract # 296
The evolution of external genitalia: sexual reproduction on dry land
Tschopp, Patrick; Sherratt, Emma; Sanger, Thomas; Groner, Anna; Aspiras, Ariel (Harvard U., USA); Pourquie, Olivier (Strasbourg Univ. Medical School, France); Gros, Jerome (Institut Pasteur, France); Tabin, Clifford (Harvard U.,USA)
The appearance of internal fertilization has been considered a major step in the emergence of terrestrial vertebrates. In mammals, the molecular pathways driving the outgrowth of the genital bud highly resemble those that are active during limb development. Here, we provide a potential explanation for these observed similarities: we show that in squamates (lizards and snakes), the external genitalia develop as a direct outgrowth of the limb bud, or the remnants thereof. CT scan analyses reveal a relative repositioning of the cloaca, an important signaling center during genitalia development, towards the limb field in all squamates investigated. Lentiviral lineage tracing using a novel ex ovo culturing system in anole lizards shows that limb and genitalia share a common embryonic origin in this species, coming from the lateral plate mesoderm. In contrast, mouse and chicken genital buds seem to originate from the tail bud. Comparative RNA-seq analysis of embryonic tissues corroborates this notion, showing more similar molecular fingerprints for the anole genital and limb buds, as opposed to their mouse counterparts. Finally, the importance of cloaca position underlying this genitalia shift is suggested by a series grafting experiments in chicken and mouse. The transplantation of cloacal tissue into the early limb bud leads to tissue outgrowth and ectopic activation of genes normally associated with genital bud development. We speculate that the recruitment of a mesenchymal cell population, namely the early limb bud, by the cloacal signaling center might initially have fostered the emergence of external genitalia, preserving key ancestral gene circuitries thereafter, regardless of subsequent repositioning.

Program/Abstract # 297
Moleculars mechanism control cytoskeletal activities during inner ear invagination
Sai, Xiaorei (Riken CDB, Japan)

The inner ear contains the sensory organs for the hearing and balancing and is embedded within the cephalic mesoderm of the embryo. However it is induced by fibroblast growth factor signals as an epithelial placode in the surface ectoderm adjacent to the posterior hindbrain. In the chick, the inner ear placode first becomes morphologically apparent as a thickening of the ectoderm. It then gradually invaginates to form an otocyst within the head mesenchyme. We have previously showed that basal fibroblast growth factor (FGF) signalling acting through phospholipase Cγ activates basal myosin II. Myosin II exhibits a non-canonical activity that results in the local depletion of actin filaments and thus causes the basal expansion of the otic placode. Subsequent to basal expansion of the inner ear placode, apical constriction drives to complete invagination. We find that the small GTPase protein, RhoA is localized apically and activation of RhoA increases gradually during inner ear morphogenesis. Our data provide evidence that RhoA play bi-functional roles to trigger apical constriction of the otic epithelia and likely, this is FGF independent process. Firstly, RhoA regulates the assembly of junctional actin filaments. Secondly, RhoA controls the apical activation of myosin light chain, the active component of Myosin II. I describe the detail molecular pathway involved in RhoA-coordinated apical constriction during otic morphogenesis.

Program/Abstract # 298

Convergent evolution of cellular immunity in jawless fish

McCurley, Nathanael; Guo, Peng; Cooper, Max (Emory University, USA)

Adaptive immune reactions require a complex choreography of molecules, cells, and tissues, yet this form of immunity appeared suddenly in evolution with the emergence of the vertebrates. The jawless vertebrates (lampreys and hagfish), however, lack the key antigen receptor genes that mediate adaptive immunity in the jawed vertebrate lineage. Instead, jawless vertebrates use a novel gene family encoding leucine-rich repeat proteins that are somatically diversified, monoallelically expressed on lymphocytes, and used as antigen receptors in adaptive immune reactions. These variable lymphocyte receptors (VLRs) are generated during lymphocyte development by a stochastic gene assembly process resulting in a potential antigen receptor repertoire of $>10^{14}$. Our lab identified a VLR lymphocyte population in lampreys (termed VLRA cells) that resembles the T cells of jawed vertebrates by numerous parameters that include development in the recently characterized lamprey thymus equivalent. The nature of the antigen recognized by VLRA receptors remains unknown, though current data suggest that they bind processed antigens. We hypothesize that VLRA cells are involved in cellular immune responses, possibly through recognition of antigen presented in the context of histocompatibility determinants. We devised several experimental approaches to test this hypothesis and our data indicate that VLRA cells respond preferentially to allogeneic stimulation, suggesting a role in cellular immunity. We furthermore used allogeneic immunization to develop antisera that recognize lamprey allodeterminants on myeloid leukocytes, consistent with the hypothesis that these cells express the antigen that drives allogeneic responses.

Program/Abstract # 299

Comparative analysis of the colon in the vertebrate lineage.

Theodosiou, Nicole; Wechter, Todd; Jain, Meaghan (Union College, USA)

During the aquatic to terrestrial transition, vertebrates were challenged by dehydration once on land. The development of a colon allowed tetrapods to maintain water homeostasis and prevent desiccation. In contrast to beliefs that a colon evolved after tetrapod evolution, a rudimentary colon exists in the cartilaginous fish *Leucoraja erinacea* (skate) and *Squalus acanthias* (dogfish). This is surprising because elasmobranchs appeared 450 mya, and thus predate the transition from aquatic to terrestrial life (approximately 370 mya). To understand if elasmobranchs were pre-adapted for conserving water prior to the tetrapod transition, the digestive tracts of representative species from gnathostomes and ray-finned fish are being examined for colon developmental markers and histology. The lamprey *Petromyzon marinus* contains a discreet region in the distal intestine with elevated levels of acid mucin-producing goblet cells, cell markers for the colon. A similar region with colon-like cells is found in the distal intestine of the ray-finned fish *Polyodon*. In addition, there is a dorsal-ventral asymmetry to the acid mucin distribution in the lamprey and paddlefish. The dorsal-ventral asymmetry in lamprey and paddlefish suggests a common origin for the colon in vertebrates. However, the asymmetry is not observed in different species of elasmobranchs suggesting that a rudimentary colon may have been advantageous to elasmobranchs, allowing them to adapt to different environments with changing salinities. Together these data suggest that the colon arose prior to the tetrapod transition and expanded in the vertebrate lineage.

Program/Abstract # 300

The role of lbx1 during *Xenopus* and *Nematostella* embryogenesis – a comparative study of myogenesis in metazoans

Strobl, Anna-Christina (NIMR MRC, UK) Steinmetz, Partick; Fredman, David; Technau, Ulrich (University of Vienna, Austria); Smith, Jim (NIMR MRC, UK)

In vertebrates, one of the main tissues derived from the mesodermal germ layer is muscle. The early branching metazoans, however, lack mesoderm, and their muscle derives from the endoderm. We are studying the evolutionary relationship between cnidarian and vertebrate myogenesis to improve our understanding of the mechanisms regulating muscle development. Although the cnidarian *Nematostella vectensis* does not possess mesoderm, many 'mesodermal' transcription factors are encoded in its genome. We dissected the function of one such gene, the NK-homeobox transcription factor *ladybird* (*lbx1*), which is involved in muscle formation in both *Nematostella* and *Xenopus*. Morpholino-mediated knockdown of *Nvlbx1* results in changes of endodermal morphology, probably due to loss of mesenteric muscle cells. This indicates that *Nvlbx1* regulates endoderm differentiation and in particular muscle formation in *Nematostella*. By using RNA-Seq to identify genes that are up- or downregulated in *Nematostella* Nvlbx1 morphants, we have gained

insights into the ancestral role of *Nvlbx1* during endoderm differentiation in *Nematostella*. In *Xenopus*, our vertebrate model, we are using a combination of ChIP-Seq and RNA-Seq to determine potential Lbx1 binding sites and targets. The conserved gene regulatory network identified in our study should shed light on the evolution of myogenesis in metazoans.

Program/Abstract # 301

The TLR co-receptor TRIL is required for Spemann organizer function in *Xenopus*

Xie, Yuanyuan (University of Utah, USA); Mimoto, Mizuho; Kwon, Sunjong (Oregon Health & Science University, USA); McKnite, Autumn; Christian, Jan (University of Utah, USA)

Toll-like receptor (TLR) signaling plays an evolutionarily conserved role in innate immunity. The first identified TLR, *Drosophila* Toll, has an essential role in dorsal-ventral patterning, whereas a similar role for vertebrate TLRs has not been uncovered. I have shown that the novel transmembrane protein, TRIL, which was originally identified as a TLR co-receptor necessary to activate NF-kB in immune cells, is required for embryonic patterning in *Xenopus*. *Xenopus* TRIL is expressed in dorsal mesodermal cells that make up the Spemann organizer during gastrulation. Targeting TRIL antisense morpholinos to dorsal cells in *Xenopus* embryos causes gastrulation defects and spina bifida as well as loss of head and eyes at the tailbud stage. In situ hybridization and immunostaining analyses suggest that TRIL is required for formation of the notochord and the central nervous system. Gene expression analyses show that TRIL regulates expression of a subset of organizer genes, including BMP antagonists, during gastrulation. We hypothesize that TRIL activates a TLR/NF-kB signaling pathway during gastrulation to regulate expression of BMP antagonists and other organizer genes required for neural induction and dorsal mesoderm formation. These findings suggest that the role of Toll/NF-kB signaling in regulating expression of BMP antagonists required for dorsal-ventral axis formation is conserved from flies to vertebrates.

Program/Abstract # 302

Fuz mutant mice reveal shared mechanisms between ciliopathies and FGF related syndromes

Tabler, Jacqueline Marie (UT Austin, USA); Liu, Karen (King's College London, UK); Wallingford, John (UT Austin, USA)

Ciliopathies are a broad class of human disorders, with craniofacial dysmorphology as a common feature. Among the hallmarks of ciliopathies is high arched palate, a condition that impairs speech and reduces quality of life. We present here the ciliopathic *Fuzzy* mutant mouse as the first animal model of high arched palate. Using mouse and frog, we show that this defect arises not, as commonly suggested, from midface hypoplasia, but rather from increased neural crest expanding the first branchial arch, resulting in maxillary hyperplasia. High arched palate is also common in fibroblast growth factor (FGF) hyperactivation syndromes, and we find that craniofacial *Fgf8* gene expression is significantly expanded in *Fuz* mutant mice. Moreover, genetic reduction of *Fgf8* levels in *Fuz* mutant mice ameliorates the maxillary phenotypes. Finally, the mouse model of oral-facial-digital syndrome-1 (*ofd1)* also shows expanded domains of *Fgf8* expression accompanied by an enlarged maxillary process, suggesting that aberrant FGF regulation is a common feature of ciliopathies. Thus, our findings reveal a cause for a common craniofacial anomaly and identify a novel etiological link between two classes of human disease: FGF-hyperactivation syndromes and ciliopathies.

Program/Abstract # 303

SUMOylated Sox3 is associated with chromatin and affects Sox3 function during zebrafish development

Lam, Chi Man; Laghari, Zulfiqar Ali; Shih, Yu-Huan; Kuo, Cheng-Liang; Struebing, Silke; Scotting, Paul John (University of Nottingham, UK)

Sox3 is a transcription factor participating in many developmental processes. However, it remains unclear how it is modified and regulated to function differently at certain times and locations in developing embryos. Our study investigates the role of Sox3 SUMOylation. SUMOylation has been shown previously to have diverse effects on the activity of several transcription factors. Our previous results suggested that Sox3 directly represses organizer genes in zebrafish. Here we look at how the function of Sox3 on organizer formation is regulated by SUMOylation. Our cell fractionation and western blot results demonstrated that SUMOylated Sox3 is associated with chromatin. Luciferase assays also demonstrated that SUMOylation of Sox3 alters its transcriptional activity and therefore enhances the repressing function of Sox3 on organizer formation in zebrafish. Overall, these results suggest that SUMOylation of Sox3 is critical to control of its different activities during zebrafish development.

Program/Abstract # 304

Determining the role of an uncharacterized tubulin in the development of multiciliated epithelial cells in *Xenopus laevis*.

Wills, Airon (University of Texas, Austin, USA), Turk, Erin (Stanford, USA); Sedzinski, Jakub (University of Texas, Austin, USA); Howes, Stuart; Nogales, Eva (UC Berkeley, USA); Stearns, Tim (Stanford, USA); Wallingford, John (University of Texas, Austin, USA)

The tubulin superfamily is a well-conserved and ancient protein family. The most well-known members of this superfamily are alpha, beta, and gamma tubulin, which are found in all eukaryotes and form the structure of the microtubule, and nucleate microtubules, respectively. However, a number of other tubulin family members have been variably inherited during evolution. Here, we present our study of a vertebrate tubulin that we have termed "eta-tubulin" due to its similarity with the eta-tubulin protein described in *Paramecium*. To our knowledge, eta-tubulin is the only member of the tubulin superfamily that remains completely uncharacterized in vertebrates. Here, we find that morpholino knockdown of eta-tubulin in *Xenopus laevis* embryos significantly shortens axoneme

length in multiciliated cells of the epidermis, and is associated with disorganization of the basal bodies. Currently, we are working to determine the mechanisms by which eta-tubulin affects ciliogenesis and basal body trafficking in *X. laevis*.

Program/Abstract # 305

Misregulation of osteoblast differentiation underlies abnormal skull growth and suture formation in sp7 mutants

Kague, Erika; Fisher, Shannon (University of Pennsylvania, USA)

During skull growth, fibrous sutures unite the edges of the skull bones. They allow expansion and movement of individual bones as the brain grows, and also regulate the rate of bone growth. Sutures consist of a central region of undifferentiated mesenchymal stem cells (MSCs) separating the edges of the bones, where osteoblasts differentiate and new bone is deposited. This spatial organization is conserved across species, yet remains a poorly understood aspect of suture biology. The transcription factor Osterix/Sp7 is essential for normal osteoblast differentiation and implicated in the maintenance of bone mineral density. Zebrafish *sp7* mutants have a fundamental defect in bone mineralization. The larval skeleton is patterned normally, but older mutants show severe mispatterning of the sutures and frequent formation of ectopic intrasutural bones. We followed the process of skull growth through sequential imaging of live fish carrying a transgene labeling early osteoblasts. In mutants, the edges of the bones are irregular, and adjacent areas of bone formation remain separate, resulting in irregular sutures. At the microscopic level, there is a dramatic increase in MSCs coupled with abnormal persistence of early osteoblasts at the edges of the bones. At normal sutures, the early osteoblasts at the edges of the bone are the most highly proliferative cell population, and their rate of proliferation is dramatically increased in the mutants. These represent non–autonomous functions of *sp7*, since MSCs and early osteoblasts do not express the gene. The features of the *sp7* mutants suggest a feedback signal linking the state of mineralization in the skull bones to the rate of bone formation, through regulating the behavior of adjacent MSCs and early osteoblasts. Our analysis of skull formation in *sp7* mutants has revealed a previously unknown aspect of suture regulation, and provides evidence for feedback linking the downstream process of mineralization with the earliest steps of osteoblast induction.

Program/Abstract # 306

Evolution of a tissue-specific silencer underlies diversification of paralogous genes

Haruki, Ochi (Yamagata University, Japan); Kawaguchi, Akane (Nara Institute of Science and Technology, Japan); Ogino, Hajime (Nagahama Institute of Bio-Science and Technology, Japan)

During the early chordate evolution, whole genome duplications (WGD) have produced many duplicated genes, called paralogs. These paralogs are often showing overlapping expression, yet distinct expression patterns in modern vertebrate. The evolutionary mechanisms for divergent expression of paralogous genes have been explained with the duplication-degeneration-complementation model. This model predicts that parts of duplicated enhancers were lost reciprocally from sibling paralogs because of degenerative mutations. But at least one enhancer copy remains in either of the paralogs, resulting complementary expressions cover the original full expression of the progenitor gene. However, involvement of innovative *cis*-regulatory changes has still remained elusive. *pax2* and *pax8* arose from a single progenitor following the WGDs. *pax8* is mainly expressed in the kidney, ear and thyroid gland during development. *pax2* shows expression not only in the *pax8*-expressing tissues but also in other tissues such as the eye, pharyngeal arches, midbrain-hindbrain boundary, hindbrain and spinal cord. We revealed that both *pax2* and *pax8* retain ancestral enhancers capable of directing *pax2*-like, multi-tissue expression. However, a silencer with in the *pax8* proximal promoter suppresses pleiotropic enhancer activity outside the *pax8*-expressing tissues. These results indicate that the silencer innovation was crucial for the divergent expression of paralogs with pleiotropic enhancers inherited from their common progenitor.

Program/Abstract # 307

Temporal and Spatial Expression of the Wnt Gene Complement in a Spiral-Cleaving Embryo

Pruitt, Margaret M.; Letcher, Edward; Bastian, Benjamin; Chou, Hsien-chao; Schneider, Stephan (Iowa State Univ, USA)

The Wnt/β-catenin signaling pathway is highly conserved in metazoans and is involved in many developmental processes, such as cell-fate determination and axis formation. Thirteen distinct *wnt* subfamilies are common between cnidarians and bilaterally symmetric animals. To gain insights into conserved functions of this ancient *wnt* gene complement, we aimed to unravel roles for each *wnt* gene within embryos that utilize a broadly conserved mode of development, spiral cleavage. Spiral-cleaving embryos use a series of stereotyped asymmetric cell divisions that allows for the identification of individual cells by their positions and size and for prediction of their fate. To study the *wnt* gene complement in a spiralian model, we made use of the marine annelid *Platynereis dumerilii* whose genome retained 12 of the 13 *wnt* subfamilies, and whose spiral cleavage pattern has been determined. Furthermore, it was found that in early *Platynereis* embryos, the β-catenin signaling pathway specifies cell fates in a reiterative binary manner. Here, we employ transcriptional profiling and *in situ* hybridization to determine the temporal and spatial regulation of *wnt* gene expression in the early *Platynereis* embryo. RNA-Seq results suggest that only a subset of *wnts* is expressed in early development. *In situ* hybridization further shows that each of these *wnts* exhibits a similar but distinct cellular pattern of gene expression that can be traced back to individual cells. This is the first analysis of the expression of all *wnt* genes encoded by a spiralian genome in early spiral cleavage stages, and provides the first comprehensive view of Wnt signaling inputs into embryos utilizing a spiral-mode of cell divisions to segregate cell fates.

Program/Abstract # 308
Mechanistic Diversification of the Hedgehog Signaling Pathway
Warner, Jacob (Duke University, USA); McCarthy, Ali; Morris, Robert (Wheaton College, USA); McClay, David (Duke University, USA)
A relatively small number of signaling pathways govern the early patterning processes of metazoan development. Since most animals use the same pathways, the architectural changes made over time to these few signaling pathways offers unique insights into the evolutionary process. In the case of Hh signaling, two very divergent mechanisms of pathway transduction have evolved. In vertebrates, effective signaling relies on the primary cilium, a specialized cell-surface organelle. In sharp contrast, protostomes, including flat worms and fruit flies, cilia are not necessary for Hh signal transduction, yet much of the transduction apparatus is the same for both animal groups. How divergent lineages could have adapted such a dramatically different way of activating the signaling pathway is an unanswered question. Here we present evidence that in the sea urchin, a basal deuterostome, cilia are required for embryonic Hh signal transduction. We found that inhibiting cilia assembly generates phenotypes nearly identical to those of Hh morphants, and we were able to visualize the Hh receptor, Smoothened, localize to cilia during active Hh signaling. This is the first evidence that Hh signaling requires cilia outside of the vertebrate lineage. Our findings support a model in which a complex signaling pathway may have evolved by co-option of components from a common single-celled ancestor and diverged mechanistically within protostome and deuterstome lineages.

Program/Abstract # 309
Withdrawn

Program/Abstract # 310
Comparison of the developmental transcriptomes of three marine Spiralians reveals the evolution of trochophore
Xu, Fei, (Institute of Oceanology, Chinese Academy of Sciences, China), Fan, Dingding (BGI-Shenzhen, China); Domazet-Loso, Tomislav (Ruder Boškovic Institute, Croatia); Li, Li (Institute of Oceanology, Chinese Academy of Sciences, China); Fang, Xiaodong (BGI-Shenzhen, China); Zhang, Guofan (Institute of Oceanology, Chinese Academy of Sciences, China)
Both Annelida and Mollusca, as the two largest phyla within Lophotrochozoa, pass through trochophore during development. However, it is not clear if morphological similarity of trochophores reflects their common evolutionary origin or a convergent evolution. Under the assumption of common evolutionary origin of trochophore one would expect to see a conserved developmental mechanisms in all trochophores. At the same time, there has been long standing debate on the origin of marine larva. The "larvae-first" hypothesis pointed that the original bilaterian life cycle included pelagic adult which was similar with the modern trochophore larva. Opposing view is given in the "intercalation" hypothesis which proposes that pelagic larvae evolved secondarily was intercalated into ancestral direct life cycle. We compared the transcriptomes of typical development stages in three spiralians, the Pacific oyster *Crassostrea gigas* (Bivalvia, Mollusca), the Pacific abalone *Haliotis discus hannai* (Gastropoda, Mollusca), and the polychaete worm *Alitta succinea* (Polychaeta, Annelida), by calculating the transcriptome age index (TAI). The results indicated that the trochophore stage of polychaete worm is expressing the phylogenatically oldest transcriptome across all ontogeny, and that overall TAI pattern supports an hourglass model of development. This TAI profile does not support the "intercalation" hypothesis. However, the TAI values for the two molluscs at the trochophore stage had the highest values, indicating that the molluscan trochophore is greatly influenced by younger genes. Indeed, morphological observations show that the early trochophore in molluscs has already some elements of the adults body plan, such as the shell fields.

Program/Abstract # 311
Glomerular development process in the Chinese experimental miniature pig
Xie, Yuansheng; Li, Xuyan; Shen, Shanshan; Cui, Shaoyuan; Li, Qinggang; Bai, Xueyuan; Chen, Xiangmei (Chinese PLA General Hospital, China)
Pig, which shares more similarities in renal structure and functions with humans than many other species, is an excellent animal to study kidney. However, little focus has been put on the glomerular development in pigs. In this study, the morphological changes of glomerular development process and the development of glomerular podocytes, endothelial and mesangial cells as well as their interrelation was observed in Chinese experimental miniature pigs at 18 time points from embryo 28days (E28d) to postnatal day 28 (P28d). The result revealed that the ureteric bud, cap mesenchyme, renal vesicle, comma-shaped body and S-shaped body were found in porcine metanephros at E28d. The glomeruli including immature and mature ones were formed at E35d. The pig was birthed at E112d, its nephrogenic zone still existed at P14d, and disappeared at P21d. Immunofluorescence staining showed that diffuse WT1 (podocyte marker) expression was observed in the metanephric mesenchyme and then in the renal vesicle, the whole comma-shaped body, the tail of the comma-shaped body, the lower aspect of the S-shaped body and the glomerular podocytes in succession. CD31, a marker for endothelial cells, was scattered in early fetal kidneys and then surrounded the developing renal vesicle and comma-shaped body. At the S-shaped stage, CD31- positive cells migrated into the vascular cleft to form precapillary cords of the immature glomeruli and finally localized in the endothelia of mature glomeruli. α-SMA (a marker for mesangial cells) did not expressed in renal vesicle or comma-shaped body, but appeared near the periphery of the S-shaped body, streamed into the vascular cleft by the late of the S-shaped stage, aggregated at the root of immature glomeruli and finally localized in the mesangial region of mature glomeruli. Our results indicate that the glomerular podocytes arise from metanephric cap mesenchyme, and the development of podocytes and

endothelial cells precedes the mesangial cells in pigs. The cross-talk between these cells might promote the development of the porcine glomerular tuft.

Program/Abstract # 312

Embryonic origin of cartilaginous elements of the axolotl visceral skeleton

Davidian, Asya (St. Petersburg State Univ, Russian Federation), Epperlein, Hans-Henning; Tanaka, Elly (Technical University Dresden, Germany); Malashichev, Yegor (St. Petersburg State Univ, Russian Federation)

Traditionally, the cartilaginous viscerocranium of vertebrates is considered as neural crest-derived. However, transplantation of cranial mesoderm to a position of trunk somites carried out by Stone (1932) had led to the formation of a heterotopic heart anlage and a small rod-like cartilage, presumably a medial element of the pharyngeal arches. Since then, the embryonic sources of the visceral skeleton had not been investigated by exact long-term labeling. We performed bilateral homotopic transplantations of neural folds along with up to 95% of cells of the presumptive neural crest from transgenic embryos into white (d/d) host embryos, or fragments of the GFP+ head lateral plate mesoderm. In these experiments the neural crest-derived GFP+ cells contributed to all elements of the gill arches, except for basibranchiale 2, whereas the grafting of GFP+ head mesoderm led to a reverse labeling result. The grafting of only the distal parts of the GFP+ head lateral plate mesoderm resulted in marking the basibranchiale 2 and the heart, implying that both these structures originate from a common mesodermal region. Co-mapping of the contralateral sides of head mesoderm with differently colored (GFP+ and Cherry+) transgenic cells showed that basibranchiale 2 develops from a paired anlage, similarly to the heart. If compared to fish (Kague et al., 2012), in which all branchial elements are of neural crest origin, axolotl demonstrates a deviation, in which the medio-posterior element of the pharyngeal apparatus consists of mesodermal cells. This might be due to an evolutionary loss of a part of neural crest derived skeleton in amphibians, in which the head mesoderm replaces neural crest cells in some elements of the pharyngeal arches.

Program/Abstract # 313

Transgenic axolotls (*Ambystoma mexicanum*) as an emerging system for the study of organ and tissue embryonic origin.

Malashichev, Yegor, (St. Petersburg State University, Russian Federation)

In the XX century quail-chick chimeras were a most routine tool for mapping the fate of individual cell populations in the avian embryos and led to important discoveries of the embryonic origin of all major organ types in this model system. Recent decade has seen a dramatic increase in the interest to the embryonic origin of organs in other organisms, the interest, which was technically supported mostly with injections of lipophilic dyes, fluorescent dextran conjugates, or retroviruses. A disadvantage of these was the loss of the marking signal due to cell divisions, or to mark the tissue of interest precisely. Most recently, the lentiviral and other transgenic marking systems along with transplantation of tissues from the transgenic donor bearing a fluorescent marker to the wt host became the symbols of a renaissance of the fate mapping of vertebrate embryos, allowing life-long marking. In my talk I present several examples of use and disuse of these methods from my own experience and the work of others, performed on amphibian and avian embryos. In particular I will stop on the fate mapping of the neural crest and mesoderm in the anterior trunk and the head, demonstrating dual embryonic origin of the shoulder girdle (somitic and lateral plate mesoderm), viscerocranium (neural crest, head paraxial and lateral plate mesoderm), and embryonic kidney (lateral plate vs. somitic and intermediate mesoderm) as well as endodermal origin of pharyngeal teeth in axolotl. A special reference will be given to dual colour (e.g. GFP+ and Cherry+) multiple tissue (mesoderm+neural crest or multiple somites) marking techniques. All examples are provided with the discussion of appropriateness of the methods used and evo-devo speculations.

Program /Abstract # 314

Withdrawn

Program/Abstract # 315

Identification of a novel embryonic signaling peptide essential for mesendoderm migration

Pauli, Andrea; Ma, Jiao; Mitchell, Andrew; Gagnon, James (Harvard, USA); Joung, Keith (Massachusetts General Hospital, USA); Saghatelian, Alan; Schier, Alexander (Harvard, USA)

Using a combination of computational and genomics approaches, we have identified hundreds of genes encoding un-annotated short peptides that are expressed during zebrafish embryogenesis. TALEN-induced loss-of-function mutants of one of these genes are embryonic lethal and have severe cardiovascular defects. This novel developmental regulator encodes a highly conserved secreted peptide that has been mis-annotated as a non-coding transcript in zebrafish, mouse and human. Detailed characterization of loss- and gain-of-function phenotypes suggests that this signal regulates mesendodermal cell migration. To uncover the downstream signaling pathway, we are currently using genetic, biochemical and cell biological approaches to identify the peptide receptor. Our studies identify a novel migration signal and highlight the potential of genomics approaches for identifying novel developmental signals.

Program/Abstract # 316

Molecular pathogenesis of Joubert Syndrome and related disorders

Caspary, Tamara; Mariani, Laura (Emory University, USA); Higginbotham, Holden (UNC School of Medicine, USA); Fritz, Julie (Emory University, USA); Anton, Eva (UNC School of Medicine, USA)

Patients with Joubert Syndrome and related disorders (JSRD) suffer from a wide array of symptoms with variable clinical presentation, including intellectual disability. While JSRD is a rare, autosomal recessive congenital disorder, causative mutations for JSRD have been identified in the small GTPase, *ARL13B*, the inositol phosphatase, *INPP5E* and 16 additional genes, all of which code for proteins related to primary cilia – thus, JSRD are members of the class of diseases known as ciliopathies. Primary cilia are essential for Sonic hedgehog (Shh) signaling, and we previously showed that Arl13b regulates Shh signaling in mouse. Here we investigate the pathogenesis of JSRD in mouse models using a conditional *Arl13b* allele and a novel, ENU-induced *Inpp5e* allele. We found that Arl13b is critical for the localization of Inpp5e to cilia. We also found that Inpp5e regulates Shh signaling in an overlapping, yet distinct, manner to Arl13b. Together these data are consistent with Inpp5e acting as a specific Arl13b effector. Through Arl13b conditional deletion, we observed defects in the migration and placement of postmitotic interneurons in the developing cerebral cortex. We found several guidance cue receptors known to be important for interneuron migration localize to interneuronal cilia, but their concentration and dynamics were abnormal in the absence of either Arl13b or Inpp5e. While wild type Arl13b could rescue Arl13b-deficiency, Arl13b variants identified in Joubert patients or an Arl13b variant that fails to localize to cilia could not compensate. Taken together our data indicate that defects in cilia-dependent signaling in interneuron development may contribute to the neurological deficits in JSRD patients.

Program/Abstract # 317

Invasive adhesion polarizes heart progenitor induction

Davidson, Bradley (Swarthmore College, USA); Norton, Jennifer; Cooley, James (University of Arizona, USA); Cota, Christina (Swarthmore College, USA)

Cell-matrix adhesion strongly influences developmental signaling. Resulting impacts on cell migration and tissue morphogenesis are well characterized. However, the in vivo impact of adhesion on fate induction remains ambiguous. Here we employ the simple chordate *Ciona intestinalis* to delineate an essential, in vivo role for matrix adhesion in heart progenitor induction. In *Ciona* pre-cardiac founder cells, invasion of the underlying epidermis promotes localized induction of the heart progenitor lineage. We found that these epidermal invasions are associated with matrix adhesion along the pre-cardiac cell/epidermal boundary. Through targeted manipulations of RAP GTPase activity, we were able to manipulate pre-cardiac cell-matrix adhesion. Targeted disruption of pre-cardiac cell-matrix adhesion blocked heart progenitor induction. Conversely, increased matrix adhesion generated expanded induction. We were also able to selectively restore cell-matrix adhesion and heart progenitor induction through targeted expression of either Ci-Integrin Beta 2 or its predicted partner Ci-Integrin Alpha 2. These results indicate that matrix adhesion functions as a necessary and sufficient extrinsic cue for regional heart progenitor induction. Additionally, tandem manipulations indicate that adhesion and invasion regionalize signaling through synergistic, cross-regulatory interactions. Thus, it appears that reciprocal adhesive/protrusive circuitry associated with directed migration has been co-opted to generate robust, regional induction. Furthermore, time-lapse imaging indicates that cytokinesis acts as an intrinsic regulator of heart progenitor specification by facilitating localized maturation of adhesive foci. These findings have profound implications for vertebrate heart development and stem cell biology.

Program/Abstract # 318

Dynamic membranes mediate heart progenitor induction in Ciona.

Cota, Christina (Swarthmore College, USA)

Integrin receptors play an essential role in matrix adhesion during morphogenesis. Integrins are also known to regulate activation and trafficking of receptor tyrosine kinases. However, the potential impact of integrins on cell fate induction remains largely unexplored. In the model chordate, *Ciona intestinalis*, FGF/MapK signaling differentially activates the Ets1/2 transcription factor in founder cells to induce cardiac cell fate. Previous work from our lab has found that integrin-mediated matrix adhesion is both necessary and sufficient for asymmetric heart progenitor induction. To begin to address the role of integrin-mediated receptor trafficking in asymmetric fate induction, we have focused on the predominant regulator of clathrin-independent receptor endocytosis, caveolin-1 (Cav1). We have found that over-expression of *Ci-Cav1* is sufficient to rescue heart progenitor induction in founder cells where adhesion has been disrupted. Furthermore, targeted expression of a dominant negative form of *Ci-Cav1* in cardiac founder cells significantly decreased heart progenitor induction. These results suggest that caveolin-rich membranes choreograph inductive signaling in response to diverse extrinsic cues.

Program/Abstract # 319

Multiple Catenins Contribute to Development: Emerging Roles of Plakophilin-3 Catenin

Munoz, William; Miller, Rachel; Lee, Moonsup (MD Anderson Cancer Center, USA); Kloc, Malgorzata (The Methodist Hospital, USA); McCrea, Pierre (MD Anderson Cancer Center, USA)

The catenin family has undergone a significant expansion during the evolution of vertebrates, resulting in varied functions that have yet to be discerned or fully characterized. Catenins contain an Armadillo domain, bracketed by less conserved amino- and carboxy-terminal tails. The most prominent family member is beta-catenin, which acts at the adherens junction and in the nucleus, and is a key player in both normal development and human disease. My work focuses upon Plakophilin-3 (Pkp3), a catenin that we hypothesize provides key functions in differing cellular contexts. Members of the Pkps are found throughout the cell with little known about their functions aside from that within desmosomal junctional plaques. Examples of Pkp3's less understood activities include putative functions in the cytosol, and further, my recent data intriguingly points to Pkp3 acting in the nucleus. I will present our

characterization of Pkp3 in early vertebrate embryos of *Xenopus laevis*. Pkp3 knock-down phenotypes include hyposensitivity to touch and altered PNS-staining, showing that Pkp3 is required in amphibian development. At the molecular level, I will present a novel interaction resolved between Pkp3 and ETV1, an ETS-family transcription factor, and preliminarily on Wnt-pathway regulation of Pkp3 stability / activity. My goal is to deepen our cellular and developmental understanding of Pkp3, especially with regards to its poorly understood roles in the nucleus. This will provide the basis to ultimately address if Pkp3's role in gene regulation is linked to its roles at desmosomal cell-cell junctions.

Program/Abstract # 320

Bidirectional Notch-Delta signaling in Nematostella vectensis suggests that Delta activation is a key component to this signaling pathway in animals.

Layden, Michael; Martindale, Mark (Whitney Laboratory for Marine Bioscience, USA)

Notch signaling is redeployed throughout animal development to govern cell fate decisions in developing tissues. Traditionally, Notch signaling has been described as one directional, and it is initiated by the Delta ligand on one cell interacting with the Notch receptor on the adjacent cell. Notch and Delta are single pass transmembrane proteins that undergo a proteolytic cleavage that releases the intracellular domain (ICD) of upon activation. However, only function of the Notch ICD has been extensively studied. The Notch ICD regulates gene expression and generally promotes an undifferentiated cell fate, which is often associated with maintaining proliferative potential. Cell culture and limited genetic studies suggest that the Delta ICD also undergoes nuclear translocation and that Delta promotes cell differentiation and loss of proliferation. We characterized Notch signaling during neurogenesis in *Nematostella*. We show that Delta activates Notch signaling and Notch activation inhibits differentiated neural gene expression. Currently, our data suggests that Notch does not promote cell proliferation. Conversely, Delta inhibits cell proliferation and increases differentiated neural gene expression. We also demonstrate that the Delta ICD localizes to the nucleus and is likely to regulate gene expression. Taken together our data supports the mounting evidence that Notch signaling is more likely to be bidirectional Notch-Delta signaling. The role of Notch-Delta signaling in regulating the delicate balance of growth and differentiation and the link between defects in Notch-Delta signaling various forms of cancer supports future work focused on understanding the Delta signaling component.

Program/Abstract # 321

Thermal stability regulates fibroblast growth factor signaling

Krejci, Pavel; Vesela, Iva (Masaryk University, Czech Republic); Buchtova, Marcela; Zajickova, Renata (University of Veterinary and Pharmaceutical Sciences, Czech Republic); Zakrzewska, Malgorzata (University of Wroclaw, Poland); Wiedlocha, Antoni (University of Oslo, Norway); Martin, Jorge (Cedars-Sinai Medical Center, USA)

The fibroblast growth factor (FGF) system represents one of the fundamental tools of cell communication. Eighteen FGFs act as tissue growth factors or metabolic hormones to regulate many important processes throughout development, life, and disease. We report that biological activity of FGF1, FGF4, FGF5, FGF6, FGF8, FGF9, FGF16-18, and FGF20 is severely limited *in vitro* and *in vivo*, manifested as failure to activate downstream FGF-receptor (FGFR) signaling over a long period of time, and to influence specific cell behavior. This phenotype is not caused by FGFR specificity or the absence of appropriate low affinity FGF co-receptors (the heparan sulfate proteoglycans) at the cell surface. Instead, failure to signal stems from thermal instability in at least 10 different members of FGF family. We further demonstrate that stabilization via exogenous heparin binding, introduction of stabilizing mutations or lowering the cell cultivation temperature rescues the biological activity of unstable FGFs in both in vitro and in vivo environments. Our data suggest that limited thermal stability may regulate biological activity of extracellular signaling molecules.

Program/Abstract # 322

Trachea-derived Dpp controls adult midgut homeostasis in Drosophila

Lin, Xinhua; Zhouhua, Li; Zhang, Yan; Han, Lili; Shi, Lai (Chinese Academy of Sciences, China)

Homeostasis in adult tissues is maintained by resident stem cells and their progeny. Little is known about the regulation of tissue homeostasis by organ-organ interaction. With the use of the *Drosophila* model, we demonstrate that trachea-derived Decapentaplegic (Dpp), the main BMP ligand in *Drosophila*, is essential for adult midgut homeostasis. We show that Dpp signaling is primarily activated in enterocytes (ECs). Depletion of Dpp signaling in ECs results in excess amounts of intestinal stem cell (ISC)-like cells and their progeny, similar to the human juvenile polyposis (JP) syndrome harboring mutations in BMP pathway genes. Importantly, we find that Dpp is expressed specifically in tracheal cells. Tracheal cells reach the intestinal cells through the visceral muscles (VMs). We show that depletion of *dpp* expression in tracheal cells phenocopies the Dpp loss-of-function defects in ECs. Finally, we demonstrate that loss of Dpp signaling in EC cells causes apoptosis and elevated JNK signal activity while ectopic expression of anti-apoptotic *p35* or *Diap1* was able to greatly suppress the defects. Our data demonstrate that the *Drosophila* trachea not only exchanges air for bodily needs, but also produces a Dpp morphogen essential for neighboring tissue homeostasis. On the basis of our observations, we propose that trachea-derived Dpp activates Dpp signaling in ECs to protect ECs from cell death and counteract environmental insults. Stabilized ECs in turn restrict ISCs from excessive proliferation, thus maintaining intestinal homeostasis. The identification of the trachea as the signal source for midgut homeostasis will provide important insight into our understanding of the mechanisms of tissue homeostasis control by inter-organ communication.

The identification of the trachea as the signal source for midgut homeostasis will provide important insight into our understanding of the mechanisms of tissue homeostasis control by inter-organ communication.

Program/Abstract # 323

Drosophila glypicans Dally and Dally-like are essential regulators for JAK/STAT signaling and Unpaired distribution in eye development

Lin, Xinhua (Cincinnati Children's Hospital, USA); Zhang, Yan (Chinese Academy of Sciences, China); You, Jia (Cincinnati Children's Hospital, USA); Ren, Wenyan (Chinese Academy of Science, China)

The highly conserved janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is a well-known signaling system that is involved in many biological processes. In *Drosophila*, this signaling cascade is activated by ligands of the Unpaired (Upd) family. Therefore, the regulation of Upd distribution is one of the key issues in controlling the JAK/STAT signaling activity and function. Heparan sulfate proteoglycans (HSPGs) are macromolecules that regulate the distribution of many ligand proteins including Wingless, Hedgehog and Decapentaplegic (Dpp). Here we show that during *Drosophila* eye development, HSPGs are also required in normal Upd distribution and JAK/STAT signaling activity. Loss of HSPG biosynthesis enzyme Brother of tout-velu (Botv), Sulfateless (Sfl), or glypicans Division abnormally delayed (Dally) and Dally-like protein (Dlp) led to reduced levels of extracellular Upd and reduction in JAK/STAT signaling activity. Overexpression of *dally* resulted in the accumulation of Upd and up-regulation of the signaling activity. Luciferase assay also showed that Dally promotes JAK/STAT signaling activity, and is dependent on its heparin sulfate chains. These data suggest that Dally and Dlp are essential for Upd distribution and JAK/STAT signaling activity.

Program/Abstract # 324

The zebrafish diencephalic glial bridge is made up of a heterogeneous population of astroglial cells

Zaman, Paula; Velez, Carla; Bashiruddin, Sarah; Dimova, Kalina; Alligood, Kristin; Doris, Rosemarie; Sinha, Risha; Husain, Tanya; Mahlanza, Tatenda; Devoto, Stephen; Barresi, Michael (Smith College, USA)

In the developing Zebrafish brain, axons are guided across the midline by attractant and repellent protein cues to form commissures. The postoptic commissure (POC) of the diencephalon is the first forming commissure in the zebrafish brain. POC axons contact a group of astroglial cells that express Glial fibrillary acidic protein, (Gfap) and form what has been called the "Diencehphalic Glial Bridge". The temporal and spatial association of POC axons with these astroglial cells suggests this glial bridge may serve to provide a supportive substrate for axonal growth across the midline. However, little is known about the molecular and cellular characteristics that define these astroglial cells. We have combined embryological, transgenic and molecular analyses to characterize the cells that make up the diencephalic glial bridge in zebrafish. Using transgenic lines that express cytoplasmic, membrane tethered, or nuclear localized fluorescent proteins under the Gfap regulatory sequences we have been able to visualize astroglial cells in the zebrafish forebrain. By employing gastrula staged cell transplantation procedures with these lines, we have been able to describe at least three distinct Gfap+ cell morphologies. Previously, Trevarrow and colleagues conducted an unbiased screen for antibodies that showed distinct labeling patterns in the zebrafish brain, and four antibodies were shown to mark radial glial-like cells, and were termed Zebrafish Radial Fiber 1-4 (Zrf). While Zrf1 has been shown to recognize zebrafish Gfap, the identity of the proteins recognized by Zrf2-4 are unknown. All four Zrf antibodies exhibit protein labeling that overlaps with the location of the POC. We demonstrate here that anti-Zrf2 and anti-Zrf3 display similar patterns of expression in the zebrafish forebrain but are distinct from Zrf1 labeling. Interestingly, anti-Zrf4 shows a restricted expression pattern that overlaps only the area of the glial bridge associated with commissural axon crossing. We are now conducting experiments that combine our transgenic and cell transplantation approach with Zrf immunoreactivity to correlate astroglial morphology with Zrf labeling. We are also carrying out biochemical analyses to identify the proteins recognized by the Zrf2,3,4 antibodies. Our data is building a model that supports the presence of a heterogenous population of astroglial cells make up the diencephalic glial bridge, which may provide an instructive environment of commissural midline crossing.

Program/Abstract # 325

Axial specification in mice is controlled by an extra-embryonic Wnt3 signaling event

Rivera-Perez, Jaime A.; Tortelote, Giovane; Huang, Tingting (University of Massachusetts Medical School, USA); Wakamiya, Maki (The University of Texas-Galveston, USA); Hadjantonakis, Anna-Katerina (Sloan Kettering Institute, USA); Behringer, Richard (M. D. Anderson Cancer Center, UT-Houston, USA)

Genetic evidence has revealed a fundamental role for the Wnt signaling pathway in the process of axial specification in mice. However, the question of how the Wnt pathway controls this process remains an open question. Here we provide evidence that axial specification in mice is the result of a Wnt3-directed inductive event initiated by the posterior visceral endoderm, an extra-embryonic tissue. We show that Wnt3 signals through the canonical Wnt signaling pathway and suggest that a feedback loop controls the maintenance of Wnt3 expression in the posterior visceral endoderm allowing gastrulation to proceed. Our results suggest an evolutionary conserved role of the canonical Wnt signaling pathway in the process of axial specification across vertebrates. However, since multiple Wnt ligands appear to act as inducers in different vertebrates, it appears that different Wnt ligands have been co-opted for gastrulation during vertebrate evolution.

Program/Abstract # 326

Wnt5a and Wnt5b function redundantly via noncanonical pathways to extend the embryonic axis

Barrow, Jeffery; Allen, John (Brigham Young University, USA); Long, Fanxin (Washington University in St. Louis, USA); McMahon, Andrew (University of Southern California, USA)

Wnt5a has long been known to signal via β-catenin-independent Wnt pathways. It has been demonstrated to play a crucial role in outgrowth of the face, limbs, genital tubercle and embryonic axis. Much less is known about its closely related family member Wnt5b. Wnt5b mutants are phenotypically normal. When Wnt5b mutant alleles are crossed onto a Wnt5a mutant background we observe severe truncations of the body axis resembling those of the Wnt3a mutant which signals through the β-catenin pathway. Double mutant embryos also exhibit craniorachischisis at high penetrance similar to Wnt /PCP mutants. Taken together, these defects suggest that Wnt5a and Wnt5b signal through multiple pathways. Here we demonstrate that that Wnt /β-catenin target gene expression is normal in Wnt5a/Wnt5b mutant embryos demonstrating that the axis truncation defects are not due to defects in canonical Wnt signaling. We compare convergence and extension defects of the Wnt5a/Wnt5b mutants with those of the PCP mutant Vangl2$^{LP/LP}$ mutants and demonstrate that Wnt5a/Wnt5b mutants have more severe CE defects than Looptail mutants. We conclude that either Wnt5a/Wnt5b signal through additional pathways to influence axis elongation or that Vangl$^{LP/LP}$ mutants only partially disrupt the Wnt /PCP pathway.

Program/Abstract # 327

Short-Range Wnt5 Signaling Specifies Posterior Ectodermal Fate in the Sea Urchin

McIntyre, Daniel C. (Duke University, USA); Seay, Winn (Harvard Medical School, USA); Croce, Jenifer (Observatoire Océanologique de Villefranche-sur-Mer, France); McClay, David (Duke University,USA)

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

Program/Abstract # 328

Regulation of vertebrate Wnt secretion and gradient formation by Wntless

Burrus, Laura W; Galli, Lisa (San Francisco State Univ, USA); Szabo, Linda (Stanford Univ, USA); Sean, Allen (San Francisco State Univ, USA); Li, Lydia (Johns Hopkins Univ, USA); Htaik, Yin Min (Roseman University of Health Sciences, USA)

The proper formation of Wnt gradients is essential for embryonic development in metazoans. Perhaps the best-characterized vertebrate Wnt gradient is in the spinal cord, where a dorsal to ventral gradient of Wnt1 and Wnt3a regulate cell fate specification and proliferation. Wntless (Wls) is an upstream regulator of Wnt secretion that is thought to carry palmitoylated Wnt from the ER to the cell surface. Loss of function studies show that Wls is required for Wnt secretion. Here, we used gain of function to further examine the role of Wls in HEK293T cells. We show that overexpression of Wls does not influence the palmitoylation of Wnt1, but promotes the association of Wnt1 with lipid rafts in HEK293T cells. Overexpression of Wls also increases Wnt secretion and signaling. We then used loss and gain of function studies to explore the role of Wls in the developing chick spinal cord. To knockdown Wls, we introduced a siRNA construct into the spinal cord of developing chicks by electroporation. Wls siRNA caused a significant increase in apoptosis, suggesting that Wls is required for cell survival in the spinal cord. Regrettably, this phenotype impeded our ability to assess Wnt1/3a gradient formation. To further explore the role of Wls in Wnt gradient formation, we turned to gain of function studies. Though it is not possible to directly assess Wnt secretion in vivo , we expected that an increase in Wnt1/3a secretion would promote beta-catenin dependent Wnt signaling and extend the Wnt1/3a gradient. Surprisingly, analysis from two different readouts of the Wnt1/3a gradient provided evidence that overexpression of Wls inhibits Wnt activity in the spinal cord. We are currently testing several hypotheses to explain our results.

Program/Abstract # 329

Primary cilium-mediated signalling is essential for normal gut patterning

Delalande, Jean Marie; Campbell, Alison; Thapar, Nikhil; Burns, Alan J (UCL - Institute of Child Health, UK)

Background: The *Talpid*^{3} gene encodes a centrosomal protein essential for primary cilium formation. In vertebrates, mutations in Talpid3 result in the absence of primary cilia, which leads to a wide range of defects including facial, skeletal and vascular abnormalities. Many aspects of the mutant phenotype can be linked to defective Hedgehog (Hh) signalling, which requires the primary cilium to function. Here, our aim was to examine *Talpid*$^{3-/-}$ chicken embryos to gain further insight into the role of cilia-mediated

signalling during gut and enteric nervous system (ENS) development. **Methods:** *Talpid*^{3} embryos were fixed at different time points, and processed for immunohistochemistry to identify cell types and morphology, and in situ hybridization for genetic pathway analyses. To assess the cell autonomous requirement of the cilia in ENS development, we used intra-species grafting between *Talpid*^{3} and GFP chickens. **Results:** *Talpid*$^{3-/-}$ gut showed significantly reduced length, tracheoesophageal fistula and open hindgut. Although enteric neural crest cell (ENCC) derivatives were distributed along the gut, ENCC were scattered throughout the gut wall, rather than arranged in typical plexuses. Transplantation of wild type ENCC to *Talpid*^{3} mutants did not rescue this phenotype. Defects in the patterning of the Hh pathway components in the *Talpid*^{3} mutants correlated with the defects in smooth muscle. **Conclusions:** We describe a number of phenotypic defects in *Talpid*^{3} mutant gut suggesting it is a useful model to study the genetic basis underlying related human gut abnormalities. We demonstrate that cilia-mediated Hh signalling is not necessary for ENCC migration, but is essential for normal smooth muscle formation and ENS patterning.

Program/Abstract # 330

The dynamic right-to-left translocation of Cerl2 is involved in the regulation and termination of Nodal activity in the mouse node

Belo, José A.; Inácio, José M.; Marques, Sara (Universidade do Algarve, Portugal); Nakamura, Tetsuya; Shinohara, Kyosuke (Osaka University, Japan); Meno, Chikara (Kyushu University, Japan); Hamada, Hiroshi (Osaka University, Japan)

The determination of left-right body asymmetry in mouse embryos depends on the interplay of molecules in a highly sensitive structure, the node. Here, we show that the localization of Cerl2 protein does not correlate to its mRNA expression pattern, from 3-somite stage onwards. Instead, Cerl2 protein displays a nodal flow-dependent dynamic behavior that controls the activity of Nodal in the node, and the transmission of the laterality information to the left lateral plate mesoderm (LPM). Our results indicate that Cerl2 initially localizes and prevents the activation of Nodal genetic circuitry on the right side of the embryo, and later its right-to-left translocation shutdowns Nodal activity in the node. The consequent prolonged Nodal activity in the node by the absence of Cerl2 affects local *Nodal* expression and prolongs its expression in the LPM. Simultaneous genetic removal of both *Nodal* node inhibitors, *Cerl2* and *Lefty1*, sustains even longer and bilateral this LPM expression.

Program/Abstract # 331

Cilia, Flow Sensing, and Polycystins: How the Embryo Determines Left From Right

Grimes, Daniel T. (Princeton University, USA), Keynton, Jennifer; Beunavista, Maria (MRC Harwell, UK); Hamada, Hiroshi; Shinohara, Kyosuke (Osaka University, Japan); Norris, Dominic (MRC Harwell, UK)

The left-right asymmetry of the internal organs of vertebrates is determined during development by a biophysical mechanism; asymmetric fluid flow. In the mouse embryo, a leftward flow is generated in the node by polarized cilia that rotate around 10 times per second. Flow induces asymmetries in gene expression in crown cells around the periphery of the node. These subtle asymmetries are then transmitted to the lateral plate mesoderm where the Nodal signaling cascade is established in the left side of the embryo only. However, the connection between leftward flow and asymmetric gene expression has not been mechanistically established. Our work on the sensory polycystin proteins PKD1L1 and PKD2 has revealed a novel pathway linking the generation and sensation of flow to the initiation of gene asymmetries around the node. Rather than being required to initiate left-sided Nodal signals, the flow sensor PKD1L1 is instead needed to restrict Nodal to the left side. It has been a matter of continued debate whether symmetry is broken by direct mechanosensation of the force of flow or by the perception of an asymmetrically positioned chemical within the node. We find that a small extracellular domain of PKD1L1 is essential for function; a mutation within this domain alters its structure and causes severe left-right defects in the mouse. Together, our findings favor a model in which flow is mechanically sensed in a PKD1L1-dependent manner to derepress left-sided Nodal signaling.

Program/Abstract # 332

Sp5l is a novel transcription factor involved in the establishment of left-right asymmetry in early zebrafish development

Inglis, Rachael (University of Cambridge,UK); Nelson, Andrew; Soong, Daniel (King's College London, UK); Amack, Jeffrey (SUNY Upstate Medical University, USA); Wardle, Fiona (King's College London, UK

The zebrafish gene *sp5l* (*sp5-like*) encodes a zinc finger transcription factor of the SP1 family. It is expressed in both ectodermal and mesodermal tissues during early development, including the dorsal forerunner cells (DFCs) which subsequently develop into Kupffer's vesicle: the ciliated organ of asymmetry in zebrafish. Using a morpholino knockdown approach, we have found that *sp5l* is required for the proper establishment of left-right asymmetry in the early embryo. This role is not shared with the related gene, *sp5*, despite their co-expression in the DFCs. Our functional characterisation of *sp5l* has revealed roles in both the formation and function of Kupffer's vesicle, primarily in control of the anti-clockwise fluid flow generated by motile cilia within the vesicle. As *sp5l* encodes a transcriptional regulator, we have attempted to identify downstream target genes that could mediate its effects on Kupffer's vesicle development by performing genome-wide expression analysis by microarray. We have also investigated the upstream regulation of *sp5l* by various signalling pathways, with particular focus on the control of its expression in the DFCs and Kupffer's vesicle.

Program/Abstract # 333

ATRX function during zebrafish early development

Ibarra Morales, Dafne Andrea; Schnabel Peraza, Denhi; Salas Vidal, Enrique; Lomeli Buyoli, Hilda; Zurita Ortega, Mario (Instituto de Biotecnologia UNAM, Mexico)

ATRX protein belongs to the SWI/SNF2 family of chromatin remodelers. It consists of an N-terminal ADD domain and a C-terminal Helicase/ATPase motif. ADD is a histone H3 binding module that recognizes a double mark: H3K9me3 and H3K4me0. The Helicase/ATPase motif is associated with alteration of DNA-histone interactions through ATP hydrolysis. The role of this protein during development is evident by the effects of mutations in the gene, known to cause ATR-X syndrome in humans which is characterized by severe mental retardation, alpha thalassemia, skeletal and urogenital abnormalities, and facial hypotonia. Zebrafish, our study model, has two copies of the gene (*atrx* and *atrxl*). Both transcripts are maternally inherited, have a similar spatiotemporal expression, and are localized asymmetrically during the early cleavage stage (4-512 cells). Atrx has the greatest identity to the human protein and at least one isoform that only contains the ADD domain, similar to one previously found in human and mouse. Interestingly, this isoform is expressed only after the midblastula transition. Embryonic injection with an *atrx* morpholino oligonucleotide that inhibits translation produces a weakly ventralized phenotype. Both the pattern of expression and the knockdown phenotype suggest an implication of Atrx in dorsoventral axis determination. By the study of early dorsal markers in morphant and wild type embryos our aim is to find whether Atrx is involved in this process.

Program/Abstract # 334

Irx1 and Irx2 are Coordinately Expressed and Regulated by Retinoic Acid, TGFbeta, and FGF Signaling during Chick Hindlimb Development

Díaz-Hernández, Martha; Bustamante, Marcia; Galván-Hernández, Claudio; Chimal-Monroy, Jesús (Instituto de Investigaciones Biomédicas UNAM, Mexico)

The Iroquois homeobox (Irx) genes play a crucial role in the regionalization and patterning of tissues and organs during metazoan development. Since the Irx1 and Irx2 gene expression regulation during hindlimb development has not been investigated yet, the aim of this study was to evaluate the gene expression pattern of Irx1 and Irx2 as well as their regulation by important development regulators such as retinoic acid (RA), transforming growth factorβ TGFβ) and fibroblast growth factor (FGF) signaling during chick hindlimb development. Irx1 and Irx2 were coordinately expressed in the interdigital tissue, digital primordia, joints and in the boundary between cartilage and non-cartilage tissue. Down-regulation of Irx1 and Irx2 expression at the interdigital tissue coincided with the onset of cell death. RA was found to down-regulate their expression by a bone morphogenetic protein-independent mechanism before any evidence of cell death. TGFβ protein regulated Irx1 and Irx2 in a stage-dependent manner at the interdigital tissue, inhibiting their expression when it was administered to the interdigital tissue at developing stages before their normal down-regulation. TGFβ administration at developing stages after normal down-regulation of Irx1 and Irx2 evidenced that expression of these genes marked the boundary between cartilage tissue and non-cartilage tissue. It was also found that at early stages of hindlimb development FGF signaling inhibited the expression of Irx2. In conclusion, the present study demonstrates that Irx1 and Irx2 are coordinately expressed and regulated during chick embryo hindlimb development. This study was supported by CONACyT grants 53484 and 168642, DGAPA-UNAM grants IN214511 and IN220808.

Program/Abstract # 335

Application of TGFß leads to enhanced chondrogenesis and impairment of posterior element formation in the developing chick limb.

López-Bayghen, Bruno, Medina-Vázquez, Georgina; García-Cruz, Carla; Chimal-Monroy, Jesús (UNAM, Mexico)

Chondrogenesis is one of the most important events that occur during vertebrate limb morphogenesis. At final stages of limb development, cells in the autopod (distal-most part) differentiate to give rise to either digits or interdigital areas. However, it is known that the initiation of a chondrogenic program in cells of the distal interdigit, by ligands of the TGFβ/Activin family, gives rise to an ectopic digit. To evaluate the effect this could have in stages were the digit/interdigit pattern is being established, beads soaked in TGFβ were implanted in the posterior distal mesenchyme of chick posterior early limb buds. Limbs were then analyzed by *ISH* to determine Sox9 and Tbx3 mRNA distribution and by Alcian blue/Alizarin red staining to show skeletal element formation. Around the site of implantation, an area of Sox9-positive cells was observed. A similar pattern was observed for Tbx3-negative cells. This inhibition of Tbx3, a transcription factor highly relevant in posterior element formation, is associated to deficiencies in the formation of digits III and IV and in some cases tarsal bones. To test the involvement of the Wnt canonical pathway, beads soaked in DKK1, an inhibitor for this pathway, were implanted in this region. This led to a similar phenotype, characterized by an induction of Sox9, albeit with less marked effects. These results suggest the importance of Wnt signaling-mediated inhibition of chondrogenesis in the formation of posterior elements. As such, exogenous application of TGFβ can disrupt this balance leading to impaired development of the limb. This study was partially supported by CONACyT grants 53484 and 168642, DGAPA-UNAM grants IN214511 and IN220808.

Program/Abstract # 336

Retinoic acid effectors functions during axolotl limb regeneration

Correa Gallegos, Donovan; Chimal Monroy, Jesús (UNAM, Mexico)

Retinoic acid (RA) participates on several developmental and regenerating systems patterning. During axolotl limb regeneration, an excess of RA delays regeneration and induces the formation of more proximal structures from the amputation site which do not

normally form, hence producing proximal-distal axis duplications (PDd). The molecular mechanism behind these observations and the natural role of RA during limb regeneration are still unknown. RA exerts its effects through several nuclear receptors (RARs) which are associated with specific functions, furthermore, RARs dimerization with the retinoid "X" receptors (RXRs) might contextually affect RAR function. The present work aims to determine the possible specific functions of the RA effectors, RARs and RXRs, during the axolotl limb regeneration. By using specific RAR agonists and antagonists we could determine that RARgamma is important for autopod morphogenesis during normal regeneration and it is also responsible of the PDd and delay in regeneration when there is an excess of RA. Besides, panagonist and panantagonist of RXRs did not have any major effects suggesting a meaningless function during limb regeneration, although the antagonisms of RXRs increase the effects of the RARgamma agonist, suggesting that RXRs might act by regulating the activity of RARs. This study was partially supported by CONACyT grants 53484 and 168642, DGAPA-UNAM grants IN214511 and IN220808.

Program/Abstract # 337

Visualizing endogenous morphogen gradients and their modulation in vivo.

Sosnik, Julian; Zheng, Likun; Digman, Michelle; Nie, Qing; Gratton, Enrico; Schilling, Thomas (UC Irvine, USA)

During development, morphogens provides cells with critical information that determines their final fates. Retinoic Acid (RA), a vitamin A derivative, functions as a morphogen to establish the anterior-posterior (A-P) axis in early embryogenesis. Defects in RA signaling in animal models and humans lead to birth and developmental defects and cancer. In early development, RA is thought to form a morphogen gradient, and although in the past we have been able to describe such distribution and formulate mathematical models, we lack direct evidence of RA distribution. In this work we make use of the intrinsic fluorescence of RA and analyze RA distribution by combining Fluorescence Lifetime Imaging Microscopy (FLIM) with a phasor approach to FLIM data analysis. Using this approach we were able to detect and measure the wildtype endogenous distribution of RA *in vivo* without dyes or labels. The observed distribution is consistent with models that predict RA forming a gradient that extends anteriorly during the second half of the embryo's gastrulation. In addition, using this technique, we can semi-quantitatively measure the endogenous fluctuations in the system in the spatial and temporal domains. This is important in light of resent work suggesting that cells don't respond immediately to signals from morphogens, but rather temporally integrate this signals, with fluctuations playing an important role in accuracy of the outcome. We were also able to generate mathematical models that predict these behaviors. In all, this work constitutes the first time we can directly observe the graded distribution of RA and provides a novel mathematical model that allows us to make testable predictions beyond the limitations of our measurements.

Program/Abstract # 338

Retinoic acid regulates musculoskeletal patterning in the zebrafish head

McGurk, Patrick; Swartz, Mary; Eberhart, Johann (University of Texas-Austin, USA)

Proper regulation of the morphogen all-trans retinoic acid (RA) is essential for head and craniofacial development. The distribution of RA is mediated by its synthesis in Raldh-expressing cells and its degradation in Cyp26-expressing cells. In zebrafish, loss of the RA-catabolizing enzyme Cyp26b1 causes reduction of the anterior neurocranium and midline fusions of Meckel's cartilage and ceratohyals, ventral first and second arch cartilage elements, respectively. These cartilages and the tendons joining them to head muscles are cranial neural crest cell derivatives. The muscles themselves are mesoderm-derived, and in cyp26b1 mutants, muscle fibers that attach to the neural crest-derived skeleton display disrupted bundling and terminate ectopically. Restoration of cyp26b1 function in neural crest rescues ventral cartilage and muscle defects in cyp26b1 mutant embryos. We predict that the specification of neural crest derivatives is sensitive to RA signaling during craniofacial development. Consistent with this model, cyp26b1 mutant zebrafish embryos express higher levels of the tendon specification transcription factor scxa in the head. Chemical inhibition of RA synthesis during facial cartilage morphogenesis rescues muscle phenotypes and reduces scxa expression in cyp26b1 mutants. We are currently investigating the differentiation of neural crest over time in cyp26b1 mutants. We are also developing transgenic models for observing the coordination of growing muscle, tendon, and skeleton in the head. Further results will help shed light into the molecular mechanisms that direct musculoskeletal development.

Program/Abstract # 339

Identifying the mechanism of action for Dispatched-mediated Hedgehog ligand release.

Bodeen, William (Univ of TN HSC - St. Jude Children's Res Hosp, USA), Ogden, Stacey (St. Jude Children's Research Hospital, USA)

The Hedgehog (Hh) signal transduction pathway plays a conserved patterning role during metazoan development. In Hh ligand-producing cells, Hh protein is synthesized as a ~45 kDa precursor that undergoes an auto-catalytic processing reaction upon its entry into the ER. This results in formation of a ~20 kDa mature peptide ligand that is modified by palmitate on its amino-terminus and cholesterol on its carboxyl-terminus. To function as a morphogen, lipid modified Hh must be solubilized and released from its site of synthesis to travel multiple cell diameters across developing tissues. One protein that is required for this release is the 12-pass transmembrane protein Dispatched (Disp), a member of the resistance-nodulation division superfamily. Despite much effort, the exact mechanism by which Disp functions to facilitate Hh ligand release is not known. We have initiated biochemical, cell biological and Drosophila genetic studies aimed at dissecting Disp recognition and solubilization of mature, lipid modified Hh. We describe an *in vitro* assay system designed to dissect Disp activity, and provide evidence that Disp may function as part of a large molecular weight complex. Future experiments will be aimed at identifying Disp-interacting partners and ascertaining their role in Hh release.

Program/Abstract # 340

Mapping the functional domains in LRP2, an auxiliary SHH receptor in the developing neuroepithelium

Christa, Anna; Christ, Annabel ; Hammes, Annette; Willnow, Thomas (Max-Delbrueck-Center for Molecular Medicine, Germany)

Sonic hedgehog (SHH) is a key morphogen in mammalian brain development. It acts by binding to its cognate receptor patched 1, resulting in down-stream signal transduction through smoothened and GLI transcription factors. Intriguingly, several membrane-associated proteins have been identified recently that act as SHH binding proteins and as co-receptors to patched 1 in the developing nervous system. LRP2, a member of the LDL receptor gene family, is such an auxiliary SHH receptor expressed on the apical surface of the neural tube. Loss of LRP2 expression in mouse models results in the inability of the neuroepithelium to respond to SHH despite proper expression of patched 1 and smoothened, whereas overexpression of LRP2 variants in cells increase SHH signaling. Using biochemical studies and cell-based reporter assays, we now have characterized the functional domains in LRP2 required to promote SHH signaling. Thus, we mapped several bindings sites for SHH to the complement-type repeats in the extracellular domain of LRP2, suggesting multivalent ligand interaction. In addition, we document the ability of LRP2 to directly interact with patched 1 through its EGF precursor homology domains, an interaction that proceeds in the absence of SHH. Generation of an LRP2 mini receptor variant that carries a single SHH binding site and one EGF precursor homology domain for patched 1 interaction is sufficient to enhance SHH signaling in cells. Our data suggest a mechanistic model whereby LRP2 and patched 1 exist as a preformed co-receptor complex at the neuroepithelial cell surface and that this co-receptor complex initially interacts with SHH through multiple bindings sites located in the extracellular domain of LRP2.

Program/Abstract # 341

LRP2 is an auxiliary SHH receptor required to condition the forebrain ventral midline

Christ, Annabel; Christa, Anna; Willnow, Thomas; Hammes, Annette (Max-Delbrueck-Centrum, Germany)

Sonic hedgehog (SHH) is a regulator of forebrain development and genetic defects in *Shh* and in components of its cellular signaling machinery lead to a broad spectrum of brain malformations. Anomalies observed in patients and in rodent models include holoprosencephaly (HPE), a failure in midline induction resulting in the lack of forebrain separation into two hemispheres. Still little is known about the mechanisms at early neurulation whereby SHH from the prechordal plate governs specification of the rostral diencephalon ventral midline (RDVM), a major forebrain organizer. Here, we identified LRP2, a member of the LDL receptor gene family as intricate component of the SHH signaling machinery in the RDVM. LRP2 acts as apical SHH binding protein that sequesters the morphogen in this target field. Binding to LRP2 at the base of the primary cilium enables interaction of SHH with its receptor patched 1, resulting in internalization of SHH/patched 1 complexes and subsequent signal transduction through smoothened. As well as mediating SHH and patched 1 interaction, LRP2-dependent internalization delivers SHH molecules to the recycling compartment of the cell suggesting re-secretion as means to further increase local morphogen concentrations. In line with a critical role for LRP2 in SHH trafficking and signaling in the RDVM, lack of this receptor in mice and in patients results in failure to respond to SHH, and in forebrain formation defects and HPE. Our data substantiate the emerging concept that auxiliary receptors are critical modulators of morphogen signal reception in target tissues, and identified an important role for LRP2 in SHH action in the forebrain.

Program/Abstract # 342

Transcriptional regulation of Shh target genes in the developing spinal cord

Kurdija, Sanja, KI; Oosterveen, Tony; Alekseenko, Zhanna; Uhde, Christopher; Sandberg, Magnus; Andersson, Elisabet; Bergsland, Maria; Dias, José; Muhr, Jonas; Ericson, Johan (Karolinska Institutet, Sweden)

Secreted protein Sonic hedgehog (Shh) acts in a graded fashion at long-range to establish cell pattern in several tissues but little is known about how these concentration dependent mechanisms function on a transcriptional level. We have identified the cis-regulatory modules (CRM) of neural Shh-target genes, which we use as tools to elucidate the mechanisms imposed by Gli proteins, the bifunctional transcriptional mediators of Shh gradient. We find that Gli activators have a non-instructive role in long-range patterning and in synergy with SoxB1 proteins activate Shh target genes in a largely concentration independent manner. Instead, Gli repressors are interpreted at transcriptional level into precise spatial gene patterns in combination with regional homeodomain co-repressors. Moreover, the local interpretation of Shh displays lower CRM context sensitivity and requires Gli activators to accumulate to a threshold level sufficient to counteract Gli repressors. Thus our data propose a novel mechanism for transcriptional interpretation of Shh gradient.

Program/Abstract # 343

Following a transient dose, Sonic Hedgehog function in normal digit formation is dispensable and can be substituted entirely by enforced cell survival

Mackem, Susan; Zhu, Jianjian (National Cancer Institute, USA)

Different approaches have led to very different models of how Sonic Hedgehog (Shh) functions as a limb morphogen. Pharmacological studies in chick and some genetic analysis in mouse support a requirement for sustained Shh activity integrated over time to specify digit pattern. However, timed genetic deletion of *Shh* in mouse indicates only a very transient requirement in patterning. In both models, Shh is required for limb bud expansion to produce a normal number of digits; they differ in how these two roles are integrated. To directly evaluate the contribution of temporal integration to Shh function in digit patterning, we restored cell

survival in *Shh* mutant embryos following a short burst of Shh activity. Cell death is intercepted by concomitant removal of the pro-apoptotic genes *Bax/Bak*, and a conditional *Shh* allele is removed after a very short burst of activity that still results in a *Shh* null phenotype in all embryos with a single copy of *Bax* ($Bax^{+/-}$;$Bak^{-/-}$). In contrast, enforced cell survival ($Bax^{-/-}$;$Bak^{-/-}$ genotype) rescues digit formation in these phenotypically *Shh* 'null' embryos, which form up to 5 normally patterned digits. However, a transient Shh pulse is necessary for normal patterning; rescue of cell survival in germ-line $Shh^{-/-}$ embryos has no effect on the null phenotype. Complementary experiments, using a knock-out first approach, are underway to determine if an early time window is critical for this transient Shh patterning function, or if delayed activation of Shh can also rescue normal digit formation and patterning. Uncovering the downstream patterning relays that are activated by transient Shh function and become rapidly Shh-independent is currently under investigation.

Program/Abstract # 344

Shh is required to distally pattern mesenchyme to form digits

Crawford, Derrick M.; Robertson, Christopher; Mayberry, Ryan; Martinez, Chad; Ford, Andrew; Barrow, Jeffrey (Brigham Young Univ, USA)

Members of the Fgf family are expressed in the apical ectodermal ridge (AER) of the vertebrate embryonic limb and play a key role in the distal outgrowth and patterning of the adjacent limb mesenchyme. Their removal results in distal truncations of the limb whereas simple beads soaked in Fgf protein are sufficient to compensate for loss of the AER. Our lab has demonstrated that one of the mechanisms whereby the AER regulates outgrowth of the limb mesenchyme is to orient growth of the mesenchyme toward itself. It would be predicted therefore that the dimensions of the AER would be crucial for shaping the mesenchyme that it recruits and ultimately underlying the shape of the limb skeleton. Indeed, we have found that the shape of the AER changes over time in a manner that corresponds to the shape of limb elements as they form along the proximodistal (PD) axis. Given this observation it would be predicted that a bead being of fixed spherical dimensions would only be capable of forming a cylindrical, rod-shaped limb. Consistently, when an Fgf4 soaked bead is placed on the distal, posterior margin of chick embryonic limbs, one observes the formation of a rod-shaped ulna and a cylindrically arranged bundle of digits. While the autopod is cylindrical as expected, it condenses to form digits as opposed to a single element as was the case for the zeugopod. This suggests that regardless the shape of the source of Fgf signals, the distal-most mesenchyme is properly patterned to form digits. We have tested the hypothesis that Shh is required to distally pattern the mesenchyme to form digits. Consistent with this hypothesis, we report that chick limbs treated with the Hedgehog pathway antagonist, cyclopamine, res ult in fusions of autopod condensations. We also demonstrate that cylindrically arranged digits also condense to form a single skeletal element.

Program/Abstract # 345

Noggin can mimic BMPs effects on early neural crest-derived mesenchyme

Buchtova, Marcela (University of Veterinary and Pharmaceutical Sciences, Czech Republic), Cela, Petra (Institute of Animal Physiology and Genetics, Czech Republic); Balek, Lukas; Prochazkova, Jirina (Masaryk University, Czech Republic); Richman, Joy M (University of British Columbia, Canada)

Bone morphogenetic protein (BMP) antagonist, Noggin has previously been shown to induce a transformation of the maxillary prominence into the frontonasal mass (Lee et al. 2001) consisting of an ectopic cartilage in the palate, a supernumerary egg tooth and transformation of the maxillary bone to a premaxilla. Here we have extended this work to show that antagonists such as Follistatin or Chordin were unable to induce the striking skeletal changes. Since the original study showed that a feedback loop was induced where *BMP2* and *BMP7* were induced, we tested whether implanting *BMP2/7* beads would induce transformations. Only a few embryos formed small cartilage rods and small keratinized growths on the edge of the beak. We uncovered temporal differences in Noggin responsiveness, first ectopic cartilage was induced at stage 15 but not at stage 20 and second Sox9 RNA expression and reporter activity were induced at stage 15 but not stage 20. It was unclear whether the cartilage induction and upregulation of *Sox9* activity were secondary to the induction of BMPs by Noggin or due to the Noggin itself. We found that Noggin is repressing the BMP pathway as shown by decreased pSMAD1,5,8 in western blots. Although the canonical BMP pathway is shut down, it is possible that Noggin has activated target genes by unique pathways. Indeed, p38-MAPK levels were increased by Noggin. Our results have demonstrated that Noggin at very early stages of craniofacial development directly promotes chondrogenesis in the maxillary mesenchyme. This tendency to form cartilage in the palate is repressed while our experiments may be unmasking an ancient trait since a palatoquadrate cartilage is present in lower vertebrates but lacking in amniotes.

This work was supported by GACR (grant 304/09/0725) to MB and CIHR grants to JMR.

Program/Abstract # 346

A computational model suggests that diffusion alone does not account for BMP2/4 movement in sea urchin embryos.

Schatzberg, Daphne; Hardway, Heather; Ferrell, Patrick; Core, Amanda; Murray, Ian; Ross, Erik; Li, Christy; Kaper, Tasso; Bradham, Cynthia (Boston University, USA)

Spatial restriction of bone morphogenetic protein (BMP) signaling is critical for dorsal-ventral axis specification in developing embryos. Extracellular BMP proteins relocalize to spatial regions distinct from those in which BMP is expressed in both sea urchin and fly embryos. In *Drosophila* embryos, extracellular BMP movement requires interaction with the secreted BMP inhibitors Sog/Chordin and Twisted Gastrulation (Tsg). In the sea urchin embryo, BMP 2/4 is expressed in the ventral ectoderm, but signals

broadly in the dorsal ectoderm. In this study, we explore the movement of BMP in developing sea urchins using a combination of experimental and computational approaches. We demonstrate that LvTsg is required for BMP signaling and dorsal specification. In contrast, increasing LvTsg levels does not inhibit dorsal specification, but does contract the dorsal region and expand the ventral region, consistent with perturbed BMP movement. Using a reaction-diffusion-based mathematical model, we demonstrate that dorsal BMP relocation can occur, but only with 5.17% of the 10,000 realistic parameter sets tested. Importantly, none of these parameter sets recapitulate the empirical behavior of the system when Tsg levels are increased, since the dorsal BMP signaling domain fails to contract. Taken together, these results suggest that diffusion alone cannot account for BMP relocation to the dorsal domain in sea urchin embryos.

Program/Abstract # 347

Notch2, BMP5-8 and Alk4/5/7 signaling are required for skeletal patterning in sea urchin embryos

Piacentino, Michael L.; Patel, Vijeta; Hewitt, Finnegan; Ramachandran, Janani; Yu, Jia; Chaves, James; Reyna, Arlene; Hameeduddin, Hajerah; Bardot, Evan; Lee, David; Coulomb-Huntington, Jasmin; Heilbut, Adrian; Core, Amanda (Boston University, USA); Poustka, Albert (Max-Planck Institut fuer Molekulare Genetik, Germany); Bradham, Cynthia (Boston University, USA)

Skeletal patterning in sea urchin embryos requires communication between the skeletogenic primary mesenchyme cells (PMCs) and the adjacent pattern-dictating ectoderm; however, the molecular basis for this process remains unknown. From an RNA-seq screen, we identified Notch2 and BMP5-8 as ectodermal genes that are required for normal skeletal patterning. Morpholino-based (MO) loss-of-function analyses demonstrate that Notch2 and BMP5-8 morphants exhibit skeletal defects with right- and left-side biases, respectively. Immunofluorescent labeling of ectodermal structures indicates that ectodermal specification and development are normal in both morphants. The asymmetric defects seen in these morphants suggested that both signaling pathways interact with Nodal, which specifies the left-right (LR) axis in sea urchin larvae. We employed an LvNodal::GFP BAC to assay Nodal expression in live embryos, and the results show that LvNotch2 and LvBMP5-8 are each required for Nodal expression during LR patterning. Next, we inhibited the Alk4/5/7 receptor with SB431542 at different time points, to by-pass the early dorsal-ventral requirement for Nodal. These experiments demonstrate that, surprisingly, SB431542 inhibits bilateral animal skeleton development, but not formation of the associated oral hood or mouth, and the effects do not overlap with Notch2 or BMP5/8 LOF. These results suggest that a distinct TGFß signal is involved in animal skeletal patterning and development, since Nodal function is asymmetric during this period, and thus indicate that at least four signals are involved in defining the late skeletal pattern.

Program/Abstract # 348

BMP signaling requires an inwardly rectifying K+ channel to pattern the developing fly wing

Bates, Emily Anne (Brigham Young University, USA)

All cells maintain an ion gradient across the cellular membrane called a membrane potential. Excitable cells alter membrane potential for neuronal activity and muscle contraction, but membrane potential is thought to remain stable in non-excitable cells. Inwardly rectifying potassium channels (Irk2/Kir2) set resting membrane potential in excitable and non-excitable cells. We showed that Irk2/ Kir2.1 plays an essential role in developmental signaling in mice and in flies. Loss of Kir2.1 channel function causes craniofacial and digit defects in humans and in mice. These defects resemble defects that occur with reduced bone morphogenetic protein (BMP) signaling, leading to the hypothesis that Kir2.1 function is required for BMP signaling. Similarly, in the fruit fly, loss of Irk2 causes BMP/Dpp-like defects. Furthermore, loss of Irk2 blocks smad/Mad phosphorylation and expression of its transcriptional target, Spalt. Irk2 is specifically required for BMP/Dpp signaling; wingless/Wnt and hedgehog targets are intact in Irk2 deficient flies. We found that Irk2 is required downstream of BMP/Dpp translation and upstream of smad/Mad phosphorylation. The requirement for Irk2 is not cell-autonomous; Irk2 is required in Dpp producing cells or in the developing tissue as a whole. These results present the provocative idea that there may be evoked release of BMP. Since alcohol directly binds and inhibits the Kir2.1 channel and developmental phenotypes of people with mutations in Kir2.1 are similar to features of fetal alcohol syndrome patients, I propose that Kir2.1 is a target of alcohol causing morphological defects of fetal alcohol syndrome.

Program/Abstract # 349

Axis determination in amniotes

Bertocchini, Federica; Carrera, Lucia (IBBTEC, Spain)

In amniotes, the molecular device that regulates the positioning of the axis of bilateral symmetry is still unknown. In chick, although the polarity is specified by the time of egg-laying, the embryo maintains highly regulative properties: when a blastula-stage embryo (about 20,000 cells) is cut in half both halves can develop an embryonic axis spontaneously. This implies that normal embryos possess inhibitory mechanisms that prevent formation of multiple axes. While many genes are expressed posteriorly at these early stages and are involved in axis formation, only two so far, the transcription factor *Gata2* and the signaling molecule *Bmp4*, show a stronger expression anteriorly, decreasing towards the posterior region. Gata2 inhibits axis formation in a non-cell autonomous way anteriorly. We investigate Bmp4 role as inhibitor of axis formation anteriorly, and Bmp4 relationship with Gata2. We propose that Gata2 and Bmp4 are part of an anterior signaling centre inhibiting axis formation and therefore, opposing the posterior axis-forming embryonic region, contributing to the positioning of the embryonic axis. Is this proposed role of Bmp4/Gata2 in positioning the embryonic axis

conserved in amniotes other than chick? We are currently exploring axis formation in reptiles, focusing on chameleon (Squamata) and turtles (Testudines).

Program/Abstract # 350

Building a Vertebrate Embryo Using a Combination of Morphogenetic Gradients

Xu, Peng-Fei; Ferri, Karine; Thisse, Christine; Thisse, Bernard (University of Virginia, USA)

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

Program/Abstract # 351

Role of FGF signaling in maintenance of cardiac chamber identity in zebrafish

Pradhan, Arjana; Zeng, Xin-Xin (Univ of California, San Diego, USA); Marques, Sara (Skirball Institute of Biomolecular Med, NYU School of Med, USA); Chi, Neil; Yelon, Deborah (Univ of California, San Diego, USA)

The heart is composed of two types of cardiac chambers, atria and ventricles, each of which behaves as a distinct functional subunit with unique morphological, electrophysiological, and contractile properties. Hence, the proper chamber-specific differentiation of atrial and ventricular cardiomyocytes is crucial for the formation of a functional heart. Although there is some understanding of the pathways important for initiating chamber-specific differentiation, little is known about the pathways required to maintain identities of differentiated cardiomyocytes. In previous studies, we have demonstrated that fibroblast growth factor (FGF) signaling facilitates the initial formation of ventricular cardiomyocytes. Here we show that FGF signaling is also required after the initial differentiation of ventricular cells in order to preserve ventricular identity. We find that both pharmacological and genetic inhibition of FGF signaling can generate ectopic atrial cardiomyocytes within the already differentiated ventricle. Analysis using chamber-specific reporter transgenes suggests that these ectopic cells are produced through transdifferentiation of ventricular cardiomyocytes. In addition, we find that administration of retinoic acid (RA) can disrupt ventricular chamber identity, suggesting the possibility of a genetic interaction between the FGF and RA pathways. Together, our data suggest a model in which differentiated ventricular cardiomyocytes retain some plasticity and require continuous FGF signaling to preserve their chamber-specific identity. Ongoing work will identify the molecular pathway through which FGF signaling acts in this context.

Program/Abstract # 352

Modulation of fungiform papillae patterning by Fgf signaling

Prochazkova, Michaela (UCSF, USA), Häkkinen, Teemu (University of Helsinky, Finland); Prochazka, Jan; Jheon, Andrew (UCSF, USA); Jervall, Jukka (University of Helsinky, Finland); Klein, Ophir (UCSF, USA)

Introduction: Fungiform papillae are epithelial structures that house taste buds on the anterior tongue. Many molecular pathways are known to influence the patterning of the fungiform papillae. Canonical Wnt signaling was shown to initiate fungiform papillae formation, whereas other pathways triggered by Shh, Egf or Bmp have inhibitory effects on papilla size and number. However, mesenchyme-derived signaling factors have not yet been identified, hampering the development of a comprehensive model of papilla patterning. *Results:* We have examined the role of *Fgf10* in fungiform papilla development. Evaluation of *Fgf10* and *Spry2* knockout phenotypes showed that *Fgf10*, which is expressed in the tongue mesenchyme underlying the papillary field, functions as a negative regulator of fungiform papilla size. Using BATGAL mice, we demonstrated that this role is mediated by inhibition of canonical Wnt signaling, which is the main activator of papilla development. A gene expression analysis using qRT-PCR revealed interactions between *Fgf10* and the Wnt and Shh pathways. We then represented these results mathematically using a reaction-diffusion model to simulate activator-inhibitor dynamics in the system. *Summary: Fgf10* functions as a negative regulator of fungiform papilla size during embryonic development, suggesting a role in fine-tuning taste sensitivity. The proposed mechanism of *Fgf10* action is via inhibition of canonical Wnt activity. FGF10 is the first mesenchyme-derived factor to date involved in fungiform papillae patterning.

Program/Abstract # 353

The Facial Neural Crest Controls Fore- and Midbrain patterning by Regulating Foxg1 Expression Through Smad1

Creuzet, Sophie; Aguiar, Diego (CNRS- Institute of Neurobiology, France)

In Vertebrate embryo, the Facial Neural Crest (FNC) provides the forebrain with a skeletal, meningeal protection and a functional vasculature. It also controls the activity of brain organizers and stimulates cerebrum growth, but the repertoire of the molecules produced by the FNC to mediate its effect is unknown. To understand how FNC conveys its trophic effect, we have studied the role of *Smad1*, expressed in premigratory FNC cells—an intracellular transducer to which multiple signaling pathways converge — in the regulation of *Foxg1*. *Foxg1* is a transcription factor essential for telencephalic specification, the mutation of which leads to microcephaly and mental retardation in Atypical Rett Syndrome. *Smad1* silencing, based on RNA interference (RNAi), was performed in premigratory FNC cells. Soon after electroporation of RNAi molecules, *Smad1* inactivation totally abolished the expression of *Foxg1* in the telencephalon, and resulted in dramatic microcephaly and partial holoprosencephaly. Besides, the depletion of *Foxg1* activity altered the expression *Otx2* and *Foxa2* in di/mesencephalic neuroepithelium. However, when mutated forms of *Smad1*mediating Fgf and Wnt signaling were transfected into FNC cells, these defects were overcome. We also revealed that *Dkk1*, a Wnt antagonist produced by FNC, initiated the specification of the telencephalon by regulating *Foxg1* activity. Additionally, the activity of Cerberus in FNC-derived mesenchyme synergized with *Dkk1* to control Foxg1 expression and maintained the balance between *Otx2* and *Foxa2*. Our present results strongly suggest that some syndromic neurological disorders, which involve misregulations of *Foxg1* and *Otx2* are neurocristopathic in origin.

Program/Abstract # 354

The specification of jaw identity in avian embryos

Richman, Joy; Nimmagadda, Suresh; Geetha-Loganathan, Poongodi; Fu, Kathy (University of British Columbia, Canada)

Hox-negative neural crest cells migrate from the brain into the face and give rise to the facial skeleton. With the exception of the joint, the majority of jaw patterning is determined after neural crest cells reach their destination. Local interactions with the forebrain, foregut and facial epithelium restrict the fate of cells and specify their identity. We previously identified two signaling pathways that together specify frontonasal identity, BMP and retinoids. In avian embryos, implantation of beads soaked in Noggin, a BMP antagonist, and retinoic acid transform maxillary jaw elements to those typical for the midline. A microarray study was carried out and here we are focusing on the genes upregulated by Nog-RA that may be mediating the change in identity in maxillary mesenchyme. Peptidase Inhibitor 15 (PI15) is expressed in the normal facial midline and codes for a poorly characterized, secreted protein. We performed gain and knock-down experiments and determined that *PI15* is a mediator of the transformation phenotype and an RA target. A major part of the phenotype is the formation of cartilage in the palate which necessitates a change in fate of ectomesenchyme. We found that like Nog-RA, PI15 protein is able to induce a reporter for the chondrogenic program (Sox9-luciferase). The second striking aspect of the phenotype is the identity change. *EMX2*, which is normally expressed in the forebrain, is induced in the maxillary region in Nog-RA treated embryos. We propose that the beak phenotype is due to simultaneous induction of cartilage and alteration of the maxillary environment to take on characteristics of the mesenchyme adjacent to the forebrain. Funded by CIHR grants to JMR and a CIHR PDF to SN.

Program/Abstract # 355

Fibulin-7 is expressed in mouse early development and its C-terminal fragment shows anti-angiogenic activity

Forcinito, Patricia (National Institutes of Health, USA); de Vega, Susana (Juntendo University Graduate School of Medicine, Japan); Yamada, Yoshihiko (National Institutes of Health, USA)

The extracellular matrix (ECM) plays critical roles in many aspects of cellular behavior during development; and in tissue functions, regeneration, and diseases. The fibulins are a family of secreted glycoproteins associated with other matrices[1]. Mutations on fibulins have been related to genetic disorders in humans[2] (i.e. limbs malformations, eyes disorders and connective tissue abnormalities). We previously identified fibulin-7 (Fbln-7) as a new member of the ECM fibulin family[3]. Fbln-7 is expressed in teeth and acts as a cell adhesion molecule for odontoblasts[3]. Fbln-7 is also expressed in avascular tissues such as cartilage, eyes, and placenta. We found that the recombinant C-terminal Fbln-7, named fibulistatin, inhibited human umbilical vein endothelial cell (HUVEC) tube formation, suggesting a role of this fragment in anti-angiogenesis, which plays an essential role in implantation and placentation processes. To study the role of Fbln-7 in development, we analyzed the expression pattern of Fbln-7 in various stages of mouse development including the blastocyst stage, the implantation stage, and late embryonic stages. We found that Fbln-7 was expressed in the extraembryonic tissue at early embryonic tissues and in the placenta at later stages. We also found that Fbln-7 was expressed in the pregnant female uterus, which normal function is essential for successful implantation and placentation. These results suggest that Fbln-7 may play a role in peri-implantation, placenta formation and finally, in normal embryonic development.

Program/Abstract # 356

Establishing the border between the Intermediate and Paraxial mesoderm during chick embryonic development

Schneider, Jenny; Yelin, Ronit; Schultheiss, Thomas M. (Technion-Israel Institute of Technology, Israel)

The kidney is a vital organ functioning mainly as the body's excretory system. In vertebrates, all kidney tissue develops from the intermediate mesoderm (IM), a strip of mesodermal tissue lying between the paraxial mesoderm (PSM) and the lateral plate mesoderm (LPM). Differentiation of IM cells and their interaction with adjacent PSM cells at early developmental stages are crucial for kidney

maturation and function. We strive for better understanding of the mechanisms underlying IM formation, establishment, and separation from the PSM. We aspire to understand the interactions between IM genes (Osr1, Pax2) and PSM genes (Tbx6, Mesogenin), and study their effect on border formation between the IM and the PSM. Using single and double staining in situ hybridization, we have characterized normal IM and PSM gene expression. We show that Osr1 and Tbx6 overlap in a posterior region of the embryo, in an area that expresses Pax2, the specific IM marker. One possibility for establishing the border between the PSM and IM is mutual repression between genes expressed in these two regions. Using electroporation in the chick embryo, we found that misexpression of Osr1 results in repression of Tbx6 in the PSM. A regulatory element was isolated that directs Tbx6 expression to the PSM. This element contains a conserved Osr1 binding site and is repressed by Osr1 in a luciferase reporter assay. In reciprocal experiments, misexpression of Tbx6 in the IM did not result in IM gene repression. Experiments are currently in progress to test whether other PSM genes, including mesogenin, can repress expression of IM genes.

Program/Abstract # 357

Molecular characterization of the *Arabidopsis* twisted mutant

Reyes, Irepan; Escobar-Guzmán, Rocio (Langebio, Mexico); Chalfun-Junior, Antonio (Universida de Federal de Lavras, Brazil); Pereira, Andy (Virginia Bioinformatics Institute, USA); Angenent, Gerco C (Plant Research International, Netherlands); Marsch Martinez, Nayelli; de Folter, Stefan (Langebio, Mexico)

The correct temporal and spatial coordination of the division and elongation of cells is important for plant growth. Helical growth pattern is the result of a continuously tilted growth axis as cells grow. Different factors regulate helical growth such as auxins, microtubules, and microtubule associated proteins. Twisted (*twt1-D*) is a gain-of-function mutant obtained by activation-tagging using the En-I transposon system. In this mutant all organs are twisted with a more severe phenotype in the siliques. In the present study we present morphological and molecular analyses of *twt1-D*. The expression and recapitulation analyses demonstrate that *TWT1* is responsible for the twisted phenotype. Results obtained for double mutants and crosses with marker lines, indicate a change in auxin homeostasis, possibly due to altered auxin transport and microtubule stability. The latest results will be presented.

Program/Abstract # 358

The phytohormone cytokinin defines and restores specific tissues of developing gynoecia and fruits in Arabidopsis

Marsch-Martinez, Nayelli; Ramos-Cruz, Daniela (CINVESTAV-IPN Irapuato, Mexico); Reyes-Olalde, Irepan; Lozano-Sotomayor, Paulina; Zuñiga-Mayo, Victor; de Folter, Stefan (CINVESTAV-IPN Irapuato, Langebio, Mexico)

The phytohormone cytokinin has many essential roles in plant embryonic and postembryonic growth and development, but its role in fruit patterning and morphogenesis had not been explored. Moreover, information about the spatio-temporal localization pattern of cytokinin signaling in gynoecia and fruits was lacking. Therefore, the synthetic reporter line TCS::GFP was used to visualize cytokinin signaling during gynoecium and fruit development. Fluorescence was detected at medial regions of developing gynoecia and, unexpectedly, at the valve margin in developing fruits, and was severely altered in mutants that lack or ectopically acquire valve margin identity. Interestingly, comparison to the phytohormone auxin signaling reporter DR5rev::GFP developing gynoecia and fruits showed that the transcriptional responses to cytokinin and auxin were frequently located in complementary patterns during gynoecium and fruit development. Moreover, cytokinin treatments in early gynoecia produced conspicuous tissue- specific overgrowth in gynoecia, while treatment of valve margin mutant fruits restored this tissue. The results suggest that the phytohormone cytokinin is an important player involved in gynoecium and fruit patterning and morphogenesis, playing at least two roles: an early proliferation-inducing role at the medial tissues of the developing gynoecia, and a late role in fruit patterning and morphogenesis at the valve margin of developing fruits.

Program/Abstract # 359

Proteomics approaches to Identify the function of PI15, a putative embryonic morphogen

Drain, Stephen; Nimmagadda, Suresh; Richman, Joy (University of British Columbia, Canada)

A chicken microarray study revealed that Peptidase Inhibitor 15 (PI15) was strongly induced in a an experiment in which a second set of midline skeletal elements plus an egg tooth formed on the side of the upper beak. *PI15* was further shown to be expressed in developing embryonic tissues including the frontonasal mass, limb and paraxial mesoderm. PI15 is a small, secreted protein (approximately 25kDa) that could be involved in a novel cell-cell signaling mechanism. PI15 protein was first purified from glioma cell lines and is upregulated in a wide variety of human tissues and several human cancers. The principal aim of my study is to identify interacting proteins to PI15 in order to learn more about its function. A construct expressing PI15-flag-IRES-GFP or a control plasmid expressing GFP was used to stably transform avian cell lines. The culture media from these lines, containing the secreted protein, was run over beads bound to flag antibody and the presence of the PI15 bait protein on these beads was confirmed by Mass Spectrometry. Furthermore, the concentration of exogenous bait protein secreted from these cells is approximately 225 ng per 150 mm plate per day. The beads bound to the bait protein, or to proteins from control cells, were reacted with lysates of tissue dissected from the frontonasal mass of stage 28 chicken embryos and duplex labeling was done to differentiate control versus experimental preparations. Proteins which interact with PI15 in the frontonasal mass can therefore be specifically identified by mass spectrometry. We will now use this peptide discovery approach to infer putative molecular pathways through which PI15 exerts its biological effects. Funded by CIHR grants to JMR.

Program/Abstract # 360

Differential Expression of Extracellular Matrix Proteins During Posterior Commissure Development

Stanic, Karen; Gonzalez, Melissa; Montecinos, Hernán; Caprile, Teresa (Universidad de Concepcion, Chile)

The function of the mature nervous system depends upon the right formation of highly complex neuronal circuits. Accordingly, a critical phase of early nervous system development is the establishment of appropriate connections between neurons and their target cells. The formation of these precise "wiring" patterns is controlled by the ability of the leading edge of an axon, termed the growth cone, to sense a combination of environmental cues and make choices based on the extracellular information consisting on soluble, membrane-bound and extracellular matrix molecules. The posterior commissure is an axonal tract located in the roof plate of the pretectal region between the most caudal prosomere (prosomere 1) and the mesencephalon. Previous result from our laboratory identify SCO-spondin as a major contributor on axonal behavior among prosomere 1 commissural roof plate, never the less how these axons arises from the pretectal nucleus present in the ventral region towards the dorsal zone is still to be elucidated. In this work we provide immunohistochemical evidence about differential expression of extracellular matrix protein (EMP) in the alar plate and floor plate of the commissural region of the prosomere 1 during PC development. We have identified so far 8 different EMP in this region: Chondroitin sulphate, Decorin, Fibronectin, HKN-1, Laminin, Perlecan, Osteopontin and Tenascine, all of them with specific trails of expression, i.e. tenascine and HKN-1 posses the same expression pattern in the alar plate opposite to SCO-spondin, however laminin present a highly similar pattern of this latter. Taken all together this work generates a topographic expression map where commissural axons are able to navigate in order to form the PC. Grant Sponsor: FONDECYT 1110723.

Program/Abstract # 361

The Ubiquitin ligase activator APC/C-Cdh1 (Rap/Fzr) regulates retinal axon targeting in the developing *Drosophila* eye

Venkatesh, Tadmiri; Gronska, Marta (City College of New York, USA)

The precise targeting of axons and formation of specific connections during development is critical to nervous system functioning and the mechanisms that regulate axon targeting are not fully understood. During development of the *Drosophila* compound eye photoreceptor (R cell) axons target stereotypically to specific layers of the optic ganglia (medulla and the lamina). To test for the involvement of ubiquitin ligases in axon targeting, we have examined the role of Drosophila–Cdh1 (Rap/Fzr) in retinal axon growth and targeting in the developing eye. Drosophila-Cdh1 (Rap/Fzr) is the activating subunit of the conserved ubiquitin ligase, anaphase promoting complex/ cyclosome (APC/C). In loss-of-function Drosophila–Cdh1 mutants retinal axons fail to terminate properly leading to aberrant axonal patterning in the optic ganglia. Experiments using *ro-tau-lacZ* constructs show that, in loss of function Cdh1 (Rap/Fzr) mutants photoreceptor R2-R5 axons fail to stop in the lamina and miss-target to the medulla layers. Conversely, gain-of-function of Cdh1 (Rap/Fzr) leads to premature termination and clumping of R cell axons. In addition, genetic mosaic analyses experiments using FLP-FRT and GAL4-UAS techniques show that Cdh1 (Rap/Fzr) functions in a cell autonomous manner. Our studies suggest that Cdh1 (Rap/Fzr) functions as a regulator of axon targeting during *Drosophila* visual system development. These results are consistent with other mammalian studies reporting a role of Cdh1 in axon growth and targeting and provides further insights into the conserved neuronal functions of the ubiquitin ligase complex APC/C^{Cdh1}.

Program/Abstract # 362

Withdrawn

Program/Abstract # 363

Cell-lineage analysis and localization of the embryonic sinoatrial node precursor cells during early mouse development

Molero Abraham, Mª MAgdalena; Franco, Diego; Aránega Jiménez, Amelia; Dominguez Macias, Jorge Nicolas (Universidad de Jaén, Spain)

The early heart forms from two mesodermal cell populations, called the First and Second Heart Fields (F&SHF). C ell lineage tracing experiments using the SHF marker islet1, have revealed that the left ventricle derives exclusively from FHF, whereas the outflow tract, right ventricle and the atria are of mixed F&SHF origin (Cai et al, 2003). More recently it has been demonstrated that sinoatrial (SAN) and atrioventricular (AVN) nodes are partially SHF-derived (Moretti et al, 2006; Sun et al, 2007). However the contribution of SHF-islet1 cells to the distinct components of the ventricular conduction system, as well as, the localization of SAN and AVN islet1-precursor cells, are still unknown. Using dye-injections within the posterior portion of the SHF (pSHF), we previously demonstrated that atria and atrioventricular canal are pSHF-derived (Domínguez et al, 2012). Now, we are interesting to determine whether SAN and AVN islet1+ precursor cells are placed at the pSHF and, moreover, whether islet1+ progenitor cells contribute as well to His-Purkinje system development. Preliminary results from dye-injections experiments reveal that, in most cases, right and left pSHF-derived cells populate ipsilaterally the embryonic atria. However, some dye-labelled cells were placed at the right atria-sinus venous junction, where the pacemaker marker, HCN4, is expressed and define the putative SAN precursor. These results would suggest that islet1+ progenitors cells within the pSHF participate in the developing of both, the working and conduction system myocardium, and open new ways to explore the putative SHF origin of the distinct CCS elements.

Program/Abstract # 364

Dissecting the roles of the proepicardium, Fgf10 and Fgf3 in cardiac development

Urness, Lisa D; Bleyl, Steven B (University of Utah, USA); Moon, Anne M (Weis Center for Research/Geisinger Clinic, USA); Mansour, Suzanne L. (Univ of Utah, USA)

The heart forms from multipotent progenitors and abnormalities within the progenitors or in their interactions with surrounding cells lead to congenital heart defects. One progenitor, the proepicardial organ (PEO), forms in mesothelium caudal to the developing heart. PEO cells migrate across pericardial space, attach to the myocardium and migrate over the myocardial surface to form the epicardium by E11.5 in mouse. Some epicardial cells undergo an epithelial-to-mesenchymal transition and enter the heart, generating coronary smooth muscle, endothelium and fibroblasts. The epicardium also provides trophic signals supporting expansion of the myocardium during mid-gestation. Fibroblast growth factors (FGFs) provide critical communication within and between developing heart progenitors and surrounding tissues. Global *Fgf3/Fgf10* double null mutants exhibit a spectrum of heart defects not found in either single null mutant. Defects include reduced epicardial cell ensheathment, detachment of epicardial cells from the myocardium, and thinned myocardium, with double mutants dying of heart failure by E11.0. *Fgf10* is expressed in cardiac mesoderm and the PEO, but the *Fgf3* expression domain relevant to the epicardial phenotype is unclear. To determine the earliest role of PEO-derived cells in murine cardiac development, we are genetically ablating *Tbx18*+ PEO cells and have found that their loss causes heart failure and death by E11.5. To determine the tissue-specific requirements for *Fgf10* and *Fgf3* in heart development we are conducting conditional mutant analyses. Our data suggest that *Fgf10* is required in the proepicardium and cardiac mesoderm and that there may be a mesodermal source of *Fgf3* important for heart development.

Program/Abstract # 365

Arid3b is required for the formation of heart poles and patterning of the atrioventricular canal (AVC)

Uribe Sokolov, Veronica; Badia-Careaga, Claudio (CNIC, Spain); Casanova, Jesus (Monash University, Australia); Sanz-Ezquerro, Juan Jose (CNB, Spain)

ARID3b is a member of the conserved ARID family of gene regulators, which is known to have important roles in both embryonic development and cancer. ARID3b null-mice die during early stages of embryonic development, but its roles in development are not completely understood. The purpose of our study is to address the function of Arid3b in the developing heart. During mouse embryo development Arid3b is expressed from early stages in the myocardium of the tubular heart and in the precursors situated in the pharyngeal mesoderm (second heart field), but later it gets restricted to the poles of the heart. Using Arid3b knock-out mice, we observed that mutants display cardiac abnormalities. Three main defects are seen – a noticeable shortening of the outflow tract, a reduction of the size and abnormal shape of the inflow region and a premature maturation of the myocardium of the AVC, as well as a fail to form the AV cushions. Expression of several molecular markers of both secondary heart field (SHF) and chambers are altered in mutant embryos. To address the cause of the defects in the heart poles, we performed DiI labelling of heart precursors and *in vitro* embryo culture, observing a reduction in the addition of cells to the heart tube. An RNA microarray comparing wild type versus mutant hearts revealed a set of differentially expressed genes, which are now being analyzed. Our conclusion is that Arid3b plays an important role in different aspects of heart development. *In vivo* embryonic phenotypes, DiI labelling experiments and *in vitro* cell culture data suggest that Arid3b could control cell addition from the SHF to the heart by regulating cell motility. Moreover, Arid3b is involved in proper chamber and valve formation.

Program/Abstract # 366

Elucidating Mechanisms Underlying Epicardial Development

Khan, Sana; Holtzman, Nathalia (Queens College, USA)

Heart organogenesis requires the coordination of cells and development of three cardiac tissue layers. The muscular myocardium is internally lined by the endocardium and externally covered by the epicardium. The stem-cell-like quality of epicardial cells is essential for development and regeneration of some cardiac tissues. The epicardium signals to and contributes cells to coronary vessels, myocardium, and endocardium. Despite its importance, the process required to form and mature the epicardium is poorly understood. Previous studies suggest that cells from the proepicardial organ (PEO) at the base of the sinus venosus (SV) migrate onto the myocardium via direct contact of PEO derived multicellular structures and indirectly through cellular cysts that float across to the myocardium to form epicardium. Taking advantage of the optical clarity, cardiac morphology and molecular tools available in zebrafish, we find evidence for two mechanisms of PEO migration. The primary PEO population sits at the SV. A subset of cells from SV PEO crawl directly onto the atrial myocardium, the direct migrators. A second set of cells, indirect migrators, travel along the pericardial surface posterior to the myocardium. In the region posterior to the AV myocardium, we observe clusters of cells, the AV PEO. We found that AV PEO cells form mulitcellular villi that transfer cells via cardiac contractions and differential adhesion to the ventricular myocardium. We tested this mechanism by disrupting cardiac contractility with drug treatment. Significantly fewer PEO villi cells transferred to the ventricle surface when cardiac contractions were abolished. PEO villi cell migration to the ventricular myocardium is cardiac contraction dependent.

Program/Abstract # 367

The role of Fosl2 in zebrafish Second Heart Field Development

Jahangiri, Leila; Guner-Ataman, Burcu; Adams, Meghan; Burns, Caroline E; Burns, C. Geoffrey (MGH, Harvard Medical School, USA)

Congenital outflow tract (OFT) malformations (e.g. conotruncal defects) are significant causes of newborn morbidity and mortality in the US and worldwide. In mammals, the embryonic OFT and primitive right ventricle arise by accretion of newly differentiated cells to the arterial pole of the heart tube from multi-potent progenitors residing in the anterior second heart field (SHF). While severe SHF deficiencies cause embryonic lethality, intermediate defects cause misalignment of the outflow tract relative to the ventricles, a defining feature of double outlet right ventricle (DORV) and Tetralogy of Fallot (TOF). In 2011, the Burns Laboratory reported that zebrafish cardiogenesis relies on LTBP3-mediated TGFβ signaling (Zhou 2011). Using microarray profiling followed by in situ analyses, we learned that transcriptional regulator, *fos-like antigen 2* (*fosl2*), is expressed in a pattern overlapping with *nkx2.5* in the heart field prior to becoming restricted to the junction between the myocardium and SHF at linear heart tube stages. Like *ltbp3* morphants that show a SHF deficiency, morpholino-mediated *fosl2* knock-down results in embryos with severe arterial pole defects characterised by reductions in the number of ventricular cardiomyocytes and multi-lineage loss of the outflow tract (OFT) derivatives. However, unlike *ltbp3* morphants that fail to maintain a SHF progenitor pool, *fosl2* morphants show a dramatic accumulation of SHF progenitor cells. Using photoconvertible reporter transgenic lines, we learned that the build-up of undifferentiated second heart field cells was due to defective myocardial accretion and differentiation in *fosl2* morphants. In addition, using RNA-seq, we have shown that markers of differentiated myocardium are downregulated in *fosl2* morphants. Furthermore, we report our findings on the genetic interactions between *fosl2* and *ltbp3* in second heart field development.

Program/Abstract # 368

Cadm4 restricts the production of cardiac outflow tract progenitor cells

Zeng, Xin-Xin I.; Yelon, Deborah (University of California, San Diego, USA)

Proper development of the cardiac outflow tract (OFT) is essential for connecting the heart to the vasculature, and abnormal OFT growth or morphogenesis can cause congenital heart defects. Given the importance of appropriate OFT assembly, the embryonic origins of OFT progenitor cells are of great interest. A variety of recent studies have illustrated that the heart is built through two major sources of progenitors: first heart field (FHF) and second heart field (SHF) progenitors, which differ in their timing of differentiation and in their contributions to specific regions of the heart. Notably, the OFT is built by the addition of late-differentiating, SHF-derived cardiomyocytes to the arterial pole of the primitive heart tube. We have identified an intriguing gene, *cell adhesion molecule 4* (*cadm4*), that is expressed in a region near the arterial pole where the SHF-derived OFT progenitor cells reside. Strikingly, loss of *cadm4* function causes a dramatic enlargement of OFT size: a surplus of SHF-derived cells aggregates around the arterial pole and ultimately results in the addition of nearly twice the normal number of OFT cardiomyocytes. Conversely, overexpression of *cadm4* reduces the size of the OFT by decreasing the number of OFT progenitor cells clustered at the arterial pole. Analyses of the dynamics of cell division and differentiation suggest that *cadm4* influences the proliferation and maintenance of OFT progenitor cells before they migrate into the heart tube and contribute to the OFT myocardium. Together, our data support a novel model in which Cadm4 activity limits the production of OFT progenitor cells, potentially through an adhesion-based mechanism for the regulation of cardiomyocyte differentiation.

Program/Abstract # 369

Mutant Shp2 from Noonan Syndrome and LEOPARD Syndrome induced similar defects during early heart development.

Bonetti, Monica (Hubrecht Institute, Netherlands)

Germline mutations in the human gene *PTPN11*, encoding Shp2, cause Noonan syndrome (NS) and LEOPARD syndrome (LS), two multisymptomatic developmental disorders that are characterized by cardiac defects, short stature, craniofacial defects and mental retardation. Interestingly, Shp2 catalytic activity is enhanced by NS mutations and reduced by LS mutations. The heart defects appear to be distinct between NS and LS patients, in that NS patients usually develop pulmonary stenosis and hyperthrophic cardiomyopathy is observed more in LS patients. We generated the most common NS/LS Shp2 mutations and expressed these in zebrafish embryos to analyse their function in vivo . The resulting embryos were shorter than control wild-type Shp2-expressing embryos and they displayed cardiac and craniofacial defects, reminiscent of human symptoms. In this study, we investigated the cardiac defects that were induced by expression of mutant Shp2. We found that the developing heart in NS/LS embryos failed to undergo looping morphogenesis. These defects were indistinguishable between NS and LS during early embryogenesis. Expression of NS and LS mutants induced MAPK hyperactivation at bud stage, which may explain why the early developmental defects were indistinguishable. The cardiac anomalies occured during the elongation of the heart tube and consisted of reduced cardiomyocyte migration, coupled with a reduced heart rotation. Furthermore, the expression of specific laterality markers was randomized in NS/LS embryos, which might be caused by defective ciliogenesis that we observed at early developmental stages. These results indicate a direct correlation between NS and LS mutations and their ability to cause defects in early heart development.

Program/Abstract # 370

The androgen receptor is differentially expressed in the atrium and ventricle tissue of mouse embryo.

De Ita Ley, Marlon; Pedernera Astegiano, Enrique Antonio; Meneses Morales, Iván; Gómora Herrera, María José; Méndez Herrera, María del Carmen (UNAM, Mexico)

The androgen receptor is differentially expressed in the atrium and ventricle tissue of mouse embryo De Ita Ley, M; Pedernera Astegiano, E; Meneses Morales, I; Gómora Herrera MJ; Méndez Herrera, MC. The presence of androgen receptor (AR) was described in the heart of adult and neonatal mice; however the pattern of expression of this receptor during the prenatal heart

development is already unknown. Embryos of 8.5-18.5 days post coitum (dpc) were obtained from pregnant mice -CD1 strain- 2 postnatal days (dpn) mice were also evaluated; atrium and ventricles were dissected and studied separately. Reverse transcriptase PCR was performed to analyze the expression of mRNA of AR, Nppa and Myh6; beta actin was used as endogenous control. The presence of the protein was evaluated by immunohistochemistry. Results: The mRNA and the protein of AR were expressed from 12.5 dpc to 2 dpn mice. The AR mRNA is differentially expressed in atrium and ventricles at 16.5 dpc, 18.5 dpc and 2 dpn mice with the highest expression in atrial tissue. The expression of mRNA of Nppa and Myh6 displayed a similar pattern with prevalence in atria. These results indicate that androgen receptor could have a role in the functional differentiation of cardiomyocytes. Also, the differential expression between atria and ventricle suggest that androgen receptor could be involved in the physiology and development of atria. Partly supported by PAPIIT IN226811

Program/Abstract # 371
Withdrawn

Program/Abstract # 372
A heterogeneous cellular origin of the cardiac lymphatic vasculature.
Klotz, Linda; Ruhrberg, Christiana (University College London, UK); Riley, Paul (University of Oxford, UK)
The lymphatic vasculature is a blind-ended network crucial for tissue fluid homeostasis, immune surveillance and lipid adsorption from the gut, as well as being the main route of cancer metastasis. The heart is the first organ to develop in mammals, however little is known about the lymphatic vasculature in the heart. The mechanisms regulating the development of the cardiac lymphatic vessels, as well as their cellular origin, are yet to be described. In this study we shed light on the development and origin of the cardiac lymphatic vasculature from mid-gestation to early adulthood in mice. We show that lymphatic vessels first sprout at embryonic day 14.5 (E14.5) in the outflow region of the heart and develop alongside coronary blood vessels, completing development and largely encompassing the heart by postnatal day 15 (P15). We have utilized various Cre transgenic mouse lines to give a comprehensive overview of lineage contributions to the cardiac lymphatic vessels. Contrary to the current dogma of the field suggesting that veins give rise to the entire lymphatic network, our lineage tracing analyses suggest a heterogeneous cellular origin for developmental de novo lymphvasculogenesis in the heart – with the involvement of both blood endothelial as well as other lineages. O ur characterization of the cardiac lymphatic vasculature offers novel insight into a largely overlooked, but arguably very important part of the lymphatic system. Future studies will focus on applying the findings from lymphatic development in the embryonic heart to adult cardiovascular pathology and disease.

Program/Abstract # 373
Elucidation of the molecular mechanism of Rasip1 and Arhgap29 in blood vessel development.
Koo, Yeon (Univ of Texas Southwestern Med Ctr, USA), Xu, Ke (Harvard, USA); Davis, George (Univ. of Missouri, United States); Cleaver, Ondine (Univ of Texas Southwestern Med Ctr, USA)
Cardiovascular function depends on patent, continuous blood vessel formation by endothelial cells (ECs). Blood vessel development initiates during 'vasculogenesis' via the aggregation of ECs into linear aggregates, which then form tubes with a central lumen that allows blood flow. However the mechanisms underlying vascular 'tubulogenesis' are only beginning to be unraveled. We recently showed that a novel GTPase-interacting protein called Rasip1 and its binding partner the RhoGAP Arhgap29 are required for formation of continuous blood vessel lumens. Rasip1 null embryos showed disrupted localization of EC polarity and junctional complexes, and loss of adhesion of ECs to extracellular matrix (ECM). In vitro studies also showed that depletion of either Rasip1 or Arhgap29 in cultured ECs lead to failed tubulogenesis and abrogation of integrin-dependent adhesion contact maturation. From these studies, we propose that Rasip1 and Arhgap29 regulate multiple cellular processes required for functional vascular tubulogenesis.

Program/Abstract # 374
Annexin A3 is required for early blood vessel formation
Meadows, Stryder M.; Fletcher, Peter (UT Southwestern Medical Center, USA); Sacharidou, Anastasia; Davis, George (University of Missouri, USA); Cleaver, Ondine (UT Southwestern Medical Center, USA)
Annexins are a unique class of proteins that bind to membrane phospholipids in a calcium-dependent manner. They are able to function in several different capacities that include organizing membrane domains and cytoskeletal linkages, as well as regulating both exocytic and endocytic transport and the flow of ions across cell membranes. While annexins are linked to many cellular processes, the function of Annexin A3 (Anxa3) during development is completely unknown. Our studies show that Anxa3 expression is conserved in the endothelial cell (EC) lineage of mice, frogs and fish, suggesting an important role during vascular development. Morpholino (MO) studies demonstrate an essential requirement for Anxa3 during early blood vessel formation, as Anxa3 MO-treated *Xenopus* embryos lack the tight junction molecule Claudin-5 and exhibit severe disruptions in EC-EC adhesion and failure of vascular cord formation. Furthermore, Anxa3 siRNA-treated ECs fail to assemble into vessel-like networks in 3D collagen matrices *in vitro*, suggesting adhesion defects. Overall, these studies demonstrate a requirement for Anxa3 in assembly and coalescence of ECs, and are the first to indicate a role for Anxa3 during vascular development.

Program/Abstract # 375

Identification of Cell Motility Genes Specific to Primitive Myeloid Linieage in *Xenopus laevis*

Kenny, Alan; Jagpal, Amrita; Allbee, Andrew; Prewitt, Allison; Shifley, Emily; Zorn, Aaron (Cincinnati Children's Hospital, USA)

Vertebrate blood development occurs in 2 conserved spatially and temporally distinct waves: primitive and definitive hematopoiesis. In *Xenopus* neurula and tail bud stages, *spib*-expressing primitive myeloid cells emerge in the anterior ventral blood islands (aVBI), the equivalent of the mammalian yolk sac. Over time these cells later migrate throughout the embryo. Despite gains in understanding myeloid development, an important question remains: what genes are induced specific to the primitive myeloid lineage allowing it to differentiate and migrate. The aims of this research are to: (1) identify the genes from the developing myeloid lineage and (2) determine the dynamic expression profiles of these genes during myeloid differentiation and migration. We examined expression of selected genes from 3 anterior ventral explant microarray sets in relation to myeloid-specific expression through neurula and early tailbud stages and compared their expression to *spiba*, a myeloid marker. We also used morpholino to generate aVBI-targeted *spiba* loss-of-function and examined the loss-of-function effect on these genes' expression by *in situ*. Compiled microarray experiments identified 10 genes specifically expressed in the aVBI-derived myeloid cells, all of which are implicated in cell migration. Dorsal-targeted Morpholino knockout of *spib* specifically in the foregut area produced a decrease in the expression of actin-binding proteins *coronin* and *cofilin* consistent with them being downstream of myeloid-specific *spiba* transcriptional regulation. Together, these results suggest a number of genes involved in cell motility including are specifically expressed in differentiating primitive myeloid cells regulated by *spiba*.

Program/Abstract # 376

The Role of BMPs in Digit Number Regulation

Norrie, Jacqueline; Li, Qiang (University of Texas-Austin, USA); Bouldin, Courtney; Harfe, Brian (University of Florida, USA); Vokes, Steven (University of Texas-Austin, USA)

Bone morphogenetic proteins (BMPs) are essential for many aspects of limb development including limb bud outgrowth, axial specification, and skeletogenesis. The collective temporal roles of BMPs have been difficult to determine because there are multiple, partially redundant ligands. To observe how and when BMPs regulate digit formation, we generated a mouse model that drives Cre-inducible transcription of the BMP inhibitor *Gremlin*, allowing temporal regulation of BMPs. Activation of *Gremlin* throughout the limb bud mesenchyme results in the generation of polydactylous hindlimbs and nearly absent forelimbs. By inhibiting BMPs at various timepoints we demonstrate that they are essential for restricting digit number during an interval between E10 and E11. The ectopic expression of Gremlin reduces but does not eliminate endogenous BMP signaling. This inhibition results in increased and sustained Fgf and Shh activity. Our current efforts are focused on elucidating the role of BMP inhibition in regulating cell growth.

Program/Abstract # 377

Transient Inhibition of FGFR2b Signaling Leads to Irreversible Loss of Cellular Beta-Catenin Organization and Signaling in AER During Mouse Limb Development

Danopoulos, Soula; Al Alam, Denise; Parsa, Sara; Tabatabai, Reza; Bellusci, Saverio (USC/CHLA, USA)

The vertebrate limbs develop through coordinated series of inductive, growth and patterning events. Fibroblast Growth Factor receptor 2b (FGFR2b) signaling controls the induction of the Apical Ectodermal Ridge (AER) but its putative roles in limb outgrowth and patterning, as well as in AER morphology and cell behavior have remained unclear. We have investigated these roles through graded and reversible expression of soluble dominant-negative FGFR2b molecules at various times during mouse limb development, using a doxycycline/transactivator/ tet(O)-responsive system. Transient attenuation (≤24 hours) of FGFR2b signaling at E8.5, prior to limb bud induction, leads mostly to the loss or truncation of proximal skeletal elements with less severe impact on distal elements. Attenuation from E9.5 onwards, however, has an irreversible effect on the stability of the AER, resulting in a progressive loss of distal limb skeletal elements. The primary consequences of FGFR2b attenuation is a transient loss of cell adhesion and down-regulation of P63, beta 1-integrin and E-cadherin, and a permanent loss of cellular beta-catenin organization and WNT signaling within the AER. Combined, these effects lead to the progressive transformation of the AER cells from pluristratified to squamous epithelial-like cells within 24 hours of doxycycline administration. These findings show that FGFR2b signaling has critical stage-specific roles in maintaining the AER during limb development.

Program/Abstract # 378

De-coupling the Hox-Shh-Fgf interaction reveals multiple inputs of Hox genes on pathways ensuring limb growth.

Sheth, Rushikesh; Grégoire, Damien; Dumouchel, Annie; Scotti, Martina; My Trang Pham, Jessica; Nemec, Stephen (Institut de Recherches Cliniques de Montréal Canada); Bastida, Maria Félix; Ros, Marian (Instituto de Biomedicina y Biotecnología de Cantabria (CSIC-UC-IDICAN) and University of Cantabria, Spain); Kmita, Marie (Institut de Recherches Cliniques de Montréal, Canada)

One of the most intriguing questions in developmental biology is how organ growth and patterning are coordinated during embryogenesis. Limb development relies on an exquisite coordination between growth and patterning but the underlying mechanisms remain elusive. Previous studies showed that A-P and P-D limb bud growth and patterning relies on a positive feedback loop between Sonic Hedgehog (Shh), the BMP antagonist Gremlin1 (Grem1), both expressed in mesenchymal cells, and Fibroblast growth factors (Fgfs) produced in the Apical Ectodermal Ridge (AER). In addition, the collinear expression of *HoxA* and *HoxD* genes has a key role

in A-P and P-D patterning by establishing positional identity along these axes. Here, we show that *HoxA* and *HoxD* genes are required at early stages for *Grem1* activation and proper *Fgf10* and *Fgf8* expression and therefore are mandatory for the establishment of the Shh-Grem1-Fgf feedback loop. Our results provide evidence that, in addition to this early function, *HoxA* and *HoxD* genes remain indispensable for proper limb growth at later stages. Together, our results reveal a dual role of *Hox* genes in controlling limb growth and establishing the limb architecture and we propose that the intricate interactions between *Hox* function and growth pathways act as the molecular network coordinating limb bud growth and patterning.

Program/Abstract # 379

Zebrafish Thrombospondin4b is essential for myotendinous ECM organization and integrin signaling

Subramanian, Arul; Schilling, Thomas (University of California, Irvine, USA)

Development of a functional musculoskeletal system requires that muscle cells attach at their ends through tendons. Muscle attachments rely on Integrin (Itg) and Dystrophin (Dys) -dependent adhesion with extracellular matrix (ECM) proteins constituting the tendon. Defects in human Itg/Dys ligands in the ECM such as Laminin (Lam) are associated with muscular dystrophy. Lam, Collagen and other ECM proteins form complex fibrillar networks at myotendinous junctions (MTJs) to bear the forces of muscle contractions, but how these structures are assembled and maintained remains unclear. Here we show that the zebrafish Itg ligand Thrombospondin-4b (Thbs4b) is essential for ECM assembly and muscle attachment at MTJs. Myoblasts initially secrete their own Thbs4b to promote attachment, but downregulate it upon differentiation, at which point Thbs4b production becomes localized to tenocytes as MTJs mature. Depletion of Thbs4b alters the architechture of the basement membrane ECM at MTJs, weakening muscle attachments, which causes muscles to detach upon contraction. Ectopic expression of thbs4b and local secretion of Thbs4b in genetic mosaics can both rescue these defects. Furthermore, Thbs4b in its homopentameric form is required to localize Laminin (Lam) and activate Itg signaling at MTJs. Thus our results reveal a novel role for Thbs4b as a regulator of muscle attachment and as a critical scaffold for assembly of other ECM components.

Program/Abstract # 380

Phenotypic Analysis of a Novel Zebrafish Mutation Affecting Juvenile Bone Development

Anderson, Rebecca (Northwestern Univ Feinberg School of Med, USA); LeClair, Elizabeth (DePaul University, USA); Topczewska, Jolanta; Topczewski, Jacek (Northwestern University Feinberg School of Med, USA)

The conserved processes of bone formation are often affected in human skeletal dysplasias. There are two types of ossification: endochondral, which develop through mineralization of cartilage, and intramembranous, which form directly from condensed mesenchymal cells. Zebrafish mutants displaying skeletal dysmorphologies are an attractive system to study human skeletal malformations. We have identified a novel mutant, *koliber*^{nu7}, characterized by a late-onset phenotype. The mutant phenotype consists of a misshapen body, reduced length and small head size first detectable by morphologic examination at six weeks. Through the use of bone and cartilage staining, examination of bone and cartilage markers and analysis of transgenic lines, we have created an in-depth characterization of the ossification timeline of *koliber*^{nu7}. Bone and cartilage analysis reveal hyperossified intramembranous bones, resulting in fused and bent vertebrae, an expanded entopterygoid and a misshapen dentary bone. Endochondral bones such as the hypurals, elements of the tail skeleton, and facial bones such as the quadrate and hyomandibular display ectopic ossification and abnormal growth plate organization. Mutant growth plates are aberrantly stratified with an expansion in the prehypertrophic zone and a dramatic loss of the hypertrophic zone. An increased thickness of the periosteum is frequently observed. Using morphometric analysis, we have analyzed malformations of both bone types in the mutants. Positional cloning indicates that the *kol*^{nu7} mutation disrupts a regulatory element in a region not previously implicated in bone development. Thus the *kol*^{nu7} mutation disrupts the function of a novel gene involved in juvenile vertebrate bone formation.

Program/Abstract # 381

Role of skeletal muscle in mandible development

Kablar, Boris; Rot, Irena (Dalhousie University, Canada)

We study developmental relationship between the mandible and the adjacent skeletal musculature. Previously, our analysis of *Myf5:MyoD* null mouse fetuses completely lacking skeletal muscle demonstrated the importance of early contraction and static loading by the muscle in skeletogenesis. The mutant phenotype displayed abnormal skeletal features, among which was the *micrognathia* (mandibular hypoplasia), where a smaller, bent and posteriorly displaced mandible was found. This phenotype was mostly due to the partial failure in secondary cartilage initiation and maintenance. Our goal here is to identify candidate molecules with a role in instructing mandibular development from the adjacent skeletal muscle. Muscle instructs mandible mechanically and biochemically (e.g., paracrine or other ways). By employing Systematic Subtractive Microarray Analysis Approach (**SSMAA**) we compared gene expression between the mandibles in amyogenic and wild type mouse fetuses. With the 3-fold cut-off value, we identified 13 up-regulated and 132 down-regulated genes in mutant mandibles. This was followed by a bioinformatics approach and detailed consultation of web-accessible mouse databases. Databases were searched for individual gene expression and distribution in the tissues of interest, and for function, by assessing the effects of mutations in a particular gene of interest. To date, our **SSMAA** revealed a list of 14 candidate genes involved in muscle-mandible developmental relationship: *Actc1, Cacna1s, Ckm, Des, Mir300, Myh1, Myog, Tnnc1, Tnni1, Tnni2, Tnnt3, Pgam2, Pvalb* and *Ryr1*. Future analysis of mouse knockouts for these genes will further elucidate their role in the mandibular development.

Program/Abstract # 382

Amplitude of growth factor signaling tunes craniofacial morphology

Szabo-Rogers, Heather L.; Cusack, Brian (University of Pittsburgh, USA)

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

Program/Abstract # 383

The role of kinin-kallikrein signaling in craniofacial development

Jacox, Laura (Whitehead Institute- MIT & Harvard GSAS, USA); Sindelka, Radek; Sive, Hazel (Whitehead Institute, USA)

The mouth is the initial opening between the gut and outside of the embryo, and forms from juxtaposed ectoderm and endoderm. In *Xenopus*, mouth formation involves multiple steps, including cell migration and death, dissolution of basement membrane, cell layer thinning and intercalation, and cell sheet perforation. Several signaling pathways are required for this process, including BMP, Wnt, Hedgehog and most recently, the kinin-kallikrein pathway. This latter pathway acts in adults as a regulator of blood pressure and inflammation, but has not been previously described in embryos. The pathway converges on production of the signaling molecule nitric oxide (NO). Using an unbiased microarray approach, three members of the kinin-kallikrein pathway were found to be preferentially expressed in the presumptive mouth region, relative to surrounding regions. These are *carboxypeptidase-N, kininogen* and neural *nitric-oxide synthase*. Requirement of all three members for mouth formation was determined by loss of function. Loss of function was associated with gross craniofacial abnormalities, histological aberrations in facial basement membranes and tight junctions, and reduced neural crest marker expression and migration, suggesting broad and diverse roles for the kinin-kallikrein pathway in craniofacial development. Further, loss of function phenotypes were rescued by downstream kinin-kallikrein peptides and exogenous nitric oxide (NO). Kinin-kallikrein signaling is a novel regulator of craniofacial development in *Xenopus*.

Program/Abstract # 384

Genetic and molecular characterization of the avian ciliopathic model Talpid2

Brugmann, Samantha A.; Chang, Ching-Fang; Schock, Elizabeth (Cincinnati Children's Hospital, USA); Robb, Elizabeth (UC Davis, USA); Snyder, Jon (Cincinnati Children's Hospital, USA); Dodgson, Jerry (Michigan State University, USA); Cheng, Hans (USDA-ARS, USA); Muir, William (Purdue University, USA); Delany, Mary (UC Davis, USA)

The chicken *Talpid2* is an autosomal recessive mutant with a myriad of Hedgehog (Hh) dependent malformations, including polydactyly and facial clefting. Although the Talpid2 has been used as a disease model for numerous studies of limb and craniofacial development, the causal genetic element has yet to be identified. To determine the genetic cause for the *Talpid2* phenotype we performed 60K SNP array and Next-gen sequencing analyses and mapped the *Talpid2* locus to a region on chromosome1 containing the avian homolog of *c2cd3*, a vertebrate specific C2 domain-containing protein essential for ciliogenesis. Reverse transcriptase-PCR (RT-PCR) using primers to the first and last exons of *c2cd3* revealed an abnormal distribution of transcripts in *Talpid2* relative to controls embryos. The molecular consequence of *c2cd3*-dependent loss of ciliary function was disrupted post-translational processing of the down-stream transcription factors of the Hh pathway, Gli2 and Gli3 in the developing facial prominences. We found that whereas both Gli2 and Gli3 processing was disrupted in *Talpid2* mutants, only nuclear Gli3A levels were significantly altered between control and *Talpid2* embryos. These results are the first to identify the causal genetic element for *Talpid2* and show that it is a distinct ciliary mutant from *Talpid3*. Furthermore, ours are the first to perform an in-depth cellular and molecular analysis of the *Talpid2* facial phenotype. Taken together, these results shed light on how craniofacial tissues utilize primary cilia to process a Hh signal and characterize the Talpid2 as a novel disease model system for ciliopathies.

Program/Abstract # 385

Mortalin plays a protective role in cell survival through the regulation of the unfolded protein response pathway during mouse embryonic development.

Frisdal, Aude (Stowers Institute, USA); Walker, Macie (University of Colorado-Denver, USA); Trainor, Paul (Stowers Institute, USA)

Neural crest cells (NCC) give rise to the majority of skeletal elements and connective tissue of the head and face. Most craniofacial malformations therefore are associated with defects in NCC development. It is thus important to understand the mechanisms that govern NCC formation, migration and differentiation. To identify new genes involved in craniofacial development, we performed an ENU mutagenesis screen in mice and here we describe *arco piccolo* which exhibits craniofacial malformation. NCC migration is

perturbed in *arco piccolo* mutants which disrupts cranial ganglia development. Furthermore, *arco piccolo* mutants display abnormal vasculature which is important for cell survival. Collectively, these neurovascular defects are consistently associated with elevated levels of apoptosis resulting in embryonic lethality. We mapped the *arco piccolo* mutation to a single base pair change in mortalin (Hspa9) which plays known roles in mitochondrial transport and chaperoning of unfolded proteins. Mortalin is expressed ubiquitously during development with elevated activity in the pharyngeal arches and yolk sac. A microarray comparing WT and mutant littermates revealed activation of the unfolded protein response (UPR) in mutants. UPR is activated through accumulation of unfolded proteins in the endoplasmic reticulum and its sustained activity leads to cell death. We hypothesize that Mortalin directly binds critical sensors of the UPR that normally suppresses activation of UPR. These data suggests that craniofacial and neurovascular defects observed in *arco piccolo* mutants is due to UPR-induced apoptosis. Mortalin therefore plays a protective role in cell survival through its novel regulation of the UPR pathway during embryonic development.

Program/Abstract # 386

Wnt signaling in mammalian craniofacial development

Zhou, Chengji; Stokes, Arjun; Wang, Yongping (UC Davins, USA)

Using conditional gene-targeting mouse approaches, we have demonstrated that beta-catenin signaling is critical in transcriptional modulation of Fgf8 in the anterior neural ridge and facial ectoderm for facial and forebrain development. We also demonstrated that Lrp6-mediated Wnt/beta-catenin signaling is required for lip/palate formation and fusion, which may act through transcriptional activation of the homeobox genes Msx1 and Msx2 in facial mesenchymal cells. Ubiquitous inactivation of Lrp6 resulted in cleft lip/palate (CLP), a common birth defect. However, conditional gene targeting of Lrp6 solely in the facial mesenchyme or in the facial ectoderm did not cause CLP, indicating that synergistic Lrp6 signaling in facial ectoderm and mesenchyme is required for lip/palate formation and fusion. Moreover, we have generated the double knockout mutants of Lrp6/Lrp5 in the facial ectoderm, and observed a severe disruption of the upper jaw and facial formation, which is similar to the beta-catenin conditional mutants. These results suggest that Lrp6 and Lrp5 are functional redundant in transducing Wnt/beta-catenin signaling for early facial development. In contrast, craniofacial ablation of Wls, a molecule required for Wnt proteins sorting and secretion, generated different types of craniofacial disorders. Together, our results suggest that both canonical and non-canonical Wnt signaling pathways are critical in craniofacial development. (Supported by NIH 1R01DE021696 and Shriners Hospitals for Children grant 87500)

Program/Abstract # 387

Growth of Meckel's cartilage and mandibular ossification in the fuzzy mutant

Yannakoudakis, Basil; Economou, Andrew (King's College London, UK); Tabler, Jacqueline (University of Texas-Austin, USA); Yeung, Yvonne; Green, Jeremy; Liu, Karen (King's College London, UK)

Development of the mandibular skeleton is unique whereby a transient Meckel's cartilage is formed. Subsequent ossification takes place within a sheath in which bone is deposited. The mechanisms governing outgrowth of the mandible have not been fully characterised. Understanding these developmental processes is important since they govern correct development of the lower jaw. Loss of the planar cell polarity gene *fuzzy*, in mice, results in a truncated Meckel's cartilage with thickening in the more anterior regions. Furthermore, the mandible undergoes excessive bone deposition within the sheath of ossification. Because *fuzzy* is important in cell polarity, our hypothesis is that in Meckel's cartilage, morphogenesis is abnormal. In order to investigate this, we are mapping cellular parameters such as orientation, spacing, and growth within the cartilage. With regards to abnormal ossification, gene expression in later stages shows an up-regulation of *runx2*, which may be driving excess mandibular ossification. We hypothesize that the balance of osteochondroprogenitor differentiation is being disturbed.

Program/Abstract # 388

The Golgi associated protein Golgb1 is required for palate development

Lan, Yu; Liu, Han; Jiang, Rulang (Cincinnati Children's Hospital, USA)

Cleft palate is a common major birth defect that requires surgical intervention shortly after birth and has significant long-term health implications for the affected individuals. Although tremendous progress has been made in the understanding of molecular regulation of palate development in the last twenty years, currently known genetic causes account for less than 30% of cleft palate pathology in humans. We genetically mapped an N-ethyl-N-nitrosourea-induced cleft palate mutation to proximal mouse Chromosome 16. Analyses of whole exome sequencing results and subsequent PCR-based genotyping of over twenty mutant mice revealed co-segregation of a splice donor site mutation in the *Golgb1* gene (*Golgb1*$^{ivs9+1G>A}$) with the cleft palate phenotype. RT-PCR and immunofluorescent staining assays confirmed that the homozygous mutant embryos lack normal Golgb1 mRNAs or protein. Instead, the mutant embryos produce an aberrantly spliced *Golgb1* mRNA that is predicted to encode a truncated protein lacking most of the structural domains of the wildtype Golgb1 protein. Further analyses showed that the *Golgb1* homozygous mutant mouse embryos exhibit failure of palatal shelf elevation. These results identify a critical role for Golgb1 mediated intracellular processes in mammalian palate development.

Program/Abstract # 389

Smad-Dependent BMP Signaling Thought Type 1a Receptor in Cranial Neural Crest Cells Directs Their Cell Fate Towards Chondrocytes to Cause Craniosynostosis.

Mishina, Yuji; Komatsu, Yoshihiro (University of Michigan, USA)
Neural crest cells (NCC) are multipotent cell populations that differentiate into numerous derivatives in the vertebrate body. In vitro experiments have demonstrated the importance of BMPs on cell fate determination of NCC. It is intriguing, therefore, how BMP signaling in NCC contributes to the formation of a craniofacial structure. For this end, we generated a transgenic mouse line that can conditionally express a constitutively active form of BMP type IA receptor (*ca-Bmpr1a*), and bred with a *P0-Cre* mouse line to activate Smad-dependent signaling in a neural crest-specific manner (*ca-Bmpr1a:P0-Cre*). The resulted mice showed short broad snouts due to the premature fusion of the anterior frontal (AF) suture. In support of a requirement for precisely regulated BMP signaling, this defect was rescued on a Bmpr1a heterozygous null background, with corresponding normalization of Smad phosphorylation. Moreover, in vivo treatment with a selective chemical inhibitor of BMP type I receptor kinases resulted in rescue of craniosynostosis. Since activation of BMP signaling in osteoblasts using *Osx1-Cre* or *Col1-Cre* did not lead the skull deformity, we hypothesized that augmentation of BMP signaling in multipotent NCC, but not in mono-potent osteoblasts, results in cell fate alterations leading to the premature fusion of the AF suture. Notably, *ca-Bmpr1a:P0-Cre* showed ectopic cartilage in the AF suture at new born stage followed by ectopic mineralization. These results suggest that transient cartilage formation may be the trigger to induce the premature fusion in the AF suture. These results also suggest that enhanced Smad-dependent BMP signaling through BMPR1A alters cell fate decision for cranial NCC towards chondrocyte lineage.

Program/Abstract # 390
Stage specific usage of Fgf signal in cochlea development
Huh, Sung-Ho Huh; Ornitz, David; Warchol, Mark (Washington Univ in St Louis, USA)
The organ of Corti (OC) is a complex mechanosensory structure that transduces sound vibrations into neuronal signals. The OC contains one row of inner hair cells (IHC) and three rows of outer hair cells (OHCs), separated by pillar cells (PCs). In addition, each sensory hair cell is associated with an underlying supporting cell (SC). The mechanisms that regulate the formation of OHCs are significant, since the loss of OHCs is a leading cause of sensorineural deafness and age-related hearing loss. Although mouse mutants lacking fibroblast growth factor (FGF) receptor 1 suggest a role for FGF signaling in OHC development, the underlying mechanisms regulating OHC development are not known. Previously, we have generated Fgf20 knockout mice and found out that mice lacking a functional Fgf20 gene are viable and healthy but are congenitally deaf. Furthermore, we identified that Fgf20 is required for OHC differentiation. The Fgf20 paralog, Fgf9, is also expressed in the developing inner ear. To investigate potential functional redundancy, double knockout mice were generated. Loss of both Fgf9 and Fgf20 resulted in a 60 percent reduction in cochlear length, suggesting decreased numbers of sensory progenitor cells. Examination of potential receptor targets of Fgf9 and Fgf20 indicates that mesenchymal FGFRs phenocopy the cochlear length phenotype and epithelial FGFR regulates epithelial differentiation and patterning. Together, these data indicate that Fgf9 and Fgf20 function together to regulate the size of the cochlear progenitor population, ultimately regulating cochlear length, and Fgf20 functions independently to induce OHC and outer SC differentiation.

Program/Abstract # 391
Odd-skipped related-1 cooperates with Six2 to maintain nephrongenic progenitor cells during kidney development
Xu, Jingyue; Liu, Han; Lan, Yu; Jiang, Rulang (Cincinnati Children's Hospital, USA)
Odd-skipped related 1 (Osr1) encodes a zinc finger protein homologous to the *Drosophila* Odd-skipped transcription factor. During metanephric kidney development, *Osr1* mRNA is highly expressed in the cap mesenchyme and down-regulated as the nephrogenic mesenchyme cells epithelialize to form renal vesicles, suggesting that Osr1 may play an important role in maintaining the nephrogenic progenitor cells. *Osr1*$^{-/-}$ null mutant mouse embryos exhibit massive apoptosis of the nephrogenic mesenchyme before metanephric kidney induction. To elucidate the role of Osr1 in metanephric kidney development, we are using Cre/loxP-mediated tissue-specific genetic analyses. We found that inactivation of *Osr1* in the cap mesenchyme after E10.5 caused premature depletion of nephrongenic progenitor cells and severe renal hypoplasia. Neprhogenic progenitor cells markers, *Cited1* and *Six2* are significantly down regulated, and the renal vesicle marker *Wnt4* is up-regulated and ectopically expressed. Moreover, we found that Osr1 physically and genetically interacts with Six2, a homeodomain transcription factor critical for self-renewal of the cap mesenchyme. These results suggest that Osr1 and Six2 act together to maintain the progenitor cell pool during mammalian nephrogenesis.

Program/Abstract # 392
Multiple roles of the transcription factor HNF1B during collecting duct morphogenesis and nephron segmentation
Desgrange, Audrey; Héliot, Claire; Umbhauer, Muriel; Cereghini, Silvia (INSERM U969, UMR 7622 CNRS UPMC, Paris, France)
The initiation of metanephros development is marked by the emergence of the ureteric bud (UB) from the Wolffian Duct (WD). Then, the UB undergoes a complex process of branching to give rise to the entire urinary collecting ducts (CD) system. As it branches, UB tip cells induce mesenchymal-to-epithelial conversion and subsequent formation of regionalized nephrons, the kidney filtering units. Here we report that HNF1B, a transcription factor required for UB branching, induction of nephrogenesis and implicated in several developmental renal pathologies, has additional later functions for specification and differentiation of CD and nephrons. We have recently shown that *Hnf1b*-specific inactivation in mouse nephron progenitors leads to rudimentary nephrons and perinatal letality. Our results uncover the requirement of HNF1B for a proximal-intermediate nephron segment fate acquisition, through the regulation of Notch components and *Irx1/2* transcription factors. Parallel studies in Xenopus embryo show that this function of *Hnf1b* appears to be conserved in vertebrates (Heliot, Desgrange *et al*., Dev 2013). In ongoing studies, *Hnf1b* removal from the WD and UB using the

Hoxb7-Cre line, leads to multiple urogenital tract abnormalities. These phenotypes are associated with the dysregulation of key regulators and in part due to an early mosaic Cre activity. Using this mosaicism we are exploring further in organ cultures, the behaviour of mutant *vs* WT cells within the UB and WD. These studies will be confronted with ChIP–seq data from embryonic kidneys, and are expected not only to uncover novel networks controlling branching and nephrogenesis but also to define how these processes are deregulated under pathological conditions.

Program/Abstract # 393

Mechanism of Wnt9b Signaling in the Regulation of Self-renewal and Differentiation of Nephron Progenitors

Ramalingam, Harini; Carroll, Thomas; Das, Amrita (UT Southwestern Medical Center, USA)

Previous studies have shown that Wnt9b/beta-catenin signaling directly regulates progenitor cell renewal and differentiation in the kidney. The mechanisms by which the same molecular pathway regulates two seemingly contradictory processes (progentior renewal vs. differentiation) are not known. We hypothesized that Wnt9b forms a gradient across the progenitor cell population and that differential responses to the level of Wnt9b elicit these two different cell fates. To test this hypothesis, we utilized a Cre inducible transgene that allows us to control the spatial and temporal expression of the Wnt. This allowed us to alter the slope and direction of the hypothesized Wnt gradient and to examine the effects on the progenitor cells. Our data suggests that a gradient of Wnt signaling does not play an instructive role in kidney progenitor cell fate. Instead, our data suggest that Wnt signaling is permissive of both fates and that other spatially restricted signals provide cues that determine progenitor cell fate.

Program/Abstract # 394

Investigating the role of planar polarity in prostate gland development

Grishina, Irina; Cisse, Yasmine (New York University School of Medicine, USA); Dean, Charlotte (Imperial College London, UK)

Defects in the planar cell polarity (PCP) pathway have been linked to disease in several branched internal organs including the lungs and the kidney. In this study, we aim to determine the role of several PCP protein components, namely Vangl2, Scribble and Rho kinase, in development of the male prostate gland. At embryonic day (E)15.5 the initial murine prostate buds form, then extend through the urogenital sinus (UGS) mesenchyme at E16.5-E17.5, and start to branch at E18.5. We examined the gland formation, and cellular localization of Vangl2 and Scribble, in wild type prostates, and in tissues homozygous for *Loop tail*^{Vangl2} (*Lp*) or *Circle tail*^{Scribble} (*Crc)* alleles. Since *Lp* and *Crc* embryos die at birth before prostate branching is visible, we analyzed *Lp/Lp* and *Crc/Crc* prostate branching in explant cultures. During prostate formation *Scribble* protein co-localized to UGS tight junctions consistent with *Scribble* function in establishing apical-basal polarity. Interestingly, localization of Vangl2 protein dynamically changed from a uniform periplasmic location at E16.5 to a more polarized localization similar to Scribble at E18.5. Further, prostate branching was severely diminished by treatment of explants with a Rho kinase inhibitor. In summary, our data points that several components of PCP pathway are important for proper prostate gland formation and branching. Further studies should determine if PCP defects can contribute to abnormal prostate differentiation and adult pathologies.

Program/Abstract # 395

Origins and plasticity of thymus and parathyroid cell fate specification in the cervical thymus

Manley, Nancy R.; Li, Jie (University of Georgia, USA)

The thymus and parathyroids develop from shared organ primordia originating from the third pharyngeal pouch endoderm in mice, but perform distinct and essential roles in adaptive immunity and calcium homeostasis, respectively. The thoracic thymus is the primary vertebrate organ for T cell generation. Accessory cervical thymi have also been identified in humans and mice, and shown in mice to be independent functional organs. However, their developmental origins and their functional significance remain unclear. Our previous work had shown that thymus and parathyroid separation during morphogenesis generates parathyroid fragments become distributed through the pharyngeal region, suggesting an origin for cervical thymi. We used genetic fate mapping to investigate the embryonic origins and developmental mechanisms underlying the formation and cell fate specification of cervical thymi in mice. Our data show that cervical thymus development requires normal thoracic thymus-parathyroid morphogenesis. We further show that cervical thymi have two distinct cellular origins: delayed differentiation of endodermal precursors, and transdifferentiation of parathyroid-fated cells. These distinct origins also have important functional consequences for their function. Compared to thoracic thymus, parathyroid-origin cervical thymi (pCT) express low levels of the thymic epithelial cell-specific transcription factor Foxn1. Consequently, pCT form a distinct microenvironment that supports an atypical thymocyte development pathway, generating T cells with unconventional phenotypic characteristics. Our data demonstrate a normally occurring case of transdifferentiation, with specific functional consequences for resulting organ.

Program/Abstract # 396

Complex tissue specific roles for HOXA3 during thymus and parathyroid development

Chojnowski, Jena L.; Trau, Heidi; Masuda, Kyoko; Manley, Nancy (University of Georgia, USA)

Hoxa3 was the first Hox gene to be mutated by gene targeting in mice, and is required for the development of multiple endoderm and neural crest cell (NCC) derived structures in the pharyngeal region. The *Hoxa3* null mutant has a loss of 3rd pharyngeal pouch (pp) derivatives, the thymus and parathyroids, thought to be due to an early failure of patterning and organogenesis. To determine the specific roles of *Hoxa3* in 3rd pp development, we used a null allele and tissue-specific deletion with endoderm or NCC-specific Cre

drivers. The *Hoxa3* null had unexpected alterations in 3rd pp patterning and delayed activation of thymus and parathyroid organogenesis, which later degenerate. *Fgf8*, *Tbx1*, and *Bmp4*, which have been implicated in patterning these organ domains, have altered expression patterns. *Fgf8* (thymus) is expanded and *Tbx1* (parathyroid) is reduced, while *Bmp4* is missing from the endoderm but normal in NCCs. Furthermore, organ-specific differentiation markers are expressed a full day later than expected. Neither the endodermal nor the NCC deletion recapitulates the null phenotype. NCC deletion results in an ectopic thymus and parathyroids due to separation and migration defects, while the endodermal deletion results in smaller, ectopic organs due to delayed thymus organogenesis and restricted parathyroid development. These data show that global loss of *Hoxa3* does not prevent thymus or parathyroid organogenesis, but plays a complex role in patterning the 3rd pp, activating the organ-specific differentiation markers, and maintaining their development. The *Hoxa3* tissue-specific knockouts further show that *Hoxa3* in either tissue is sufficient for 3rd pp development, but also has tissue-specific functions.

Program/Abstract # 397

Pdx-1 is a determinant of epithelial organization in the developing pancreas

Marty Santos, Leilani M; Cleaver, Ondine (UT-Southwestern Medical Center, USA)

During development the pancreatic epithelium undergoes a transient stratification at a time when the multipotent progenitor cells (MPCs) that give rise to the exocrine, ductal and endocrine lineages are specified. We hypothesize that this transient stratification is important for commitment of MPCs to their different lineages, and that defects in the determinants that organize the early pancreatic epithelium will impact cell fate and differentiation. The transcription factor Pdx-1 is known to be required from the earliest stages of pancreatic development and later for the specification of endocrine cell fate, particularly that of beta-cells. We have observed that the timing of failure of the homozygous Pdx-1 null pancreatic bud coincides with the transient stratification of the pancreatic epithelium. Our findings suggest that Pdx-1 is a positive regulator of the adhesion molecules E-cadherin and beta-catenin, whose expression is required for normal development and branching of the pancreatic epithelium. In addition, we find that Pdx-1 is required for proper re-establishment of cell polarity within the de-stratifying epithelium. We observe that levels of laminin are sharply decreased both at the periphery of the pancreas and within the epithelium. Similarly, apical polarity determinants are reduced within the Pdx1-null stratified pancreatic epithelium. Together, these defects result in impairment the normal 3D architecture of the pancreas epithelium, suggesting that basic epithelial adhesion and polarity determinants are targets of Pdx-1. Ultimately, understanding the stepwise processes by which endocrine beta cells acquire their fate and function will advance efforts towards cell replacement therapies to treat diabetes.

Program/Abstract # 398

Prox1 controls morphogenesis and cell fate in the mouse embryonic liver

Sosa-Pineda, Beatriz; Seth, Asha; Yu, Nanjia; Ye, Jianming; Guez, Fanny; Bedford, David C.; Neale, Geoffrey A. (St. Jude Children's Research Hospital, USA); Cordi, Sabine (de Duve Institute, Belgium); Brindle, Paul K. (St. Jude Children's Research Hospital, USA); Lemaigre, Frederic P. (de Duve Institute, Belgium); Kaestner, Klaus H. (University of Pennsylvania, USA)

The function of the liver is central to preserve homeostasis. A better understanding of the cellular and molecular processes establishing both, its complex architecture and cellular diversity, will help us prevent, diagnose, and cure human hepatic diseases. Here we report that in mouse embryos the transcription factor Prox1 is expressed in all hepatoblasts of the early hepatic diverticulum (hepatoblasts being the precursors of both, hepatocytes – the chief epithelial cells in the liver – and cholangiocytes – the epithelial cells lining the intrahepatic biliary ducts–), in developing hepatocytes, and in emergent intrahepatic bile ducts. We also show that Prox1 is a critical regulator of liver morphogenesis and hepatic epithelial cell differentiation. Specifically, we determined that Prox1 ablation in mouse hepatoblasts caused a severe phenotype characterized by formation of aberrant parenchymal epithelial structures covered with a thick basal membrane, defective bile duct morphogenesis, increased expression of cholangiocyte markers and concomitant reduction of hepatocyte markers, and widespread fibrosis. These defects were accompanied by decreased expression of specific inhibitors of TGF-beta signaling. Results of in vitro and in vivo experiments allowed us to conclude that Prox1 limits the responsiveness of hepatoblasts to TGF-beta signals. In turn, this avoids excessive biliary differentiation, promotes proper bile duct morphogenesis, and prevents fibrosis. Intriguingly, Prox1 loss-of-function also affected the expression of genes involved in hepatic metabolism, and of microRNAs regulating biliary development or cell adhesion. Ongoing efforts should address the molecular bases of Prox1 function in developing hepatic cells.

Program/Abstract # 399

The Septum Transversum Mesenchyme Induces Gall Bladder Development

Saito, Yohei; Kojima, Takuya; Takahashi, Naoki (University of Tokyo, Japan)

The liver, gall bladder, and ventral pancreas are formed from the posterior region of the ventral foregut. During ventral foregut-derived organ development, interactions between the ventral foregut endoderm and the adjacent mesenchyme are critical. For example, fibroblast growth factor (FGF) from the cardiac mesoderm and bone morphogenetic protein (BMP) from the septum transversum mesenchyme (STM) induce hepatogenesis. After hepatic induction, *Sox17*+/*Pdx1*+ pancreatobiliary common progenitor cells are present in the remaining posterior portion of the ventral foregut. These progenitor cells differentiate into *Sox17*+/*Pdx1*- gall bladder progenitors, and *Sox17*-/*Pdx1*+ ventral pancreatic progenitors, but the cell-extrinsic signals that regulate this differentiation process are unknown. This study shows that the STM grows in the posterior direction after E8.5 in the mouse, becoming adjacent to the presumptive gall bladder region to induce gall bladder development. In this induction process, STM-derived BMP4 induces

differentiation from common progenitor cells adjacent to the STM into gall bladder progenitor cells by maintaining *Sox17* expression and suppressing *Pdx1* expression. Furthermore, the STM suppresses ectopic activation of the liver program in the posterior region of the ventral foregut following hepatic induction. In particular, STM-derived FGF10, which starts to be expressed in the STM at E9.0, contributes to the suppression of hepatic gene expression in the presumptive gall bladder and ventral pancreas regions through an Fgf10/Fgfr2b/Sox9 signaling pathway. Thus, the STM plays pivotal roles in gall bladder development by both inductive and suppressive effects.

Program/Abstract # 400

EpCAM Is an Endoderm-Specific Wnt Derepressor that Licenses Hepatic Development

Huiqiang Lu, Jun Ma, Yun Yang, Wenchao Shi, and Lingfei Luo (Southwest University, China)* Mechanisms underlying cell-type-specific response to morphogens or signaling molecules during embryonic development are poorly understood. To learn how response to the liver-inductive Wnt2bb signal is achieved, we identify an endoderm-enriched, single transmembrane protein, epithelial-cell-adhesion-molecule (EpCAM), as an endoderm-specific Wnt derepressor in zebrafish. hi2151/epcam mutants exhibit defective liver development similar to prt/wnt2bb mutants. EpCAM directly binds to Kremen1 and disrupts the Kremen1-Dickkopf2 (Dkk2) interaction, which prevents Kremen1-Dkk2-mediated removal of Lipoprotein-receptor-related protein 6 (Lrp6) from the cell surface. These data lead to a model in which EpCAM derepresses Lrp6 and cooperates with Wnt ligand to activate Wnt signaling through stabilizing membrane Lrp6 and allowing Lrp6 clustering into active signalosomes. Thus, EpCAM cell autonomously licenses and cooperatively activates Wnt2bb signaling in endodermal cells. Our results identify EpCAM as the key molecule and its functional mechanism to confer endodermal cells the competence to respond to the liver-inductive Wnt2bb signal.

Program/Abstract # 401

Intestinal epithelial secretory cell differentiation is dependent on ascl 1a acting through Notch signaling.

Wallace, Kenneth; Roach, Gillian; Wallace, Rachel; Cameron, Amy; Ozel, Emrah; Hongay, Cintia; Baral, Reshica; Andreescu, Silvana (Clarkson University, USA)

Intestinal epithelial cells initially choose between the enterocyte or secretory cell fate. After this initial decision, cells continue differentiating into a variety of different subtypes. While the majority of cells within the intestinal epithelium are enterocytes, secretory cells are interspersed in a characteristic pattern along the anterior to posterior axis. Previously, Notch signaling has been demonstrated to participate in the decision between these two epithelial cell types. Here we identify *ascl1a* as the gene required to initiate specification of the intestinal epithelial secretory fate with a loss of function mutation resulting in an epithelium consisting of only enterocytes. Loss of *ascl1a* also coincides with loss of expression of the Notch ligand *deltaD*. To determine whether Notch signaling is active in specifying epithelial cell fate throughout the entire period of *ascl1a* expression, we inhibited Notch signaling using the gamma secretase inhibitor DAPT. Inhibition of Notch signaling during only two periods during the first half of embryogenesis results in increases in *ascl1a* and *deltaD* expressing epithelial cells in addition to increased numbers of secretory cells at 74 hpf. This indicates that Notch signaling is utilized for two discrete periods rather than continuously. While we observe early increases in secretory cells, continuous Notch inhibition with DAPT to the end of embryogenesis does not significantly alter overall numbers but instead changes the quantity of specific secretory cell subtypes. Lack of secretory cell increases at the end of embryogenesis may result from a combination of differential roles/responses of Notch receptors to inhibition and incomplete reduction of signaling.

Program/Abstract # 402

Molecular characterization and functional analysis associated with retinoic acid signaling pathway during gut regeneration in the sea cucumber

Viera-Vera, Jorge; Stephanie, Ortíz-Troche; Díaz-Díaz, Lymarie; García-Arrarás, José E. (Universty of Puerto Rico, Puerto Rico)

The sea cucumber, *Holothuria glaberrima*, has proven to be an important non-classical research model for understanding the cellular and molecular processes governing organ regeneration. This deuterostome regenerates most of its viscera after an induced evisceration event, where the digestive tube is the first to do so through the formation of a blastema-like structure. To determine the genetic network of intestinal regeneration, we focused on the characterization of genes related to the metabolism and function of retinoic acid (RA), a known mediator of tissue regeneration in vertebrates. We also explored the effects of interfering with RA signaling via citral and diethylaminobenzaldehyde (DEAB), two well-known inhibitors of retinaldehyde dehydrogenase (RALDH). Analysis of various genetic libraries revealed the presence of genes associated to RA metabolism (short-chain dehydrogenase reductase 7 and RALDH4) and to its function (retinoic acid receptor and retinoic acid X receptor). Additional sequence analyses including: primary structure sequencing, phylogenetic analysis, protein domain prediction, and multiple sequence alignment further confirmed their presence. Moreover, animals treated with RA synthesis inhibitors showed a smaller intestinal regenerate. These animals also showed altered cellular dedifferentiation patterns and a 50% decrease in cell proliferation. These findings contribute towards our understanding of RA function during adult regenerative organogenesis and provide a novel opportunity to determine the cellular events linked to RA signaling in this re-emerging model system.

Program/Abstract # 403

Eye Development in a Freshwater Shrimp Caridina nilotica (Crustacea Decapoda: Atidae)

Okuthe, Grace (Walter Sisulu University, South Africa)

Here a detailed study of major events in the retinogenesis in a freshwater shrimp, *Caridina nilotica* will be presented using classical histological and immunohistochemical methods. Vision is one of the most important developmental changes occurring during early development in many organisms. The eyes, which are part of the central nervous system, allow organisms to visualize their surroundings and also pursue prey. The retina is an excellent model because it serves as an easily accessible portion of the central nervpus system. In the present study, eyes of neonates, larval, juvenile and adult *C. nilotica* will be studied with light and electron microscopy. Histological sections will be used to describe the major events of retinogenesis in the laboratory from hatching time, to 65 days post hatch (dph). The aim of the current study is to elucidate key stages of retinal development in these species. These studies are relevant to an understanding of general neuronal processes, and for biomedical applications including a variety of inherited human diseases. It is envisaged that *C. nilotica* may in future constitute a convenient model organism to address relationship between structural and functional development of sensory organs.

Program/Abstract # 404

Insights into the mechanism of tooth initiation from a pre-existing tooth germ in the snake and the mouse

Gaete, Marcia; Tucker, Abigail (King's College London, UK)

Some teeth start development associated with a pre-existing tooth germ, rather than from the initiation of a new placode. This is observed in mouse molar development and in the snake, which constantly renews its teeth. In the snake, teeth develop from an epithelial dental lamina that connects several tooth generations in a chain and ends in a highly proliferative successional lamina. In the mouse, the molar placode gives rise sequentially to three molars in each mandible quadrant. The sequential molars appear to bud off from the posterior region of the predecessor molar, and the tip of the molar placode appears morphologically equivalent to the successional lamina. The cellular mechanisms responsible for sequential tooth emergence and organization are still unknown. We have identified that Wnt/beta-catenin targets are expressed in the successional lamina in mouse and snake, and the stem cell marker Sox2 is expressed in the dental lamina. Regions next to the budding tip of the successional lamina are connected to Sox2 + lingual oral epithelium. By lineage tracing, we observed that cells in the successional lamina of the mouse and snake are odontogenic while also being maintained at the posterior edge of the lamina. Wnt/beta-catenin misregulation in culture induces changes to the normal pattern of budding in both mouse and snake. Moreover, overactivation of Wnt/beta-catenin alters the molecular and proliferative pattern of the snake dental lamina, increasing the number of dysmorphic ectopic tooth germs. Our results suggest a model in which the dental lamina and successional lamina are organized in domains with specific signalling, gene expression and proliferative responses that allows organized tooth initiation.

Program/Abstract # 405

Ectodysplasin/NF-kappakB in mammary placode development

Voutilainen, Maria; Lindfors, Päivi; Rysti, Elisa; Lönnblad, Darielle (University of Helsinki, Finland); Schmidt-Ullrich, Ruth (Max-Delbrück-Center for Molecular Medicine, Germany); Mikkola, Marja (University of Helsinki, Finland)

Ectodysplasin (Eda), a member of the tumor necrosis factor superfamily is one of the key regulators of skin appendage development in vertebrates. Mammary gland development in mouse begins at E10.5. By E12 five pairs of mammary placodes, local thickenings of the epithelium, have formed at conserved positions between the fore and hind limb. We have previously shown that transgenic overexpression of Eda in developing ectoderm (K14-Eda mice) leads to NF-κB dependent, accelerated branching morphogenesis. Moreover, K14-Eda mice develop ectopic mammary placodes, which give rise to supernumerary glands in adult. We have crossed a mouse model with suppressed NF-κB signaling (I-κBα ΔN mice) with K14-Eda mice to study the dependency of extra mammary placode formation on NF-κB activation. All five pairs of mammary placodes form in I-κBα ΔN mice. However, in the compound I-κBα ΔN/K14-Eda mutants the extra placodes do not form. In addition, the expression levels of many placode markers in endogenous placodes seem lower. These transgenic embryos exhibit increased amount of apoptosis within the mammary epithelium at the bud stage. This might in part explain the reduction in gene expression levels of the placode markers and decrease in the placodal size. Taken together, these data indicate that formation of endogenous mammary placodes does not require NF-κB signalling but development of Eda-induced ectopic placodes does. A microarray analysis on Eda treated mammary buds has revealed several potent Eda target genes that might function in the formation of mammary placode and be important for the development of ectopic placodes. We are currently assessing the function of these candidate genes.

Program/Abstract # 406

Mechanistic Insight into the Pathology of Polyalanine Expansion Disorders Revealed by a Mouse Model for X Linked Hypopituitarism

Thomas, Paul Q.; Hughes, James; Piltz, Sandra; Rogers, Nicholas; McAninch, Dale (University of Adelaide, Australia); Rowley, Lynn (Murdoch Childrens Research Institute, Australia)

Polyalanine expansions in transcription factors have been associated with eight distinct congenital human diseases. It is thought that in each case the polyalanine expansion causes misfolding of the protein that abrogates protein function. Misfolded proteins form aggregates when expressed *in vitro*, however it is less clear whether aggregation is of relevance to these diseases *in vivo*. To

investigate this issue, we used targeted mutagenesis of embryonic stem (ES) cells to generate mice with a polyalanine expansion mutation in *Sox3* (*Sox3*-26ala) that is associated with X-linked Hypopituitarism (XH) in humans. By investigating both ES cells and chimeric mice we show that endogenous polyalanine expanded SOX3 does not form protein aggregates in vivo, but rather is present at dramatically reduced levels within the nucleus of mutant cells. Importantly, the residual mutant protein of chimeric embryos is able to rescue a block in gastrulation but is not sufficient for normal development of the hypothalamus, a region that is functionally compromised in *Sox3* null embryos and individuals with XH. Together, these data provide the first definitive example of a disease-relevant PA mutant protein that is both nuclear and functional, thereby manifesting as a partial loss-of-function allele.

Program/Abstract # 407

The epigenetic factor Reptin regulates zebrafish development through both cilia dependent and independent pathways

Sun, Zhaoxia (Yale U, USA)

The cilium, an antenna-like organelle, has emerged as a key center of sensation, signal transduction, growth and motility for the vertebrate cell. Consistently, ciliary defects have been linked to a growing list of human diseases, collectively referred to as "ciliopathies". Although the importance of the cilium is now firmly established, how the cilium performs its vital motility and sensory functions remains largely unclear. Addressing this question in a vertebrate model system will be critical for understanding and treating ciliopathies. In zebrafish, a number of cilia mutants have been isolated and they provide valuable research tools for studying cilia and ciliopathies. From a previous genetic screen for cystic kidney mutants, we isolated *hi2394*, an insertional mutant displaying kidney cyst formation and ventral body curvature, phenotypes typically associated with ciliary defects in zebrafish. In this study, we show that *hi2394* is a loss of function allele of *reptin*. Interestingly, *reptin* encodes an AAA ATPase that is known to be involved in epigenetic regulation. We further provide evidence that *reptin* genetically interacts with known ciliary genes and it is essential for the normal function of cilia. In addition, since recently it was suggested that in some ciliopathies, DNA damage response (DDR) is activated as shown by an increased level of phosphorylated H2AX, we examined DDR in fish mutant embryos using the same approach. Results showed that while the signal in the classic cilia mutant *ift172*^{hi2211} is comparable to wild type siblings, the level of phosphorylated H2AX is greatly increased in both the eye and the brain of *reptin*^{hi2394} mutants. Consistently, compared to IFT mutants, *reptin*^{hi2394} mutants are more neocrotic and die earlier. Taken together, these results suggest that *reptin* is involved in cilia dependent and independent pathways during vertebrate development.

Program/Abstract # 408

Impaired Folate Uptake and Neural Tube Closure Defects in Lrp2 Deficient Mice

Mecklenburg, Nora; Kur, Esther (Max- Delbrück Centrum, Germany); Cabrera, Robert (University of Texas, USA); Willnow, Thomas E.; Hammes, Annette (Max- Delbrück- Centrum, Germany)

The low-density lipoprotein (LDL) receptor-related protein 2 (LRP2) is a multifunctional cell surface receptor highly expressed in the embryonic neuroepithelium. Loss of receptor activity in the developing murine CNS causes holoprosencephaly (HPE) due to impaired SHH signalling (Christ et al. 2012). Besides the HPE phenotype, we observe additional neural tube defects (NTDs) in LRP2 deficient embryos, unrelated to SHH dependent forebrain development. Thus, the rostral neural tube is still open at somite stages 17-26 whereas in wild type controls closure of this neural tube region is completed at the 15 somites stage (E9.0). Similar NTDs have been described previously to be caused by impaired folate metabolism in mice. A function for LRP2 in endocytosis of folate bound to soluble folate receptors (FolR1) has been already suggested for the kidney (Birn, 2005). Therefore we analyzed whether LRP2 expressed in the neuroepithelium is required for delivery of folate to neuroepithelial cells during neurulation. Expression levels for FolR1 and Slc19a1, a carrier that transports folate directly across the cell membrane (the second import route for folate) are unchanged in LRP2 deficient mice. However, uptake assays in whole embryo cultures showed that LRP2 deficient neuroepithelial cells are unable to mediate uptake of soluble FolR1. Consequently, folate concentrations are significantly reduced in Lrp2$^{-/-}$ embryos compared to control littermates. Moreover, expression of the folic acid dependent gene *Alx3* is significantly down regulated in *Lrp2* mutants. In conclusion, this study suggests that LRP2 is essential for cellular folate uptake in the developing neural tube, a step crucial for proper closure of the neural tube.

Program/Abstract # 409

Wnt/catenin Signaling System Functions in Embryoid Bodies Aggregated from Human Embryonic Stem Cell

Xu, Xuehong (Shaanxi Normal Univ, China); Xu, MengMeng (Shaanxi Normal Univ/Duke University, USA); Zhou, Xin (Shaanxi Normal Univ, China); Jones, Odell (Univ of Maryland School of Med, USA); Pan, Yuexin (Case Western Reserve Univ, USA); Bryant, Joseph (Univ of Maryland School of Med, USA); Anthony, Donald (Case Western Reserve Univ, USA)

As an essential molecule in Wnt/β-catenin signaling, β-Catenin plays a crucial role in the decision making for tissue differentiation in embryogenesis and pathogenesis. Associated with other proteins such as LEF/TCF family of transcription factors, the complex of β-Catenin and LEF/TCF is accumulated in the cytoplasm and transported to the nucleus. In mouse skin, the deferential fate of the skin stem cell depends on β-catenin, which organizes stem cells into follicular or epidermal lineages. These indicate that Wnt/β-catenin signaling should also function in lineage differentiation of hESC. To study the function of β-catenin in early differentiation, we cultured H9 stem cell and aggregated them into embryoid bodies (EB). Using confocal microscopy combined with immunostaining, we revealed that in early EBs some guarding cells were first differentiated from EB stem cell aggregates. These early differentiated cells for guarding epithelial cells have strongest expression of β-catenin within EB. These cells were flattened on the surface of EB,

covering the surface by connections formed through protein interactions. At certain confocal sections of EB, instead of a round boundary, a polygonal boundary was observed even though the EB appeared round under conventional microscope. In these polygonal boundaries, β-catenin positive guarding epithelial cells were positioned on every corner of the polygon. In the inner portion of the EB, undifferentiated β-catenin positive cells express β-catenin in the nucleus. As the initially simple shape of EB becomes more and more intricate during development, we revealed that more β-catenin positive cells were also observed in this complex structure. Based on these results, we predicted β-catenin to play different roles while guarding epithelial cells or undifferentiated stem cells in the inner portion of EB in *in vitr*o culture system. In this early differentiation process, phosphorylation of β-catenin may be a critical factor for fate determination of the human stem cell.

Program/Abstract # 410
Organ-specific regulation of steroidogenesis by Hoxb9
Gardiner, Jennifer (Institute of Cancer Research, UK)
The gonads and adrenal cortex are derived from the same cell population, known as the adreno-gonadal primordium (AGP). The organ primordia separate from one another at around E11 in the mouse, and the testis and adrenal gland produce different steroidogenic enzymes from E13.5. Steroidogenesis is controlled by a master regulator transcription factor, steroidogenic factor 1 (*Sf1*). However, the genetic cues directing differential steroid production are currently poorly understood. *Hox* genes are well-documented regulators of embryonic patterning. Differential *Hox* gene expression was observed in the developing steroidogenic organs; *Hoxb9* is expressed in the adrenal gland, *Hoxd9* in the testis. In order to investigate the role of *Hox* genes in regulation of steroidogenesis, we have generated a transgenic mouse, named *Sf1:Hoxb9*, in which *Hoxb9* is misexpressed under the control of *Sf1*. *Sf1:Hoxb9* mice exhibit an increase in the number of 'adrenal-like' cells – those producing enzymes involved in adrenal steroidogenesis - in the developing testis from E13.5. We have investigated the cause of this phenotype, and further analysed the requirement for *Hoxb9* in adrenal steroidogenesis in vitro.

Program/Abstract # 411
Cell fate mapping and specification of the coelomic lining epithelium in the avian embryo
Arraf, Alaa; Yelin, Ronit; Schultheiss, Thomas M. (Technion-Israel Institute of Technology, Israel)
Background: The coelomic lining epithelium of the embryo is composed of a single cell layer that invests the external surface of the viscera and the inner surface of the body wall. Studies indicate that the coelomic lining epithelium is an active tissue which participates in organogenesis of different significant organs including heart epicardium, liver, lung and gonads, but the full extent of the developmental potential of the coelomic lining is not known. Specification of the precise differential anatomical site in the anterio-posterior axis, and the temporal sequence of the emergence of the lining cells are crucial for understanding of the molecular and cellular regulators that determines the coelomic lining cells` fate. Methods: We are using a cell tracking technique in which microinjection of the fluorescent vital DiI into the coelomic cavity of stage 12 avian embryo is analyzed after different time points of incubation. In addition we use histological methods, including in situ hybridization and immunohistochemistry to detect co-expression of DiI with specific markers of the candidate`s coelomic lining derived cells. Plastic sections of different stages embryos are used to study exact morphological development in the coelomic cavity region. Results: We have validated that coelomic injection of DiI at different stages, labels only the coelomic lining and not any of the underlying mesenchymal cells. We will present initial results regarding the cell fates of coelomic lining epithelium -derived cells and the timing of their departure from the coelomic lining.

Program/Abstract # 412
Scarb2a is essential for Notochord Development in Zebrafish
Díaz Téllez, Abigail; Carrillo Rosas S.; Ramos Balderas J.; Zampedri C.; Maldonado E. (Universidad Nacional Autonoma de México, Mexico Distrito Federal, Mexico)
Scarb2 is a membrane glycoprotein, whit two tran-membrane sites, 11 sites for N-glycosylation and a C-terminal di-leucine motif. Mutations in Scarb2 were described as a causing of Action Myclonus Renal Failure Syndrome (AMRF), which is characterized by progressive myoclonus epilepsy. Zebrafish has three copies of *scarb2* (*scarb2a, scarb2b and scab2c).* Scarb2a insertional-mutant was obtained in a large-scale forward genetic screening. This mutant is characterized by the presence of vesicular bodies in the brain at 1 dpf and hypopigmentation at 2 dpf both phenotypes are restored at 3 dpf. However, since 1 dpf *scarb2a* mutant shows defects in the notochord formation. Therefore, we are using this mutant as model to gain better understanding in how Scarb2 works during notochord formation. Through electronic microscopy, we have observed that vacuole notochord cells of *scarb2a* mutant are smalles. In situ hybridization revealed that *scarb2a* is expressed in the brain and in the notochord at early stages, also in the Scarb2a mutants there is a disordes in *collagen II* expression. Actually, experiments are ongoing to decipher if the defect in the notochord of *scar2a* Zebrafish mutant are cell autonomous or non-autonomous.

Program/Abstract # 413
Developmental hierarchy, cell fate regulation and carcinogenesis: a view from the *Drosophila* model
Sinha, Pradip (Indian Institute of Technology Kanpur, India)
Not all cells in a lineage hierarchy transform neoplastically when oncogenically targeted. Developmental disposition of a target cell, therefore, is a critical cancer determinant. A growing body of literature now shows that a majority of cancer cells of origin display

stem cell-like characteristics. However, given the complexities of tissue architecture in a tumor, identification of the developmental lineages of these transformed cells often remains a daunting task. Taking advantage of the simplicity of tissue architecture in the fly model, we have investigated the cellular determinants of neoplasia following loss of highly conserved tumor suppressors such as Lgl or Scribble (Scrib). Our findings provide a broad framework for carcinogenesis wherein the transcription factors and signalling pathways linked to primitive cell states in an organ primodium provide the cooperative drives required for neoplasia in *lgl* or *scrib* mutant clones. Conversely, those transcription factor and signalling pathways specifying terminal cell fates induce rapid elimination of oncogenically targeted cells. Switch-to-a-primitive-cell-state, therefore, is a critical driver of carcinogenesis. These findings from the fruit fly model offer novel insights to cancer mechanisms and also provide novel therapeutic opportunities.

Program/Abstract # 414
FGF signaling pathways required for lung development are essential mediators of the pathogenesis of pleuropulmonary blastoma and adenocarcinoma
Ornitz, David M; Yin, Yongjun (Washington University in St. Louis, USA); Hill, D. Ashley (Children's National Medical Center, USA); Betsuyaku, Tomoko (Keio University School of Medicine, Japan)
Mice lacking Fibroblast Growth Factor (FGF) 9 have pulmonary hypoplasia and die at birth due to respiratory failure. Investigation of the underlying mechanisms identified an early embryonic feed-forward signaling interaction between mesenchymal FGF and β-catenin-dependent Wnt signaling that is essential for lung mesenchymal growth and differentiation. To further probe the function of FGF9, we have developed an inducible mouse model in which FGF9 can be expressed in lung epithelium. We find that expression of FGF9 in embryos induces lung mesenchymal hyperplasia whereas expression in adults causes the rapid proliferation of progenitor-like cells in distal airway epithelium. Embryonic induction of FGF9 closely models the histopathology of Pleuropulmonary Blastoma (PPB), a syndromic lung cancer that is associated with heritable mutations in *Dicer1*. Genetic modeling identified FGF9 as an essential mediator of the pathogenesis of PPB. In contrast to embryos, induction of FGF9 in the adult resulted in the rapid formation of adenocarcinoma. This is relevant to human disease in that amplification of *FGFR1* occurs in 22 percent of squamous cell lung cancer, and mutations in *FGFR3* have been identified in non-small cell lung cancer. FGF ligands, including FGF9 are also expressed in a large proportion of lung cancers and activation of FGF signaling is one mechanism of escape from anti-EGFR therapies. Genetic modeling in the adult lung identified FGF receptor 3 (FGFR3) as the obligate FGFR mediating the FGF9 oncogenic signal. These data identify the FGF9-FGFR3 pathway as a primary oncogenic signal and suggest that this pathway could be exploited for therapeutic applications in some forms of human lung adenocarcinoma.

Program/Abstract # 415
3-O-sulfated heparan sulfate expands the Kit+ epithelial progenitor pool via FGFR2b-dependent proliferation.
Patel, Vaishali; Lombaert, Isabelle (NIH, USA); Xu, Yongmei; Liu, Jian (University of North Carolina, USA); Hoffman, Matthew (NIH, USA)
During organogenesis a rapid expansion of the epithelial progenitor pool is required for growth and morphogenesis. Kit and Fgfr2b signaling maintain and expand the progenitor pool in branching epithelial organs. Elucidating the cellular mechanisms that induce rapid expansion of epithelial progenitors is crucial to understand organogenesis and to expand progenitors for regeneration. Since heparan sulfate (HS) is required for Fgfr2b function we hypothesized that specific HS synthesized by Kit+ progenitors may control their expansion. Kit+ epithelial cells were isolated from fetal salivary glands and the HS biosynthetic enzymes were analyzed. Surprisingly, the enzymes that generate 3-*O*-sulfated heparan sulfate (3-*O*-HS) were specifically and highly expressed in Kit+ progenitors and Fgfr2b-signaling rapidly increases their expression. Using recombinant enzymes to specifically modify HS we show that 3-*O*-HS increases Fgfr2b signaling and the number of Kit+ progenitors. Alternatively, reducing Kit signaling decreases 3-O-sulfotransferase expression and organogenesis. Thus 3-*O* sulfated HS increases Fgfr2b and Kit signaling that feeds back to increase HS biosynthesis providing a rapid response mechanism to modify HS structures and control progenitor proliferation in response to a growth factor. The identification of specific HS structures that control localized progenitor cell proliferation will be useful to expand progenitor cells for use in regenerative therapy.

Program/Abstract # 416
An AP2/ERF transcription factor important for new organ development
Duran Medina, Yolanda; Marsch-Martinez, Nayelli (Cinvestav -IPN Unidad Irapuato, Mexico)
Plant organ production is indeterminate, organs are continuously formed in a highly controlled manner. Organ development depends on the meristematic activity. Leaves, stems and axillary meristems are produced from shoot apical meristem (SAM). The function of the SAM is to maintain itself as a source of cells and to generate daughter cells that are displaced towards the meristem periphery, where they acquire specific differentiation pathways. The maintenance of the SAM requires a precise coordination of growth and differentiation. Hormones have an important role in this balance, but it is apparent that additional signals influence hormone signalling to control meristem function and leaf initiation. Many genes are also involved in organ development. An AP2/ERF transcription factor of Arabidopsis is expressed in restricted regions in organ primordia and young organs. The overexpression of this gene promotes the development of small size organs, due to a reduction in cell size and cell number. This gene plays a role in cell differentiation reflected through ectopic callus formation in roots. According to global gene expression analysis using the overexpression mutant, some its effects may be due to hormonal signaling alteration. The overexpression phenotype and global expression data suggest that

this gene is involved at the start of organ formation. The relationship of this transcription factor with auxins has already been studied, but not other hormonal pathways, and the role of such interaction is unknown, therefore, the main objective of our project is to determine the gene interaction with those other hormones, and the role that this interaction plays in organ development in *Arabidopsis thaliana*.

Program/Abstract # 417

Optimizing Culture Conditions for M. domestica Organogenesis-stage Embryos

Lycette, Devon, (Oberlin College, USA)

The period of prenatal organ development is brief in marsupials, with birth neonates far less developed than their eutherian counterparts. Inspired by a recent study (Hickford *et al.*, 2008), we investigated the possibility of devising a protocol that would allow us to study organogenesis in somite-stage *Monodelphis domestica* embryos. We used two types of culture media: Advanced DMEM and Advanced MEM, and supplemented them with 10% fetal bovine serum, 50 μg/mL glutamine, 50 IU/mL penicillin and 50 μg/mL streptomycin. Incubation conditions for controls were 6% CO_2/air at 33°C or 35°C. A modular incubator chamber was used to maintain experimental gas conditions of 95% O_2/5% CO_2. To prevent fluctuations in pH during photography, embryos were held in HEPES-buffered DMEM. Our results thus far indicate that Day 10 (early somite stage) embryos grown in Advanced MEM and 95% O_2 and incubated at 35°C are most amenable to in vitro culture. Under these conditions, embryos survived in culture for 24 hours and showed less blistering than control embryos. Further investigation is ongoing to determine conditions that will support *M. domestica* embryos in culture for more extended periods of time and allow for cell lineage studies.

Program/Abstract # 418

Germline Regeneration in *Parhyale hawaiensis*

Kaczmarczyk, Angela; Villa, Luis; Andrade López, José; Patel, Nipam (University of California, Berkeley, USA)

The crustacean *Parhyale hawaiensis* displays a remarkable property of germline development; ablation of the germline (g) blastomere in the embryo can be compensated for during a later post-embryonic period, resulting in fertile animals and normal offspring. We hypothesize that the adult germline stem cell (GSC) niche is playing a role in this replacement. GSCs rely on their somatic stem cell niche to continuously self-renew and produce gametes. Identifying and characterizing this niche in the *Parhyale* adult gonads will provide a better understanding on variation and conservation of the GSC niche. Moreover, it will help to elucidate how germline cells in *Parhyale* are replaced post-embryonically.

Program/Abstract # 419

Chemical Activation of Wnt/Beta-Catenin Blocks Limb Regeneration at Two Different Stages

Wischin Fuentes, Sabina Citlali, Robles-Flores, Martha; Chimal-Monroy, Jesús (UNAM, Mexico)

It has been proposed that Wnt/β-catenin signaling activation favors regeneration during limb regeneration. It is important to take into account, however, that Wnt/β-catenin signaling has not just one specific function, but plays a wide variety of different roles during limb development, for instance in proliferation, in patterning and in chondrogenesis inhibition. It has been proposed that limb development is an event analogous to limb regeneration; based on such considerations we think that the activation of Wnt/β-catenin signaling during the limb regeneration process may be much more complex than generally assumed. We therefore investigated the activation of Wnt/β-catenin signaling by applying a Wnt agonist to different stages during limb regeneration (wound healing, dedifferentiation, blastema and redifferentiation), which are marked by different physiological events. We found out that limb regeneration is inhibited at two different time periods; one, when the drug is administered at the beginning of limb regeneration, before the blastema is formed and the other when the drug is applied after the blastema has been formed, just before the onset of the redifferentiation process. We also saw that animals treated at the beginning do not form a blastema. We speculate that the first inhibition phenomena is linked to innervation inhibition, while the second might be due to Sox9 inhibition.

Program/Abstract # 420

Notch signaling regulates cardiomyocyte proliferation during zebrafish heart regeneration

Burns, C Geoffrey; Zhao, Long; Guner-Ataman, Burcu (Massachusetts General Hospital, USA); Kikuchi, Kazu; Poss, Kenneth (Duke University, USA); Caroline, Burns (Massachusetts General Hospital, USA)

Ischemic heart disease is a prevalent cause of death worldwide that results largely from the human heart's inability to regenerate lost cardiomyocytes following injury . In contrast, adult zebrafish robustly regenerate their heart muscle following injury through partial dedifferentiation and proliferation of existing muscle cells. However, the genetic pathways promoting myocardial dedifferentiation and proliferation are largely unknown. Here, we report that zebrafish heart regeneration is highly dependent on the Notch signaling program. Specifically, we discovered that all four Notch receptors are highly upregulated in response to ventricular apex amputation, but are largely confined to the epicardial and endocardial cell compartments, with limited, if any, activation in the myocardium. Using a novel transgenic strain, we learned that ubiquitous Notch pathway suppression blocks heart regeneration and leads to scar formation. Surprisingly, ubiquitous Notch pathway activation also leads to scarring, indicating that successful heart regeneration is exquisitely sensitive to Notch signaling. Next, we investigated the cellular mechanisms underlying these regenerative failures. First, we learned that while cardiomyocyte dedifferentiation occurs normally in Notch inhibited hearts, cardiomyocyte proliferation is significantly

diminished. Interestingly, we found that cardiomyocyte proliferation is also dampened in Notch activated hearts. Based on our findings in zebrafish and those of others in ES cell and mouse models, we are currently testing the working model that Notch signaling is required in the endocardium to provide a non-autonomous pro-proliferation signal to the myocardium. Additionally, we speculate that aberrant Notch activation in mature cardiomyocytes results in DNA checkpoint activation and subsequent apoptosis. Taken together, these mechanistic studies highlight the indispensable role played by Notch signaling during vertebrate heart regeneration.

Program/Abstract # 421

Shh Pathway: Inhibitory Signal for Retina Regeneration

Barbosa Sabanero, Karla (Miami University, USA), Judge, Chelsey (Case Western Reserve University, USA); Luz-Madrigal, Agustin; Del Rio-Tsonis, Katia (Miami University, USA)

The embryonic chick can regenerate its retina if damaged by the transdifferentiation of the retinal pigmented epithelium (RPE), as long as there is a source of Fibroblast Growth Factor-2 (FGF2) present in the eye cup. During this process, RPE cells lose their original characteristics, such as pigmentation, they proliferate and eventually differentiate into different retina cells. Previous studies in our lab have shown that over-expression of Sonic Hedgehog (Shh) inhibits this process. Therefore, the aim of this study is to determine the mechanisms by which Shh inhibits FGF-induced transdifferentiation, analyzing if Shh is acting through its canonical pathway using its downstream target Gli-1. To evaluate if Shh is acting through its canonical pathway we over-expressed Gli-1 using a RCAS Gli-1 virus, then we analyzed how Gli-1 expression was regulated 24h after retinectomy without FGF by qRT-PCR, and finally we knocked-down Gli-1 using morpholinos. Our results show that the over-expression of Gli-1 upregulates RPE markers such as Mift, Otx-2, and down-regulates Pax-6, a master regulator for retina. At the histological level, we observed that over-expression of Gli-1 inhibited RPE transdifferentiation. qRT-PCR shows the downregulation of Gli-1 at 24h after retinectomy. Finally, RPE from retinectomized eyes treated with Gli-1 morpholinos show depigmentation, dedifferentiation of the RPE as well as upregulation of retina maker Pax-6. In conclusion, our results suggest that Shh can inhibit FGF-induced transdifferentiation by acting through its canonical pathway via Gli-1, maintaining the identity of the RPE and upregulating RPE markers. Furthermore, Gli-1 inhibition is sufficient to promote dedifferentiation of the RPE.

Program/Abstract # 422

Influence of Papillomavirus Oncogenes E6/E7 and Sex Hormones in the Regeneration of Mouse Ear Holes

García, Celina; Hernández-García, David; Valencia, Concepción; Werner, Mariana; Covarrubias, Luis (Instituto de Biotecnología, Mexico)

In the transgenic mouse line, Tg(K6b-E6/E7) (Tg), E6 and E7 papillomavirus oncogenes are highly expressed in the skin and increases during the proestrous-estrous phase in the cervix and uterus. These Tg mice regenerate their hair follicles continuously skipping the telogen resting phase, and close ear holes more efficiently than wild-type (Wt) animals by promoting re-epithelization and growth (Cell Growth Differ. 11:527-39, 2000; J Invest Dermatol. 128:2894-903, 2008). Notably, the regeneration phenotype is more obvious in females than in males, suggesting that sex hormones play a role. These observations correlate with the highest incidence of cervical lesions in Tg mice receiving a chronic estradiol treatment. In order to study how estradiol and/or testosterone influence regeneration, we analyzed ear hole regeneration under different hormonal conditions. Regeneration was increased in Wt and Tg males treated with estradiol, more evident in young and castrated mice, indicating not only that estradiol improves regeneration but also that testosterone may act as an inhibitor of the process. Accordingly, ovariectomy caused a marked reduction in regeneration; the effect was detected in both Wt and Tg mice, but ovariectomized Tg mice retain similar ability of complete regeneration as the un-treated controls. These data suggest that oncogenes and estradiol cooperate by controlling different pathways involved in regeneration. We will present molecular and cellular evidence that provide new insights about the role of metabolism and inflammation in regeneration, processes that may relate also with the susceptibility to develop cancer. Supported by DGAPA-UNAM IN225910/IN209813, and fellowship to G.C. (DGAPA and CONACYT-131031).

Program/Abstract # 423

Restoration of the brain function during brain regeneration in planarian and newt

Inoue, Takeshi (Kyoto University); Takano, Tomomi (Kobe University, Japan); Hoshino, Hajime; Akiyama, Yoshitaro; Umesono, Yoshihiko; Agata, Kiyokazu (Kyoto University, Japan)

A variety of external signals are interpreted into defined cellular functions in the brain that often result in distinct animal behaviors. Although much has been learned about some of the specific neuronal activities supporting the transformation of a neuronal stimulus into a behavioral response, much remains to be understood about the developmental, molecular and cellular processes that determine the onset and maintenance of stimulus perception and its association with a corresponding physiological response. Planarians are believed to be one of the most primitive central nervous system (CNS)-possessing organisms. Planarians display behaviors responsive to signals coming from outside such as light avoidance behavior. Furthermore, they can modulate their behavioral responses by sustained associative training and retain memory for a certain time. Because of the extraordinary regenerative capacity of planarians, the nervous system can be regenerated and its function completely restored. In order to better understand the regeneration of the brain, we analyzed the process of behavioral recovery during head regeneration using RNAi and a behavioral assay system in the planarian. The results revealed that novel neuropeptide genes might be required for the proper functional recovery during brain regeneration. Moreover, we found that the expression levels of these genes were up-regulated by light signal-induced or chemical-induced neural

activity specifically via NMDA receptors. These results suggested that the neural peptides induced by neural activity of the newly regenerated neurons might be required for the proper phototactic behavior.

Program/Abstract # 424

Primary Cell Cultures from the Regenerating Gut of the Sea Cucumber *Holothuria glaberrima*

Bello, Samir A.; García-Arrarás, José E. (University of Puerto Rico, San Juan, PR, United States)

Regeneration studies in echinoderms are hampered by the lack of suitable cell culture methodologies. We have established an in vitro model to study the cellular and molecular basis of organ regeneration in the sea cucumber *Holothuria glaberrima*. This organism has the remarkable ability to regenerate its digestive tract after evisceration. Regenerating gut rudiments at 5 days post-evisceration were dissociated and isolated cells were seeded on glass slides. Cells were incubated for 24h, 5d or 10d and their identity was established by SEM and immunocytochemistry. The proliferative capacity was evaluated by BrdU incorporation. Among the cell phenotypes observed in culture were: (1) Spherical (10 μm in diameter) labeled Meso-1 cells. These are dedifferentiated cells that probably give rise to most cells of the new intestine. (2) Phalloidin labeled cells suggesting the presence of muscle cells undergoing dedifferentiation or differentiation processes. (3) Small spherical cells (5 μm in diameter) immunoreactive for RN1 and/or calbindin (neuronal cell markers in sea cucumbers) which correspond to neuronal precursors. (4) Ovoid and spindle shaped (5-10 μm in diameter) β-tubulin labeled cells exhibiting cell projections. About 5% of the cultured cells incorporated BrdU at the three time points evaluated. Taken together, our results indicate that we have established the methodology to obtain primary cultures from the regenerating gut of *H. glaberrima*. This valuable tool is now being used to test the signaling events that determine regeneration-associated processes in sea cucumbers.

Program/Abstract # 425

Transient reduction of 5-methylcytosine and 5-hydroxymethylcytosine is caused by active DNA demethylation during regeneration of zebrafish fin

Hirose, Kentaro (Hiroshima University, Japan); Shimoda, Nobuyoshi (National Center for Geriatrics and Gerontology, Japan); Kikuchi, Yutaka (Hiroshima University, Japan)

It is well known that dedifferentiation processes such as the loss of molecular markers for differentiated cells, re-expression of molecular markers for progenitor cells, and restart of cell proliferation occur during regeneration in amphibians and zebrafish. Although epigenetic modifications are thought to be critical for the dedifferentiation processes in regeneration, the status and changes of DNA methylation during regeneration remain largely unknown. In this study, we analyzed the spatial and temporal changes of 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC) distribution during zebrafish fin regeneration by using dot blot assays and immunohistochemical analyses. We showed that during regeneration of zebrafish fin, the levels of 5mC and 5hmC are transiently reduced in blastema cells and cells adjacent to the amputation plane at 30 hours post-amputation (hpa), and the level of 5mC, but not 5hmC, is almost restored by 72 hpa. We observed that the dedifferentiated cells showed reduced levels of 5mC and 5hmC independent of cell proliferation by 24 hpa, suggesting that active demethylation pathways lead to the reduction of 5mC and 5hmC levels. Furthermore, expressions of the proposed demethylation- and DNA repair-related genes were detected during fin regeneration. Taken together, our findings illustrate that the transient reduction of 5mC and 5hmC in dedifferentiated cells is caused by active demethylation during regeneration of zebrafish fin.

Program/Abstract # 426

Extensive conversion of hepatic biliary epithelial cells to hepatocytes after extreme hepatocyte loss in zebrafish

Choi, Tae-Young (University of Pittsburgh, USA); Ninov, Nikolay (UC-San Francisco, USA); Stainier, Didier Y.R. (Max Planck Institute, Germany); Shin, Donghun (University of Pittsburgh, USA)

Biliary epithelial cells (BECs) have been considered as the source of regenerating hepatocytes when hepatocyte proliferation is compromised. However, their contribution to hepatocytes in vivo has been controversial. To resolve this issue, we established a novel zebrafish liver regeneration model in which hepatocyte-specific ablation can be temporarily, pharmaco-genetically achieved. By tracing the lineage of BECs, we show that BECs can extensively give rise to regenerating hepatocytes in larval and adult zebrafish. Upon severe hepatocyte loss, BECs highly proliferated and dedifferentiated into hepatoblast-like cells and subsequently redifferentiated into hepatocytes that were highly proliferating to restore liver mass. This BEC-driven liver regeneration was impaired in *sox9b* and *wnt2bb* mutants: upon severe hepatocyte loss, most BECs disappeared in *sox9b* mutants and the proliferation of newborn hepatocytes was greatly reduced in *wnt2bb* mutants. Our results demonstrate that BECs can extensively contribute to hepatocytes, thereby leading to full liver recovery from severe liver damage.

Program/Abstract # 427

Regeneration of the adult zebrafish jaw by bone-producing chondrocytes is distinct from jaw development

Crump, Gage DeKoeyer; Paul, Sandeep; Schindler, Simone (USC Keck School of Med, USA)

A major goal in human health is to improve the ability of large fractures and skeletal wounds to heal. Here, we present a new model of skeletal regeneration in the genetically tractable zebrafish. In a matter of just a few weeks, we find that adult zebrafish can regenerate nearly two-thirds of their lower jawbone, and they appear to do so through an unusual chondrocyte population that directly produces woven bone. During development, the majority of chondrocytes undergo hypertrophy and apoptosis, with bone matrix being produced

by invading osteoblasts. Quite differently during jaw regeneration, we find that newly formed chondrocytes co-express three types of skeletal collagens from early stages – *col2a1a* (cartilage), *col10a1* (hypertrophic cartilage) and *col1a1a* (bone) - as well as other osteoblast markers such as *runx2b*, *osterix*, and *osteopontin*. Consistent with regenerating chondrocytes adopting an osteoblast-like fate, we also observe these cells surrounding themselves with first an Alcian+ cartilage and then an Alizarin+ and Trichrome+ bone matrix. This shift of fate from chondrocytes to osteoblasts coincides with upregulation of Ihh signaling, and we are currently using genetic strategies to test the requirement of Ihh signaling for robust bone regeneration. Having distinct bone-making strategies in developing embryos versus regenerating adults makes sense, as the gradual process of endochondral ossification that is ideal for growing bones may be inadequate for the quick restoration of bone following injury. Instead, we propose that during regeneration a specialized population of chondrocytes promote fast restoration of bone by directly converting into osteoblasts.

Program/Abstract # 428
Role of Sox2+ Cells in Spinal Cord Regeneration in *Xenopus laevis*
Muñoz, Rosana; Edwards, Gabriela; Méndez, Emilio; Farías, Marjorie; Larraín, Juan (Pontificia Universidad de Chile, Chile)
Spinal cord (SC) injury induces direct damage that includes membrane disruption, vascular damage and haemorrhage. As a consequence of this injury, cell types, including microglia, macrophages and dividing progenitor cells, are recruited, culminating in the formation of the glial scar, which in turns, impedes axon regeneration. In contrast to mammals, amphibians can regenerate SC after injury. In *Xenopus* this capacity is lost at the end of metamorphosis. We hypothesized that Sox2a, protein involved in the activation of neural progenitor cells, plays an important role as a substrate for axonal regeneration. We have found that SC transection in *Xenopus* tadpoles results in increased proliferation on day 2 post-transection (dpt) in all tissues including Sox2+ cells in the ependymal zone of the neural tube stage 50 tadpoles. This proliferation decreases at 4dpt and 10dpt is not detected. We found an increase of Sox2 + cells in the gap area that accompanies the formation of axons observed by acetylated tubulin staining. Furthermore, we have observed in functional assays, which transected animals after electroporation with Sox2MO swim slow recovery compared with CoMO. Sox2+ cells are necessary for SC regeneration and lead to a model whereby SC injury activates proliferation of Sox2+ cells, contributing to SC growth or repairing the ablation gap generated by SC transection. Grants: Inserción Conicyt (79090027). Iniciación Fondecyt (11110006).

Program/Abstract # 429
A Transcriptomics Analysis of Spinal Cord Regeneration in *Xenopus laevis*
Moreno, Mauricio; Lee-Liu, Dasfne; Tapia, Victor; Almonacid, Leonardo; Munoz, Rosana; Edwards, Gabriela; Melo Francisco; Larrain, Juan (P. Universidad Catolica de Chile, Chile)
Xenopus laevis shows different regeneration ability depending on its life stages. After spinal cord injury (SCI) larval stages are able to regenerate whilst after metamorphosis juvenile frogs are not. The cellular and molecular mechanisms responsible for these differences are not completely understood. A high throughput RNA sequence analysis was performed to obtain a global transcriptomic outlook of spinal cord regeneration in *Xenopus* regenerative (tadpole) and non-regenerative (frog) stages. We have found more than 5000 differentially expressed transcripts when comparing both stages. Our results show that differential gene expression occurs earlier in the regenerative than in the non-regenerative stage. There are distinctive biological processes that show different timing in response to SCI including 'cell cycle', 'response to stress', 'metabolic processes' and 'immune response'. These differences in the expressed transcriptomes could explain the high regenerative ability shown by the tadpole. We are further characterising the biological processes and specific identified genes to show how the regeneration is achieved and then try to improve the regeneration ability of non-regenerative frogs. Funding: ICM (P07/011-F, P09/016-F), BASAL PFB12/2007, FONDECYT (11100348, 11110006).

Program/Abstract # 430
Schwann cells negatively regulate lateral line neuromast regeneration in zebrafish
Sánchez, Mario; Allende, Miguel (Universidad de Chile, Chile)
The zebrafish posterior lateral line (PLL) is a mechanosensory system composed of neuromasts (Nm) distributed along the trunk and tail connected by a continuous string of interneuromastic cells (INCs). Nms contain mechanosensory hair cells (HC), supporting cells, which are the source of newly regenerated HCs, and mantle cells, which provide structural support to the organ. The INCs are progenitor cells described to retain a dormant state by the presence of underlying Schwann cells along the PLL nerve that become reactivated later during larval development to form intercalary Nms. However, there are still no studies that describe whether these cells can be reactivated in a regenerative context after the loss of a Nm. To assess this potential, we developed a protocol to eliminate a single Nm by using a tungsten microelectrode in larvae and we followed the regeneration process. Electroablation eliminates all cell types of the neuromast. The injury results in the total disconnection of the INCs, Schwann cells and the PLL nerve. We observed that the INCs begin the reconnection process between 0-13hpd (hours post damage). At 13-36hpd we observed an accumulation of INC cells in the injury region. At 48-72hpd the regenerated neuromast recovers all cell types. The regeneration process is completed in 40% of the larvae. When we inhibit the migration of Schwann cells along the PLL nerve, we obtain over 90% regeneration. We aim to further characterize the INCs in terms of their potential fates by following their development in the neuromast during regeneration of this organ. We also aspire to understand the specific contribution of Schwann cells to this process. FUNDING: FONDECYT 1110275; FONDAP 15090007.

Program/Abstract # 431

Oncogenic K-Ras promotes basal extrusion of epithelial cells by degrading S1P through autophagy

Slattum, Gloria Mercedes; Gu, Yapeng; Rosenblatt, Jody (University of Utah, USA)

Epithelia provide a protective barrier for the organs they encase, yet the cells compromising the epithelia are constantly turning over via cell death and cell division. To maintain a protective barrier during tissue development and homeostasis, epithelia extrude cells destined to die by contracting a band of actin and myosin. Although extrusion can remove cells triggered to die by apoptotic stimuli, during homeostasis, epithelia extrude live cells, which then die by anoikis. Because transformed cells may override anoikis and survive after extrusion, the direction of extrusion has important consequences for the extruded cell's fate. As most cells extrude apically, they are eliminated through the lumen, however, cells with upregulated survival signals that extrude basally could potentially invade the underlying tissue and migrate to other sites in the body. We found oncogenic K-Ras cells extruded basally, rather than apically, in a cell-autonomous manner and can survive and proliferate following extrusion. Expressing oncogenic K-Ras^{V12} down-regulates the bioactive lipid Sphingosine 1 Phosphate (S1P) and its receptor $S1P_2$, both of which are required for apical extrusion. Surprisingly, the S1P biosynthetic pathway is not affected, as the S1P precursor, sphingosine kinase, and the degradative enzymes S1P lyase and S1PP phosphatase are not significantly altered. Instead, we found that S1P is degraded by autophagy, which is highly pronounced in extruding Ras^{V12} cells. Disruption of autophagy chemically or genetically in K-Ras^{V12} cells rescues S1P localization and apical extrusion. We propose that basal cell extrusion provides a novel mechanism for cells to exit the epithelium and initiate invasion into the surrounding tissues.

Program/Abstract # 432

Akt-p53-miR-365-cyclin D1/cdc25A Axis Contributes to Gastric Tumorigenesis Induced by PTEN Deficiency

Teng, Yan; Yang, Xiao (Institute of Biotechnology, China)

Although PTEN/Akt signaling is frequently deregulated in human gastric cancers, the in vivo causal link between its dysregulation and gastric tumorigenesis has not been established. Here we show that inactivation of PTEN in mouse gastric epithelium initiates spontaneous carcinogenesis with complete penetrance by 2 months of age. Mechanistically, activation of Akt suppresses the abundance of p53, leading to decreased transcription of miR-365, thus causing upregulation of cyclin D1 and cdc25A that promote gastric cell proliferation. Importantly, genetic ablation of Akt1 restores miR-365 expression and effectively rescues gastric tumorigenesis in PTEN-mutant mice. Moreover, orthotopic restoration of miR-365 represses PTEN-deficient-induced hyperplasia. These data demonstrate that PTEN-Akt-p53-miR-365-cyclin D1/cdc25A axis serves as a new mechanism underlying gastric tumorigenesis, providing novel potential therapeutic targets.

Program/Abstract # 433

The ciliary localization of Gli2 is important for its activation by Hh

Liu, Aimin; Liu, Jinling; Zeng, Huiqing (Penn State, USA)

Hedgehog (Hh) family of signaling proteins regulates cell growth and differentiation and requires the primary cilium for their function in mammals. The Gli family of transcription factors, major downstream effectors of the Hh signaling pathway, are localized to the tip of the primary cilium; however, how this localization is regulated and whether it is important for Gli activation remain poorly understood. By deletion and domain swapping experiments, we identified a part of the Gli2 protein as the ciliary localization domain, or CLD. A Gli2 variant lacking this domain, Gli2dCLD, is not localized to the cilium, even in the presence of activated Smoothened, or with defective retrograde intraflagellar transport. Our in vitro over-expression analysis indicated that Gli2dCLD retains intrinsic transcriptional activity and remains responsive to the inhibition of Suppressor of Fused. To investigate whether Gli2 ciliary localization is required for its activation in vivo, we replaced the endogenous Gli2 with Gli2dCLD in mouse through gene-targeting. The homozygous *Gli2dCLD* mutant embryos exhibit reduced expression of Hh target genes *Ptch1* and *Gli1*, and defects in the spinal cord patterning, suggesting that Gli2 activator activity is reduced. We found that the level of Gli2dCLD in the mutants is significantly higher than that of Gli2 in wild type, suggesting that Gli2 activation, rather than its stability, is compromised in *Gli2dCLD* mutants. Furthermore, the dampened Hh pathway activation in *Gli2dCLD;Ptch1* double mutants suggests that Gli2 has to be in the cilium to respond to upstream Hh pathway activation. In summary, our data shows that the full activation of Gli2 in vivo requires its ciliary localization.

Program/Abstract # 434

KIF17 controls ciliary localization and function of GLI2

Carpenter, Brandon S.; Blasius, Teresa L.; Verhey, Kristen J. ; Allen, Benjamin L. (U Michigan- Ann Arbor, USA)

Primary cilia are cellular organelles that are essential for Hedgehog (HH) signal transduction during vertebrate embryogenesis. The HH transcriptional effectors GLI2 and GLI3 traffic through primary cilia, which is required for proper processing of GLI proteins. However, the mechanisms that control the ciliary targeting and trafficking of the GLI proteins are largely unknown. Kinesin-2 motor proteins, 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 in cilia formation, dissecting out a HH-specific function is difficult. Unlike KIF3A and 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 versions of KIF17 perturbs GLI2 and GLI3 ciliary localization. In addition,

coimmunoprecipitation experiments demonstrate that GLI2 interacts with KIF17 via its C-terminal tail domain that is involved in cargo binding. Interestingly, expression of dominant negative KIF17 constructs with intact tail domains inhibits HH signaling in cells with constitutive HH pathway activity. These data suggest that GLI2 interacts with the tail domain of KIF17 and that this interaction regulates HH signaling.

Program/Abstract # 435
Semaphorin Receptors Promote Hedgehog Signaling
Pinskey, Justine; Allen, Benjamin; Giger, Roman (U Michigan- Ann Arbor, USA)
The Hedgehog signaling pathway plays vital roles in embryonic growth and patterning, and its de-regulation can lead to developmental defects and cancer. Neuropilins, which are receptors for the Semaphorin family of axon guidance molecules in the developing nervous system, have recently been shown to positively regulate the Hedgehog pathway. Using a luciferase reporter assay, we confirmed that Neuropilin-1 and -2 promote Hedgehog signaling when expressed in NIH/3T3 fibroblasts. Strikingly, however, over-expression of either Neuropilin-1 or -2 in the developing chick neural tube is not sufficient to alter specification of Hedgehog - responsive genes during ventral neural patterning. These data suggest that other modifiers are required to promote Hedgehog pathway function in vivo. To this end, we found that PlexinA1, a Semaphorin co-receptor with Neuropilin-1, also amplifies Hedgehog signaling in NIH/3T3 cells. This effect appears to be specific for PlexinA1, as the structurally similar proteins PlexinA2 and PlexinD1 do not promote Hedgehog signaling. Further, imaging studies with epitope-tagged proteins indicate that, unlike other Hedgehog pathway components, Neuropilin-1 and PlexinA1 are not enriched in primary cilia, a key platform in Hedgehog signal transduction. Thus, ciliary localization is not essential for Semaphorin receptor function in Hedgehog regulation. Overall, our results provide evidence for crosstalk between multiple members of the Semaphorin and Hedgehog signaling pathways, which are co-expressed spatially and temporally during development.

Program/Abstract # 436
The Role of Cytoplasmic Dynein 2 Light Intermediate Chain in Sonic Hedgehog Signaling and Ciliary Structure
Agbu, Stephanie; Anderson, Kathryn (Memorial Sloan-Kettering Cancer Center, USA)
The Sonic hedgehog (Shh) pathway is important for embryonic development as well as tissue homeostasis in the adult organism. Vertebrate Shh signaling is dependent upon the primary cilium, a microtubule-based organelle present on almost every cell of the body. Trafficking of Shh components within the cilium requires intraflagellar transport (IFT), which utilizes both anterograde and retrograde molecular motors. The cytoplasmic dynein 2 complex serves as the main retrograde motor in the cilium. The cytoplasmic dynein 2 light intermediate chain (*Dync2li1*) has been identified as a component of this complex, but its role in Shh signaling has not been elucidated. Here we examine Shh signaling and ciliary structure in *Dync2li1* null embryos. *Dync2li1* null embryos die at e11.5 with reduced Shh signaling in the neural tube. *Dync2li1* null ciliary morphology is also abnormal in the neural tube and limb mesenchyme. Shh pathway components accumulate in cilia of *Dync2li1* null mouse embryonic fibroblasts, suggesting that retrograde trafficking is defective upon loss of *Dync2li1*. In contrast to results with previously analyzed mutations in the IFT dynein heavy chain (*Dync2h1*), a hypomorphic allele of IFT172 fails to rescue Shh signaling in both *Dync2li1* and *Dync2h1* null embryos. These analyses highlight the essential role of *Dync2li1* in the maintenance of ciliary structure and thus Shh signaling in vertebrates. Because *Dync2li1* null embryos phenocopy *Dync2h1* null embryos, our studies indicate that ablation of cytoplasmic dynein 2 light intermediate chain abolishes the function of the cytoplasmic dynein-2 IFT motor in the primary cilium.

Program/Abstract # 437
Crosstalk Between Wnt and Hh Signaling Direct Extraembryonic Endoderm Formation
Golenia, Greg; Deol, Joey; Kelly, Gregory (U Western Ontario, Canada)
The earliest epithelial-to-mesenchymal transition in mouse embryogenesis is recapitulated using F9 cells, which differentiate into primitive endoderm (PrE) when treated with retinoic acid (RA). Wnt signalling is involved in the process as induction of PrE is inhibited when F9 cells are treated with Wnt6 siRNA or canonical Wnt inhibitors IWR-1 and XAV-939. Likewise, RA-induced differentiation is accompanied by an increase in Indian Hedgehog (Ihh), and we have evidence that Cyclopamine, a Hh inhibitor, blocks the ability of RA to induce differentiation. These results demonstrate that Wnt and Hh signaling are involved in PrE differentiation, but whether the pathways converge or are working independently is not known. In Wnt signaling, GSK-3β inactivation allows β-catenin to translocate to the nucleus where it interacts with TCF/LEF to regulate gene expression required for differentiation. *Wnt6* is up-regulated in response to RA, but its specific Frizzled (Fzd) receptor transducing the signal is not known. qRT-PCR results show that various *Fzd* transcripts are present in undifferentiated cells and ectopic expression of *Fzd7* is sufficient to induce PrE. To address the crosstalk between the Hh and Wnt pathways, we plan to use Fzd7 siRNA to inhibit RA-induced, canonical Wnt signalling, and then treat cells with 20(S) OHC to activate the Hh pathway and assay for PrE differentiation. Likewise, Cyclopamine-treated cells will be treated with BIO to activate canonical Wnt signaling to see if these cells can differentiate in the absence of canonical Hh signaling. Results will determine if both Hh and Wnt signaling are required for differentiation and if so, is GSK-**3**β the co nvergence point used by both pathways.

Program/Abstract # 438
Pannexin 3 inhibits Wnt/ß-catenin signaling and increases p21 activity to promote cell cycle exit of osteoprogenitor cells

through its channel activities

Ishikawa, Masaki; Yamada, Yoshihiko (NIH/NIDCR, USA)

Pannexin 3 (Panx3), a new member of the pannexin gap junction family, is expressed in the perichondrium/periosteum and in osteoblast. Previously, we reported that Panx3 is induced in the transition stage from proliferation and differentiation of osteoprogenitor cells and promotes osteoblast differentiation through its hemichannel, endoplasmic reticulum (ER) Ca^{2+} channel, and gap junction. Canonical Wnt/ β-catenin signaling is essential for osteoprogenitor cell proliferation. Here we show that Panx3 inhibits proliferation and promotes cell cycle exit of osteogenic C2C12 cells, primary calvarial cells, and newborn calvaria explants. Overexpression of Panx3 reduced osteoprogenitor cell proliferation, whereas the inhibition of endogenous Panx3 increased the proliferation. We found that this inhibition was mediated through reduced Wnt signaling by β-catenin degradation and GSK3β activation, which was caused by reduced cAMP/PKA signaling through the Panx3 hemichannel. The Panx3 hemichannel also contributes to the inhibition of proliferation, by reducing cAMP/PKA/CREB signaling which induces the expression of genes such as cyclinD1 for cell progression. Furthermore, the released ATP promotes PI3K/Akt signaling, which activates the Panx3 ER Ca^{2+} channel to increase intracellular Ca^{2+} that increases p21 transcription and phosphorylation by promoting Smad signaling through the calmodulin signaling pathway activated by Panx3 ER Ca^{2+} channel. Our results revealed multi-functional roles of Panx3 for osteoprogenitor proliferation and cell cycle exit. Thus, Panx3 plays a critical role in switching from proliferation to differentiation of osteoblasts.

Program/Abstract # 439

Identification and expression of novel Wnt signaling-associated protein kinases

Park, Edmond; Shin, Eun-Young (Korea Basic Science Inst., Rep. of Korea); Shin, Ju-Hyun (Chungnam National Univ. Hosp., Rep. of Korea); Kim, Gun-Hwa (Korea Basic Science Inst., Rep. of Korea)

The Wnt pathway is an evolutionarily conserved signaling network that is critical for mammalian development and adult tissue maintenance. In addition, aberrant activation of the Wnt signaling is implicated in driving the formation of various human cancers, particularly those of the digestive tract. Inhibition of aberrant Wnt pathway activity in cancer cell lines efficiently blocks their growth, highlighting the great potential of therapeutics designed to achieve this in cancer patients. In this study, we try to identify novel protein kinases that are associated with canonical Wnt signaling pathway by using TOPflash reporter assay system and human protein kinase (~500 genes) library. As the result, we identified numbers of protein kinases that positively regulate Wnt signaling pathway. The novel protein kinases would be possible and valuable target for regulating Wnt pathway in cancer and mammalian development.

Program/Abstract # 440

Axin-stimulated Wnt signaling in mouse embryogenesis and intestinal progenitor cells

Parrish, Angela; Mahaffey, James; Anderson, Kathryn (Sloan-Kettering Institute, USA)

The Wnt signaling pathway is essential for embryonic development and adult stem cell maintenance and is misregulated in many cancer types. Although the pathway has been extensively studied, the mechanism of pathway activation remains controversial. In the early mouse embryo, Wnt signals specify and maintain the primitive streak (the site of gastrulation) and progenitor cells in the streak and tail bud. Axin and Axin2 are considered to be negative regulators of the canonical Wnt pathway. However, we previously showed that a protein-stabilizing Axin2 point mutation (*canopus*) leads to a decrease of Wnt signaling in the head but an increase in the late primitive streak, assessed by the expression of the transgenic Wnt reporter Topgal. To confirm elevated canonical Wnt signaling in the primitive streak, we are examining the intracellular localization of β-catenin in the heads and tails of Axin-stabilized embryos. We find that limiting Wnt production with small molecules blocks the increase in Wnt signaling caused by Axin stabilization; genetic experiments to confirm that Axin-dependent pathway activation depends on ligand are in progress. Elevated Wnt signaling has a critical role in colon cancer through activation of intestinal stem/progenitor fates. We find that adult *canopus* heterozygotes show an increase in the number of β-catenin+ cells in small intestinal crypts, suggesting that stabilized Axin2 can also increase Wnt signaling in intestinal progenitor cells. We conclude that Axin proteins can tissue specifically activate or repress Wnt signaling, and this will be important for potential use of Axin-stabilizing drugs to treat cancer.

Program/Abstract # 441

Early Endocytic Trafficking in Control of Developmental Signaling

Gerstner, Norman; Zimyanin, Vitaly; Wieffer, Marnix; Zerial, Marino (MPI-CBG, Germany)

During gastrulation, cells simultaneously process different signaling inputs to pattern the early embryonic body axis. Several conserved signaling pathways, fundamental for morphogenesis and other developmental functions, are regulated by endocytosis. Ligand-activated receptors are internalized via different entry routes and compartmentalized into early endosomes. We have previously performed a genome-wide screen on endocytosis (Collinet et al., Nature 2010) and identified several genes that selectively regulate transport of signaling cargo to different types of endosomes, including early endosomes involved in signal transduction. We hypothesize that cargo sorting between distinct early endosomes is essential for the precise regulation of developmental signaling. By interfering with a novel Rab5-effector that regulates the transport of signaling cargo between endosomal compartments, we investigated the role of endosomal trafficking in modulating signaling strength and specificity during zebrafish gastrulation. Since Wnt/beta-catenin signaling and D/V patterning were strongly affected upon such perturbation, we focused on the Wnt/beta-catenin pathway and trafficking of Wnt-receptor complexes. We quantified trafficking and signaling defects at the level of the whole embryo,

cells and sub-cellular endosomal compartments. Our results suggest that the balance of Wnt-signaling components between distinct early endosomes determines signaling strength and specificity as part of the regulatory mechanisms underlying gastrulation.

Program/Abstract # 442

Detection of BMP signaling in pre-implantation mouse embryos

Reyes de Mochel, Nabora Soledad; Javier, Anna; Chiang, Michael; Luong, Mui Nhuc; Cinquin, Olivier (UC- Irvine, USA)

Mammalian development begins with fertilization, followed by multiple cell cleavage events and the transition from maternal to zygotic transcription. All these events work in concert to establish the first and thus most significant developmental event, lineage differentiation. This lineage specification gives rise to the trophectoderm (TE), the precursor for extraembryonic structures such as the placenta and yolk sac, and the Inner Cell Mass (ICM), which becomes the embryo proper. While the regulatory interactions and morphological contributions of transcription factors such as OCt3/4, Nanog, Sox2, TEAD4, and Cdx2 are extensively studied, surprisingly little is known about the roles of secreted growth factors during the first lineage differentiation. The presence of bone morphogenetic proteins (BMPs) in early mouse embryogenesis suggests a functional role for BMP signaling. In order to determine the functional significance of BMP signaling during early mouse embryogenesis, we investigate the onset of active BMP signaling and its possible functional role. Here, we report the detailed characterization of active BMP signaling in the pre-implantation mouse embryo.

Program/Abstract # 443

TGF-beta/Smad Signaling Maintains Cardiac Homeostasis by Down-regulating miRNAs Inducing Cardiac Hypertrophy

Yang, Xiao; Wang, Jian (Beijing Inst. of Biotechnology, China)

Heart failure (HF) is one of the most frequent causes of death worldwide. The underlying causes of HF are diverse but often relate to cardiac hypertrophy, which is an adaptive enlargement of the myocardium in response to altered stress or injury. The role of TGF-b signaling in cardiac hypertrophy has been extremely contradictory due to the complexity of TGF-β activation as well as its diverse effects on different type of cells. We have previously demonstrated that the endogenous cardiomyocyte Smad4-dependent TGF-b pathway protects heart from cardiac hypertrophy and fibrosis. Recently, we have revealed that the function of endogenous TGF-b/Smad signaling in maintaining cardiac homeostasis involves the downregulation of miRNAs inducing cardiac hypertrophy. We show that TGF-b1 inhibits the expression of miR-23a/miR-27a/miR-24-2 and miR-23b/miR-27b/miR-24-1 clusters at the transcriptional level. Transgenic mice with cardiomyocyte-specific overexpression of miR-27b exhibit cardiac hypertrophy and dysfunction by directly targeting the peroxisome proliferator-activated receptor-γ (PPAR-γ). Most importantly, *in vivo* silencing of miR-27b using a specific antagomir or adenovirus expressing anti-sense miR-27b in a pressure-overload-induced mouse model of HF attenuates cardiac hypertrophy and dysfunction. The function and mechanisms of other miRNAs regulated by TGF-b/Smad signaling in cardiac hypertrophy will also be discussed. All these results provide critical genetic evidence showing that TGF-b/Smad signaling maintains cardiac homeostasis by down-regulating miRNAs inducing cardiac hypertrophy, which might serve as efficient therapeutic targets for cardiac diseases.

Program/Abstract # 444

Identification and Expression Analysis of Two Homologs from Xenopus laevis of the Tumorhead Putative Binding Protein, FBXO30

Traverso, Edwin E.; Zbinden, Theodor; Flores, Noelia; Núñez, Dariana; Ayala, Jesús (UPuerto Rico-Humacao, USA); Hernández, Josué; García-Arrarás, José (U Puerto Rico-Río Piedras, USA)

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, which encode proteins that are 91% identical to each other. The FBXO30-A and FBXO30-B proteins share 64% and 63% identity with their *Homo sapiens* protein homolog, respectively. FBXO30 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. We have found through RT-PCR that FBXO30-A and FBXO30-B are maternal factors as their messages are present in the unfertilized egg. Their mRNAs persist during the cleavage stages, decrease dramatically once gastrulation starts, and reappear at the mid-tailbud stages. The FBXO30-A protein has been detected in the nuclei of cells at the gastrula (st. 12) stage. 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 # 445

The Effect of Calcium Activity Perturbation on Gene Expression in the Developing Nervous System of Xenopus

Rabe, Brian A.; Herbst, Wendy A.; Saha, Margaret (The College of William and Mary, USA)

Calcium ions serve as ubiquitous secondary messengers in a wide array of cellular processes, particularly during early neural development. While spontaneous calcium transients have been implicated in neural specification and differentiation, little is known regarding the mechanisms by which different patterns of calcium activity influence phenotype. In order to address this question, we

have investigated the effect of calcium activity perturbation on gene expression during neural development using Xenopus primary cell culture of presumptive neural tissue. Neural plates were dissected, dissociated, and plated in either normal physiological calcium (2mM Ca++) or elevated calcium (10mM Ca++) which results in significantly increased spiking activity. After culturing these cells until sibling embryos have reached late neurula, early tailbud, or early swimming tadpole stage, we extracted RNA and performed microarray analysis to investigate differential gene expression on each of these three stages. We have utilized a number of techniques to analyze the resulting data using a modified t-test, fold change analysis, and a novel mathematical algorithm employing a generalized linear model designed for low sample size. Our preliminary results indicate a wide array of genes exhibit differential expression ranging from genes whose products have known calcium binding activity to genes controlling cell cycle and stress responses. Each time point reveals a unique set of differentially expressed genes, suggesting a dynamic regulation of response to increased calcium. To complement these approaches we are also using RNAseq of similarly collected samples as well as an in vivo approach using a GCaMP.

Program/Abstract # 446

RA induced primitive extraembryonic endoderm leads to increased reactive oxygen species and a shift from aerobic glycolysis to mitochondrial biogenesis

Hwang, Jason TK; Wen, Jason; Kelly, Gregory (U of Western Ontario, Canada)

Mouse F9 cells are used to recapitulate the epithelial-to-mesenchymal transition (EMT) associated with extraembryonic endoderm differentiation. F9 cells treated with retinoic acid (RA) form primitive endoderm (PrE) and this is accompanied by an increase in reactive oxygen species (ROS). Treating cells with H2O2 induces differentiation, while treating either RA- or H2O2-treated cells with antioxidants inhibits it. Together, these results indicate that ROS are sufficient and necessary for PrE differentiation. Furthermore, that NADPH oxidase (*Nox*) genes are up-regulated in F9 cells treated with RA, and the ability of DPI, a Nox inhibitor, to block differentiation, provide evidence that the ROS source is cytoplasmic in nature. To investigate further and to examine mitochondrial function during this EMT, the levels of LDHA, PDK1 and phospho-PDH, which are elevated in cells using aerobic glycolysis, were examined in undifferentiated and RA-treated F9 cells. Results show the levels of these proteins decreased in response to RA. Furthermore, treating cells with dichloroacetate to increase mitochondrial respiration induced PrE differentiation in the absence of RA. Together, these results suggest that undifferentiated F9, like stem cells, use aerobic glycolysis to maintain their undifferentiated state. When induced, however, they undergo a metabolic shift from glycolytic to oxidative phosphorylation, which together with increased Nox activity would contribute to elevated ROS levels required for PrE differentiation.

Program/Abstract # 447

Distinct roles for isoforms of Regulator of G-protein Signalling 3 (RGS3) throughout neuronal maturation.

Fleenor, Stephen (U of Oxford, UK)

The differentiation and maturation of neurons from the neuroepithelial progenitor state is a fundamental process in nervous system development. In the forming cranial sensory ganglia, committed neuroblasts delaminate from a progenitor neuroepithelium and differentiate into mature neurons as they migrate away. Recently Regulator of G-protein Signalling 3 (RGS3) was found to be up-regulated during this process. In the chick, the RGS3 gene produces three distinct isoforms with unique functional domains. Here I present specific localisation of individual RGS3 isoforms to progressive neuro-differentiation states: from progenitor epithelium, to immature neuroblast, to mature neuron. shRNA-mediated knockdown of the longest isoform in ovo results in precocious neuronal differentiation. Consistently, overexpression produces an opposite effect. Excitingly however, distinct phenotypes emerge from knockdown of all isoforms and from overexpression of individual isoforms, suggesting separate roles for individual isoforms. These findings suggest roles for specific RGS3 isoforms in driving distinct aspects of neuronal maturation.

Program/Abstract # 448

Regulation of TNFa and COX2 by NFATc1 pathway during adipose commitment.

López-Victorio, Carlos J; Beltrán-Langarica, Alicia; Vellez-delValle, Cristina; Kuri-Harcuch, Walid (Cinvestav IPN, Mexico)

Obesity is defined as an abnormal and excessive accumulation of fat that causes weight gain and and it is considered a risk factor for several common diseases. During adipogenesis preadipocytes undergo biochemical, morphological and metabolic changes related to gene expression. The adipogenic conversion has three major steps: commitment, clonal expansion of committed cells, and phenotype expression. Our group has developed a model to study of adipose differentiation were the 3T3-F442A cell line is stimulated by a combination of staurosporine and dexamethasone (St/Dex) in absence of adipogenic serum. StDex induces two well-defined stages of commitment: induction and stabilization. In this study we analyzed the relation between Cn/NFATc1 signaling pathway, TNFα expression and the inhibition of COX2 pathway by celecoxib during the early events of adipose commitment. It has been reported that Cn acts as a anti-adipogenic pathway that negatively regulates adipogenesis by preventing the expression of critical pro-adipogenic transcription factors, this pathway includes NFATc1 participation as a nuclear effector, and Calmodulin A (CaM) as a molecule needed for Cn activity. We found increased transiently *nfatc1* and *ptgs2* mRNAs during the induction of commitment, and then it proceeds with the down regulation in the expression of these, the activation of Cn up regulates *ptgs2* and *nfatc1* and *tnfα*, furthermore the inhibition of Cn phosphatase activity in 3T3-F442A cells down-regulate the expression of TNFα and promoting progression of adipogenesis. Treatment with celecoxib, a specific COX2 inhibitor, does not change adipose conversion, however triglycerides

accumulation is decreased. This work was supported in part by grants 104350 from Consejo Nacional de Ciencia y Tecnología and PICDS08-8 from ICyTDF (Mexico). CJLV is a graduate student supported by the PICDS08-8 scholarship from ICyTDF.

Program/Abstract # 449

Importance of Intersectin1 isoforms during proper embryonic development of *Xenopus laevis*

Cheng, Cheng; Jimenez, Oscar; Thorn, Judith (Knox College, USA)

Intersectin 1 (ITSN1), located on the human chromosome 21, is associated with neurodegenerative diseases such as Down Syndrome, Alzheimer disease and Hungtington disease. Itsn1 interacts with many proteins, forming complexes implicated in endocytic and mitogenic pathways important in neurogenesis and maintenance. In this study, we reported the abnormality caused by itsn1 depletion and overexpression during the early *Xenopus* development. We microinjected the embryos at 1 cell stage with translation blocking itsn1 morpholino, in which no abnormal phenotype was observed; however, the itsn1 morpholino injection in the oocytes followed by host transfer results in the slow of blastopore closure, abnormal pigmentatation and a general shortening of the embryo axis. Similar phenotype is also observed in embryos microinjected with Intersectin1-short (itsn1-S) mRNA at the 2-cell stage. Our analysis of the relative expression of itsn1 during embryonic development along with our microinjection results indicates that sufficient itsn1 protein is necessary pre-zygotically for early embryonic development, and regulating cell movement. Further research is going to be focusing on the overexpression of itsn1 long isoform (itsn1-L) and the causality of the axis defects due to protein overexpression and depletion.

Program/Abstract # 450

Chromatin state transitions and epigenetic constraints during early Xenopus embryogenesis

Veenstra, Gert Jan (Radboud Univ, Netherlands)

Chromatin state is essential for pluripotency, competence and cell lineage commitment. It specifies how genes are marked for activation or repression by epigenetic mechanisms. Little is known however, about the developmental origins of chromatin state and its regulation. We have generated chromatin state maps of *Xenopus tropicalis* embryos by ChIP-sequencing to explore the developmental origins of chromatin state with respect to inheritance, sequence features and molecular mechanisms. To assess chromatin state dynamics we profiled promoter histone modifications, enhancer histone modifications, facultative and constitutive heterochromatin modifications and DNA methylation at multiple stages of development. We find blastula stage marking of promoters and enhancers by histone H3 lysine 4 tri- and mono methylation (H3K4me3, H3K4me1) respectively, followed by dynamic commisioning of enhancers by the enhancer-bound p300 co-activator during gastrulation and subsequent development. The Polycomb Repressor Complex 2 (PRC2) binds widely to enhancers, but the Polycomb mark H3K27me3 is only deposited at a small subset of these sites. This mark is newly deposited from blastula stages onward within constrained domains lacking prior DNA methylation. Unmethylated regions represent two epigenetically different loci: Polycomb-regulated genes and constitutive house-keeping unmethylated promoters which gain H3K4me3 but not H3K27me3. These loci can be differentiated on the basis of specific DNA sequence signatures which are conserved between humans, frogs and fish. The results imply a genetic-default model in which genomic sequence is the major determinant of unmethylated regions. Unmethylated DNA triggers H3K27me3 deposition by an allosteric loop when not opposed by transcriptional activation. The sequence signature involved provides an epigenetically marked but genetically inheritable constraint on Polycomb regulation and serves as a scaffold to guide deposition of H3K27me3 during exit of pluripotency.

Program/Abstract # 451

The lysine acetyltransferase HBO1 is essential for maintaining the poised chromatin state of neural stem cells

Tim Thomas, Andrew Kueh, Anne Voss (Inst. of Medical Research, Australia)

The five MYST proteins (KAT5-8) constitute one third of the mammalian genome's capacity to regulate transcription and chromatin conformation at the level of histone acetylation. We have reported the biological and molecular roles of the MYST histone acetyltransferases *in vivo* in mice.[1-7] MOZ (MYST3) and QKF (MYST4) are essential for the development and self-renewal of haematopoietic stem cells and neural stem cells, respectively.[2,3] MOF (MYST1) is required genome-wide histone 4 lysine 16 acetylation (H4K16ac).[4] However, MOZ is unexpectedly specific and regulates H3K9ac at *Hox* and *Tbx* loci, correspondingly, *Moz* mutation leads to homeotic transformation of the axial skeleton[5] and DiGeorge-like defects in heart development.[6] Unpublished data will be presented showing that neural stem cells lacking HBO1 (MYST2) are devoid of H3K14ac, yet undergo self-renewing proliferation for months in culture. Wild type neural stem cells are multipotent. In contrast, *Hbo1* null neural stem cells are unable differentiate into neurons, forming only astrocytes. *In vivo*, *Hbo1* null cortical neurons fail to express key regulators of cortex development. Re-expression of HBO1 in neural stem cells restores H3K14ac and multipotency when conducted 3 days, but not 5 weeks after decline in HBO1 protein, suggesting irreversible changes to the chromatin after prolonged absence of HBO1. Our data suggest that HBO1 and H3K14ac are essential for the maintenance of multipotency and the poised chromatin state. 1. Thomas et al (2000) Development 127:2537 2. Thomas et al (2006) Genes Dev 20:1175 3. Merson et al (2006) J Neurosci 26:11359 4. Thomas et al (2008) Mol Cell Biol 28:5093 5. Voss et al (2009) Dev Cell 17:674 6. Voss et al (2012) Dev Cell 23:652

Program/Abstract # 452

AMPK (AMP-activated kinase) buffers adverse transgenerational consequences on growth and reproduction following a single exposure to nutrient stress in *C. elegans*

Richard Roy, Emilie Demoinet (McGill U, Canada)

Life history events can be recorded not only in our memories, but also by altering our genes through chromatin modification. We show that during environmental challenges AMPK blocks the formation of specific histone marks on the chromatin to ensure that gene expression is not inappropriately activated under conditions of nutrient stress. Following embryogenesis in *C. elegans*, the emergent L1 larvae can survive for about two weeks in a non-developing diapause-like state until they encounter a nutrient source that will trigger post-embryonic development. In the absence of AMPK, these larvae die prematurely after 5 days, indicating that AMPK is required to survive this challenge. If mutant AMPK L1 larvae are placed on food after 3 days of nutrient stress, the animals will initiate development and subsequently arrest at various points throughout larval development. More interestingly, many of the challenged AMPK mutants that survive become sterile, although some do remain fertile (30%). If the fertile animals are left to reproduce (F1 generation), and they are cultured without any nutrient stress/challenge, they will re-capitulate a similar profile of arrested and sterile animals to that observed in the challenged parental generation. This phenomenon is reproduced at each subsequent generation and is associated with a progressive loss in reproductive capacity. This mortal "germ line" phenotype is never observed in the same AMPK mutants that were not stressed, nor in starved wild type animals. Our data suggest that AMPK protects against adverse changes to the germ line that occur following a single bout of nutrient stress and which have detrimental reproductive consequences that are multigenerational in nature.

Program/Abstract # 453

The ATRX gene is separated in *Drosophila*: description of the xnp2 gene

López Falcón Piza, Brenda Araceli; Meyer Nava, Silvia; Montero Barrera, Daniel; Hernández Rodríguez, Benjamín; Zurita, Mario (UNAM-Cuernavaca, Mexico)

The human *ATRX* gene encodes a chromatin remodeling protein that has two important domains, an helicase/ATPase domain and a domain composed of two zinc fingers called the ADD domain. The ADD domain binds to histone tails and has been proposed to mediate hATRX binding to chromatin. The putative ATRX homolog in *Drosophila* (XNP/dATRX) has a conserved helicase/ATPase domain but lacks the ADD domain. We performed a bioinformatic search in the data bank of the *Drosophila* genome and found that the annotated gene: CG8290 (which we named *xnp2*) encodes four proteins that share a common region in the amino terminal end that contains an ADD domain which is highly conserved with the ADD domain of the hATRX protein. Furthermore 3D modeling of the domain shows that the structure and aminoacids which mediate the histone tail contacts are highly conserved. These isoforms (xnp2a, b, c, and d) are generated by alternative splicing and are expressed throughout the development of *D. melanogaster*. We determined using pull-down and CoIP assays that they interact physically with xnp/dATRXL. Furthermore co-immunostaining of polythene chromosomes with specific antibodies show that they co-localize mainly in the chromocenter, with xnp/dATRXL and HP1α, although euchromatic localization can also be seen through the chromosome arms. ChIP experiments demonstrate these proteins are present *in vivo* in the same heterochromatic regions. Interestingly the xnp2b,c and d isoforms have extra domains which suggest newly acquired functions of these proteins. These results strongly support that in *Drosophila* the *ATRX* gene diverged and that the *xnp2* encoded proteins participate with xnp/dATRXL in some cellular functions such as heterochromatin maintenance.

Program/Abstract # 454

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

Basson, Michiel A.; Yu, Tian; Danielsen, Katrin; Shah, Apar (King's College London, UK); Marques, Ana (Oxford, UK); Bowler, Timothy (Monterfiore Med Ctr, USA); Ponting, Chris (Oxford, UK); Reinberg, Danny (HHMI/NYU, USA); Scambler, Peter (King's College London, UK)

The *CHD7* gene encodes a chromodomain helicase implicated in the fine-tuning of developmental gene expression. Mutations in human *CHD7* result in CHARGE syndrome. In addition to the core features of CHARGE syndrome, neurodevelopmental defects such as cerebellar hypoplasia and autism have been reported. Early embryonic development of the cerebellum requires the tight regulation of FGF8 signalling levels in the developing mid-hindbrain region. We have previously shown that reduced FGF8 signalling is associated with hypoplasia of the cerebellar vermis. To understand the causes of the cerebellar defects associated with CHARGE syndrome, we analysed brain development and gene expression in *Chd7*-deficient mice. Our analysis has identified two functions for CHD7 in cerebellar development. During early embryonic mid-hindbrain development, CHD7 is associated with several putative *Otx2* distal regulatory elements, suggesting that CHD7 might regulate *Otx2* expression directly. We find that *Otx2* is de-repressed in *Chd7*-deficient embryos, resulting in re-specification of r1 into mesencephalon in *Chd7*-/- embryos and defects in the establishment of normal levels of Fgf8 expression at the mid-hindbrain boundary in *Chd7*+/- embryos. Consistent with this finding, *Chd7* and *Fgf8* loss-of-function alleles interact during development and *Chd7+/-;Fgf8+/-* mutants exhibit severe hypoplasia of the cerebellar vermis. In addition to this early role, CHD7 regulates the expansion and differentiation of granule cell precursors in the postnatal cerebellum. Deletion of *Chd7* specifically from these neuronal precursors is sufficient to cause cerebellar hypoplasia. Gene expression and chromatin immunoprecipitation experiments suggest a role for CHD7 upstream of a key pathway that has been linked to cerebellar hypoplasia and autism in the human population.

Program/Abstract # 455

Epigenetic mechanisms involved in temperature-dependent sex determination of the sea turtle Lepidochelys olivacea

Venegas, Daniela; Marmolejo, Alejandro; Valdes-Quezada, Christian; Recillas-Targaga, Felix; Merchant-Larios, Horacio (UNAM-Mexico City, Mexico)

Sex determination in vertebrates depends on the expression of a highly conserved network of genes. However, the mechanisms that trigger the activation of male or female producing networks vary among species. Sea turtles have temperature-dependent sex determination (TSD); it means that each embryo has the complete set of genes necessary to develop either as male or female. Thus, temperature affects gene expression levels that trigger the establishment of sex determining networks. In the current study, we analyzed for the first time, some epigenetic mechanisms that might be involved in TSD. We found differences in global DNA methylation patterns between gonads of embryos incubated at male (MPT) or female promoting (FPT) temperatures using the Amplification of Intermethylated Sites. We also discovered differences in 5HmeC levels between gonads of embryos incubated at MPT or FPT, specifically in pre-Sertoli cells of the medullar cords. These differences correlated with unpaired distribution of other epigenetic marks like H3K27me3. We performed a more specific DNA methylation analysis using Sodium Bisulfite DNA conversion over promoter sequences of some genes involved in sexual differentiation, such as *Sox9* and *Dmrt1*. Current results suggest that epigenetic mechanisms are involved in regulating gene networks during TSD and/or gonadal differentiation in *L.olivacea*.

Program/Abstract # 456

Polycomb/ Trithorax group proteins collaborate with Heterochromatin protein 1 to regulate *Drosophila* sex determination

Rodriguez, Janel; Horabin, Jamila (Florida St U-Tallahassee, USA)

A combination of histone modifications, a 'histone epigenetic code', is created and altered by specific enzymes. These marks provide cues for developmental timing and regulation. Some of the well studied chromatin remodeling proteins are members of the Polycomb and Trithorax Groups (PcG and Trx-G). The two groups are primarily antagonistic and control patterning of the body during embryogenesis through the regulation of gene expression. In *Drosophila*, one of the earliest developmental decisions is that of the embryo determining its sex. This process involves regulating the expression of the X chromosome sensing promoter of *Sex-lethal* (*Sxl*) at its establishment promoter, Sxl_{Pe}. Using this sensitive system which differentiates one versus two X chromosomes, our lab has shown that heterochromatin proteins are required for proper Sxl_{Pe} regulation. We found that Heterochromatin Protein 1a (HP1a) plays both a repressive and activating role. The PcG/Trx-G proteins also genetically interact with mutations in the sex determination pathway and influence the ability of females to determine their sex; these proteins are necessary for proper histone 3 lysine 4 (H3K4) and histone 3 lysine 27 (H3K27) methylation at the promoter. Surprisingly, we find that embryos deficient in E(z), Su(z)12 or ASH1 protein also affect the binding of HP1a at Sxl_{Pe} sequences. Our data suggest that early deposition of H3K27me3 is needed to both prevent premature activation of Sxl_{Pe} as well as facilitate its robust expression, and that there is crosstalk between the PcG/Trx-G proteins and HP1a. The two heterochromatin systems coordinate repression of Sxl_{Pe} in males and facilitate its transcription in females.

Program/Abstract # 457

Polycomb determines responses to Smad2/3 signaling in embryonic stem cell differentiation and in reprogramming

Kuehn, Michael; Dahle, Øyvind (National Cancer Inst, USA)

Understanding how pluripotency is established and maintained is of fundamental importance in the fields of embryonic development and stem cell based regenerative medicine. A key concept is that embryonic stem (ES) cells exist in a naïve ground state and must transition to an epigenetically distinct state to be responsive to developmental signaling. Similarly, reprogramming of differentiated cells to induced pluripotent stem (iPS) cells requires a resetting of epigenetic states. We recently gained insight into how distinct intrinsic epigenetic states are established and how they can respond selectively to extrinsic signals by showing that the Nodal/Activin/TGFβ signaling pathway counteracts Polycomb mediated epigenetic repression at specific target genes in ES cells. Smad2 and Smad3 (Smad2/3), the intracellular mediators of this pathway, recruit the histone demethylase Jmjd3 to target loci chromatin where it erases Polycomb repressive histone marks. We now find that this regulatory paradigm also allows selective gene regulation in the transition from the naïve ground state and in reprogramming. Specifically, we find that Polycomb imposes responses to Smad2/3 mediated signaling to selectively regulate expression of the master pluripotency factor Oct4 during initiation of differentiation, but not in the self-renewing ground state. During reprogramming back to the ground state, enhancement of reprogramming efficiency stemming from blocking Smad2/3 signaling also depends on Polycomb. These context-dependent responses imposed by Polycomb action provide a mechanism for selective gene regulation that can reconcile the apparently conflicting roles of Smad2/3 signaling in pluripotency, differentiation and reprogramming.

Program/Abstract # 458

Quantifying the impact of blood flow on embryonic cardiac development in zebrafish

Garrity, Deborah M.; Johnson, Brennan M; Hammond, Sean L; Zeller, Molly J; Dasi, Lakshmi Prasad (Colorado St U-Fort Collins,USA)

In normal development, tissues in the forming heart are impacted by shear stress forces arising as blood cells pass through the heart tube. Moreover, altered shear stress has been correlated with morphological cardiac deficiencies, although these have never been systematically defined. Zebrafish offer superb optical access for live imaging of embryonic hearts. Studies suggest that altering blood viscosity or hemocrit levels adversely affects morphological development in the heart, particularly valve development in the atrioventricular junction (avj). Here, we describe recent work dedicated to quantitatively describing the impact of blood flow upon the developing heart. Hearts were imaged using bright field microscopy at 1500 frames/s at 0.76 lm/pixel. Based on imaging data, we extracted spatiotemporal plots that were used to calculate blood velocity and luminal diameter at the atrial inlet and the avj. Volume analysis was used to describe hallmark functional parameters, including flow rate waveforms, fraction of retrograde flow, stroke

volume, and cardiac output. We characterized the typical variation observed over the course of a heartbeat at 55 hours post-fertilization, and plan to extend this analysis over developmental time. Slowing the heart rate approximately 25% by decreasing temperature nevertheless left heart function (as measured by the hallmark parameters) relatively intact. However, slowing the heart rate to a similar extent by other methods (drugs) drastically altered flow patterns across the inlet region and avj, indicating heart function was differentially affected. These protocols therefore represent an opportunity to define the downstream morphological or genetic responses that occur in the embryonic heart when flow is quantitatively altered in different areas of the heart, or in different portions of the cardiac cycle.

Program/Abstract # 459

Regulation of genome architecture during heart development

Gómez Velázquez, Melisa; Badía Careaga, Claudio (Ctr Nac de Investigaciones Cardiovasculares, Spain); Galjart, Niels (Erasmus Med Coll, Netherlands); Gómez Skarmeta, José Luis (Ctr Andaluz de Biol del Desarrollo, Spain); Manzanare, Miguel (Ctr Nac de Investigaciones Cardiovasculares, Spain)

The central question in developmental biology is to understand how a single cell becomes a complex multicellular organism. For this to occur, gene expression must be highly and tightly controlled in time and space. The information regarding the genes that need to be expressed at a certain time and place is coded in regulatory elements that sit throughout the genome. The distribution and location of these elements are going to define on which genes they can act. This is one of the reasons why the genome needs to be exquisitely organized in three dimensions. CTCF, an 11 zinc finger protein, has been recently associated at multiple levels in this process, as it can act as an insulator factor, a looping factor and an enhancer-promoting factor. We are interested in understanding how genome architecture is involved in early development. Here, we focus our attention in heart development and in order to decipher the role that CTCF plays here, we are specifically deleting the gene in cardiac tissue by using a conditional *Ctcf* allele and tissue-specific Cre drivers. When doing so, the embryos die at stage E13. As a first approach to understand the underlying defects we are analyzing by in situ hybridization at E9.5 and E11.5 the expression pattern of genes that could be de-regulated by the loss of genomic structure due to lack of CTCF. More specifically, we are studying genes that are organized in tandem on the genome, that show divergent expression pattern in the developing heart, and that are separated by stable CTCF binding sites. Results will be presented for genes that show these features, including transcription factors of the *Irx* and *Tbx* families.

Program/Abstract # 460

DNA demethylation confers competence on the genome for zygotic genome activation in zebrafish embryos

Meng, Anming; Wu, Di; Jia, Shunji (Tsinghua U, China)

The zygotic genome of animal embryos is transcriptionally inactive upon fertilization, and becomes active after a certain period of development. As transcription can be controlled by the presence of 5-methylcytosines (5mC) in DNA, the global DNA methylation level in an embryo may play a role in the zygotic genome activation (ZGA). In mammals, the paternal genome is rapidly demethylated immediately after fertilization through 5mC hydroxymethylation, resulting in a decrease of the global DNA methylation level of the zygote genome. We found that the genome-wide erasure of DNA methylation upon fertilization in zebrafish embryos is not associated with 5mC hydroxymethylation. Instead, one of DNA glycosylases, hypothesized as XDG, appeared to mediate global DNA demethylation. *xdg* knockdown in zebrafish embryos causes an increase of the global DNA methylation level concomitantly with a reduction of the nuclear transcription level, ultimately resulting in embryonic lethality; conversely, *xdg* overexpression in embryos is sufficient to reduce the global DNA methylation level and induce earlier activation of zygotic genome transcription. Thus, XDG-mediated DNA demethylation through base excision and repairing is crucial for development of vertebrate embryos.

Program/Abstract # 461

MicroRNA-30a regulates zebrafish myogenesis via targeting the Six1 homeoprotein

O'Brien, Jenean H.; Hernandez-Lagunas, Laura; Artinger, Kristin Bruk; Ford, Heide L. (UColorado-Denver,USA)

Six1 is a homeodomain containing transcription factor that functions in embryonic muscle development, muscle regeneration after injury, and muscle tumor promotion. In mouse and zebrafish, knockdown of Six1 results in decreased myogenic progenitor cell activation and decreased fast-twitch fiber differentiation. However, overexpression of Six1 can also inhibit early differentiation. These seemingly paradoxical functions of Six1 suggest that precise control of Six1 expression levels is critical for directing proper myogenesis. Throughout embryogenesis, microRNAs (miRs) coordinate complex temporal patterns of protein expression, highlighting miRs as potential regulators of Six1. Several miRs have been demonstrated to be important for muscle development, however most target genes that act downstream of Six1. Here, we investigated miR-mediated regulation of Six1 in myogenesis. Prediction algorithms identify zebrafish paralogs *six1a/b* as potential targets of miR-30a, which is supported by a reciprocal expression pattern in the somites at 24 and 48 hours post fertilization. Morpholino-mediated miR-30a knockdown results in upregulation of endogenous Six1 levels, and phenocopies Six1 overexpression. Further, miR-30a overexpression leads to decreased *six1a/b* mRNA and protein levels, and directly represses GFP-six1a 3'UTR reporter expression. Importantly, abnormal somite morphology and increased cell death are observed with miR-30a overexpression, phenocopying *six1a/b* inhibition, and these phenotypes can be rescued with *six1a* RNA lacking a 3'UTR. Together, these data indicate that miR-30a regulates zebrafish myogenesis via targeting Six1, and provide a framework to examine whether miR dysregulation contributes to muscle pathologies.

Program/Abstract # 462

Retinaldehyde dehydrogenase 2 (Raldh2) and retinoic acid are crucial for nephron endowment

Li, Qinggang; Liu, Ying; Xie, Yuansheng; Chen, Xiangmei (Beijing, China)

Retinaldehyde dehydrogenase 2 (Raldh2) enzyme is responsible for retinoic acid (RA) synthesis, which is encoded by Aldh1a2. Aldh1a2 mutation or RA deficiency can cause kidney dysplasia and a reduction in the number of nephrons which is associated with increased risk of hypertension .To address the effect of Raldh2 and RA on kidney development, We observed the expression of Raldh2 during kidney development with real time RT-PCR and western blotting and examined phenotype of littermate kidney after maternal retinoic acid diet. For maternal retinoic acid administration, We replaced standard mouse chow with retinoic acid-supplemented chow for 24 h on E7.5. On E8.5 and E9.5 we increased the dose of retinoic acid in the chow to 13.3 mg per kg body weight. On E10.5, we replaced retinoic acid–supplemented chow with standard chow until the day of dissection. We found the level of Aldh1a2 mRNA, protein in kidney were high from E12.5d to E15.5d, and decreased dramatically after that, reached the lowest level at postnatal day 14. Aldh1a2 was mainly expressed in stromal mesenchyme and cap mesenchyme, not ureteric bud during early kidney development. After E14.5d, Aldh1a2 was selectively expressed in parietal cells and premature proximal tubular epithelial cells, which was disappeared after postnatal day14. Our data show increased UB branching and glomerular number after maternal retinoic acid administration in the embryonic kidneys. Meanwhile, no significant change in Aldh1a2 expression was observed. These findings indicate that Raldh2-retinoic acid induce metanephric mesenchyme differentiates into the nephron, Raldh2 might be consider as a parietal precursor cell marker.

Program/Abstract # 463

Neural Crest to neuroblastomas: a two way street for lessons on Development and Cancer

Bajpai, Ruchi; Samanta, Soma; PeliKan, Richard (Uof Southern California, USA)

Neural crest cells are among the most migratory cells of the body and neural crest derived tumors like neuroblastomas and melanomas are among the most metastatic tumors known. To uncover any underlying commonality we determined the genome wide binding profile of P300, an enhancer associated protein in human ES derived cranial neural crest cells (CNCC) and neuroblastoma (NB) cells that originated in the thoracic region. To our surprise we saw no significant overlap between the P300 binding sites but found several highly confident and statistically significant binding motifs for transcription factor that are part of the core transcriptional circuitry active in migratory neural crest cells at these sites. This suggests that at least in part, NB utilize a NCC like transcriptional circuitry for self renewal and/or metastasis. Moreover determining active enhancers (as described by Rada Iglesias et al, 2012) identified unique regions that define the molecular basis of cellular heterogeneity in neuroblastomas, predict stem cell fractions as well as determine clinical grade of a primary tumor be it a neuroblastoma (n=22) or closely related neurofibromatosis (n=15). Our data suggest that the resident tumor stem cells within a heterogeneous neuroblastoma, have a ‘unique regulatome’. These stem cells can self-renew and also generate a range of differentiated progeny with a different and distinctive ‘active regulatome’ and a gene expression profile akin to human fetal dorsal root ganglia. In vivo analysis shows that these enhancers are activated in developing peripheral nervous system revealing a distinct transcriptional circuitry active in thoracic neural crest cells.

Program/Abstract # 464

Vgl4b is a new gene expressed in the ectoderm of Xenopus laevis

Barrionuevo, M.Guadalupe; Aybar, Manuel J.; Tribulo, Celeste (Univ Nac de Tucuman, Argentina)

In vertebrates were identified four Vestigial like genes (Vgl1-4) that have been shown to be involved in a variety of developmental processes. *Vgl4* gene encodes a protein that has two tandem conserved regions called TONDU (TDU) motifs in its carboxyl-terminal domain. In *Xenopus laevis* we have identified a new *Vgl4* paralogue which we called *Vgl4b* while the previously described was appointed as *Vgl4a*. The bioinformatic analysis of Vgl4b protein sequence revealed the presence of the two TDU motifs characteristics of this cofactor. There was a strong Vgl4 homology in TDU motifs which are completely conserved between human, mouse, zebrafish and *Xenopus.* We analyzed the temporal and spatial expression of *Vgl4b* by simple and double in situ hybridization and RT-PCR and compared it with the expression of *Vgl4a*. At neurula stage, the main expression of *Vgl4b* is located in the inner layer of the ectoderm corresponding to the epidermis and the neural folds while *Vgl4a* is expressed mainly at the neural plate and weakly in the epidermis. In sectioned embryos we could observe that *Vgl4a* is expressed also in the mesoderm while *Vgl4b* remains only at the ectoderm. At tailbud stages, *Vgl4b* is expressed strongly in branchial arches, otic vesicle and trunk epidermis. In comparison, *Vgl4a* expression is detected in whole brain, optic and otic vesicles, branchial arches and somites. To determine the regulatory relationship between *Vgl4b* and other genes expressed in the inner layer of the ectoderm, we carried out functional experiments. Taken together, our results show that *Vgl4b* is a new member of *Vgl* gene family with differential expression in the ectoderm, and could be involved in the development of this tissue.

Program/Abstract # 465

maternal KLF2 regulates the expression of early pan-ectodermal activator, Foxi1e, in Xenopus development

Cha, Sang-Wook Cha; Shoemaker, Amanda; Wylie, Christopher; Kofron, Matthew (Cincinnati Children's Hospital, USA)

One of the first, and major, patterning events that takes place in all triploblastic embryos is the formation of the three primary germ layers. In the early *Xenopus* embryo, maternally encoded T-box transcription factor, VegT , is localized to the vegetal cytoplasm in the oocyte, and can initiate mesoderm and endoderm formation. But, much less is known about the formation of the ectoderm, which

arises from the most animally located cells of the blastula. Previously we shown that Foxi1e is the first known early zygotic pan-ectoderm activating gene and another Forkhead box protein, Foxi2, is key activator for its expression and other epidermal genes' expressions. Despite having maternal activator, Foxi2, in entire ectodermal tissue, Foxi1e is only expressed in deep layer of presumptive ectodermal tissue, it suggests that the existence of potential repressor in superficial layer. Here, we report that a member of Kruppel-like factor family, KLF2, as the maternal repressor for Foxi1e expression. This set of maternal activator/repressor (Foxi2/KLF2) will provide the mechanism of differential expression of Foxi1e.

Program/Abstract # 466

The WT1 protein expression in radial glia of human developmental cerebellum

Parenti, Rosalba; Puzzo, Lidia; Magro, Gaetano; Gulisano, Massimo (U of Catania, Italy)

Developmental expression of Wilms' tumor (WT1) protein is crucial for cell proliferation, apoptosis, differentiation and cytoskeletal architecture regulation. The variable WT1 expression and distribution at nuclear or cytoplasmic level, suggest different roles in the different human fetal tissues. More recently, it has been suggested also a direct role in the development of neural tissue and in neurodegenerative disorders. The aim of this study is to investigate immunohistochemically the temporal and spatial distribution of WT1 during human ontogenesis of neural tissues from the 7 week of gestational age, focusing the analysis on the developing human cerebellum. Double immunohistochemistry with markers of radial glial stem cells, astrocytes, newborn neurons and mature neurons revealed that WT1 was dynamically expressed being localized in radial glia during the first developmental step (12 gestational week) to be expressed, gradually, in Purkinje cells and most neurons of deep cerebellar nuclei. In large developing Purkinje cells WT1 immunoreactivity involved mainly cytoplasmic compartment and the short processes of Purkinje cells which are maturing along scaffold radial glia in the molecular layer. No immunoreactivity was found in the axons or axon terminals of the cells. Since radial glial cells give rise to the Purkinje cells providing a scaffold for the migration of these cells, transient WT1 expression firstly in radial glia followed in Purkinje cells, would suggest that this protein may act by specifying differentiation processes of cerebellar cortex development.

Program/Abstract # 467

A ribosomal biogenesis mutant reveals roles for BMP in asymmetric brain development

Gamse, Joshua T.; Wu, Simon (Vanderbilt, USA); Freed, Emily (Yale, USA); Leshchiner, Ignat; Goessling, Wolfram (Harvard Med Sch, USA); Baserga, Susan (Yale, USA)

Unilateral migration of neurons is one strategy for generating asymmetry in brain anatomy. The parapineal organ of zebrafish, which is specified in the middle of the brain and migrates to the left side, is an excellent tool for studying how such unilaterally-placed neurons develop. A genetic screen for mutants with excess parapineal neurons on the left side identified *utp15*, a component of the nucleolar small subunit processome (SSU). In *utp15* mutants, pre-rRNA processing is impaired and cell death occurs in the developing CNS. In addition, BMP signaling is downregulated in the region of parapineal specification of *utp15* mutants due to increased expression of the BMP inhibitor *chordin*. The phenotypes of many ribosomal biogenesis mutants are suppressed by eliminating p53-induced cell death. However, although loss of p53 in *utp15* mutants suppresses cell death in the CNS, it has no effect on the excess parapineal phenotype. The data suggest that *utp15* promotes BMP signaling by preventing activation of *chordin* expression, and thus limits the number of parapineal cells that are specified. We are using tissue-specific inactivation of BMP signaling and *utp15* to investigate how the correct number of parapineal cells is specified, in order to understand how asymmetric populations of cells arise in the vertebrate brain.

Program/Abstract # 468

Dynamic and asymmetric segregation of cells from the rhombic lip contributes to both neural tube and roof plate tissues in the zebrafish hindbrain

Campo-Paysaa, Florent (IGFL-ENS, France); Clarke, Jonathan; Wingate, Richard (King's College London, UK)

Choroid plexus is a structure of brain ventricles that regulates the internal environment of the brain by acting as both a permeable, regulated barrier with the blood system and producing the cerebrospinal fluid. Choroid plexus develops from a locally expanded dorsal tube roof plate epithelium of the lateral, third and fourth ventricles, which becomes progressively thicker and highly vascularised. During early events of choroid plexus specification of the fourth ventricle, cells arise from a proliferative region located at the interface between the roof plate and the neural tube: the rhombic lip. Whether neural tube cells (expressing atoh1a) and roof plate cells (expressing gdf7) arise from the same pool of progenitor cells or from two distinct populations of progenitors remains elusive. To address this question, we investigated the dynamics of cell division and segregation from the rhombic lip during zebrafish development, using the particular advantage of this model system for the analysis of development at a single cell resolution in vivo. Thus, we carried out mosaic lineage analysis to identify single cells at the roof plate boundary and follow their cell divisions over several cycles. These experiments were carried out in atoh1a::GFP line to allow the neural fate of daughter cells to be unambiguously assigned. In addition, we investigated the control of asymmetric segregation of cells from the rhombic lip through functional studies by interfering with atoh1a functions. Finally, taking advantage of the EtMn16 zebrafish line, reporter for choroid plexus precursors, we studied the link between the emergence of roof plate cells from the rhombic lip and early specification of the choroid plexus.

Program/Abstract # 469

Search for novel genes involved in hindbrain segmentation

Vazquez-Echeverria, Citlali; Escarcega, David (Inst Tecnol y Estudios Superiores de Monterrey, Mexico); Pujades, Cristina (Univ Pompeu Fabra, Spain)

In the hindbrain, anteroposterior (AP) patterning involves a segmentation process that leads to the formation of seven bulges named rhombomeres (r). Early events leading to the segmentation of vertebrate hindbrain into rhombomeres with distinct identities are still not well understood. Cell patterning along the AP axis has been shown to be initiated during gastrulation and subsequently refined during neurulation. Cells are specified or committed to different rhombomere fates before morphological distinctions among them could be discerned. This commitment is presumably accompanied by changes in gene expression. To identify new genes expressed in specific rhombomers in mice we have explored the use of transcriptional profiling of single pre-rhombomeric cells, in the context of hindbrain segmentation by analyzing cells when the specification of the identity of the segment is just beginning. DNA microarray analysis was combined with single-cell PCR procedure to study gene expression profiles of single cells from different pre-rhombomeric territories (pre-r4 and pre-r5). Our results showed a Rhombomeric differential gene expression for: *Tbx20, HNF-3, Wnt14b, Pbx4, Klf15, POU-III, Emx1, Olig1, Hes5, bicc1, Limk1, Crabp1*. We are currently searching whether differentially expressed genes are involved in cell decision for an specific rhombomeric identity by different bioinformatics' methods.

Program/Abstract # 470

Induction of Brn3a Through Ectopic Expression of Mash1, Ngn1, or Ptf1a

Landsberg, Rebecca L. (Coll. of St. Rose, USA); George, Angela (Springfield, USA)

The inferior olivary nucleus (ION) is involved in coordinating balance and movement by relaying inputs from the cortex and the spinal cord to the Purkinje cells in the cerebellum. The progenitors that generate the neurons of the ION have been localized to the caudal extent of the dorsal embryonic hindbrain neural tube inclusive of an anatomical region known as the lower rhombic lip (LRL). Progenitors within the LRL are arranged along the dorsoventral (D/V) axis into distinct domains predictive of future cell fate. The relative location of ION progenitors has been mapped to ventral regions of the caudal LRL that express *Ptf1a*, *Olig3*, and *Wnt1*. We have data that suggests that posterior regions of the ION might arise from more dorsal pools of progenitors that express either *Ngn1* or *Mash1*, suggesting a much larger territory of progenitors responsible for ION production. The ION is one of the few structures in the ventral medulla that has been shown to express the transcription factor Brn3a. We wished to determine if over- or missexpression of Ngn1 or Mash1 in the NB2A neuroblastoma cell line would correlate with ectopic expression of Brn3a, which is expressed by ION neurons immediately after they exit the LRL. Our studies found that cells transfected with *Ngn1*, *Mash1*, or *Ptf1a* consistently exhibited increased levels of Brn3a protein while cells transfected with *Math1* or vector alone did not express Brn3a. The data suggests that Brn3a expression can be activated by Ngn1, Mash1, or Ptf1a, supporting a model in which the neurons of the ION arise from progenitors with a history of expression *Ngn1, Mash1,* or *Ptf1a*.

Program/Abstract # 471

Determination of neural precursor cell commitment into mesencepahlic dopaminergic neurons

Guerrero, Gilda; Bastidas, Aimée; Covarrubias, Luis (UNAM-Cuernavaca, Mexico)

It is considered that cells define their fate progressively during development by going stepwise through mechanisms that restrict their plasticity (e.g., competence, specification, commitment). Although molecular markers are good indicators of specification and differentiation, functional assays are required to define the signals involved and the time at which cells become fully committed. In this work, we focus in defining when neural precursor cells of the midbrain (mNPC) determine their fate into dopaminergic neurons (mDAN). We analyzed the differentiation of ventral mNPC, the source of mDAN, from different stages after transplantation to E10.5 midbrain explants. We observed that ventral E10.5 mNPC (Lmx1a+, Ngn2+, Nurr1-, Th-) implanted in E10.5 midbrain explants behave as committed showing differentiation into mDAN even when they differentiate out of the natural milieu around the midline. In contrast, E9.5 mNPC from the ventral region (Lmx1a+, Ngn2-, Nurr1-, Th-) differentiate into TH+ neurons only when implanted around the midline. Re-specification of mNPCs failing to differentiate into mDAN out of the midline did not occur since they retained Lmx1a expression. Lmx1a+ rosette-like structures were commonly found in explants when donor mNPCs were from E9.5 but not from E10.5 embryos, and thus, appear to be a characteristic of the non-committed mNPC developmental stage (Dev.Biol. 349, 192-203, 2011). Despite this association, we propose that E9.5 mNPC are competent to differentiate into mDAN but, due to the lack of signals needed for specific differentiation (e.g., Shh, Fgf), the apparent un-committed phenotype was observed.

Program/Abstract # 472

Loss of Dll1 affects the timing of neurogenesis in the midbrain dopaminergic niche

Valencia, Concepción; Trujillo-Paredes, Niurka; Guerrero, Gilda (UNAM-Cuernavaca, Mexico); Guerra-Crespo, Magdalena (UNAM-México city, Mexico); Baizabal, José Manuel; Covarrubias, Luis (UNAM-Cuernavaca, Mexico)

Notch signaling is a well-established pathway that regulates neurogenesis during vertebrate nervous system development. However, little is known about its role in the differentiation of specific neuron types. In the present work we studied the role of Delta like 1 (Dll1) Notch ligand in the differentiation of midbrain dopaminergic neurons. We found that the midbrain of mice lacking Dll1 developed properly just before neurogenesis started. At E10.5 in the ventral region, the number and distribution of cells expressing the dopaminergic specification genes Foxa2 and Lmx1a was similar to wild-type embryos, but at E11.5 the number of Foxa2+/Lmx1a+

cells decreased without an apparent change in distribution. As expected for premature neurogenesis, the number of proliferating cells decreased and neuronal markers were detected earlier in the midbrains of embryos lacking Dll1 or with reduced Notch signaling. In a neurosphere assay, neurogenesis and dopaminergic differentiation of ventral precursor cells were more efficient when they were derived from Dll1 null than from wild-type midbrain, suggesting that premature neurogenesis prevents the characteristic loss of neurogenic and specific differentiation potential of neurosphere cells. Dopaminergic differentiation, as measured by the expression of the gene encoding tyrosine hydroxylase, emerge as neural precursor cells and become neurons in both wild-type and embryos lacking Dll1. Interestingly, Dll1+/- showed altered dopaminergic neurogenesis, but modifications in the phenotype are no apparent during adult life. We propose that neurogenesis and dopaminergic differentiation are processes coupled by Notch signaling during normal development. Supported by CONACyT 50956/131031.

Program/Abstract # 473

How Neural Cells Acquire an Identity: Role of Calcium Signaling and Voltage-Gated Calcium Channels in Neuronal Phenotype Specification

Schleifer, Lindsay (Coll of William & Mary, USA); Lewis, Brittany B. (Cornell Med Coll, USA); Ng-Sui-Hing, Albert; Anastas, Vollter; Saha, Margaret S. (Coll of William & Mary, USA)

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

Program/Abstract # 474

The role of calcium activity in neuronal phenotype specification

Herbst, Wendy A.; Rabe, Brian A.; Saha, Margaret S. (Coll of William & Mary, USA)

While spontaneous calcium activity has been implicated in neural phenotype specification and differentiation, little is known about the underlying mechanisms regulating this activity in early development. In order to understand both the role of calcium activity in neuronal phenotype acquisition and the mechanisms mediating calcium activity, we pursued two approaches. First we employed a candidate gene approach, selecting the voltage-gated calcium channels for analysis because of their early neural expression and their ability to mediate large influxes of calcium ions into the cell. Antisense morpholino oligonucleotides (MO) were employed to knock down a specific calcium channel, Cav2.1. Cav2.1-MO embryos displayed a down-regulation of glutamic acid decarboxylase mRNA, a marker of inhibitory neurons. Analysis of calcium spiking in dissociated neural tissue indicated that Cav2.1-MO embryos showed a reduction in calcium activity. The second approach was to examine the phenotype, calcium activity, and to correlate it with the expression of specific genes. Calcium activity was imaged in vivo during neurula stages in *Xenopus* embryos using a genetically encoded calcium indicator, GCaMP6m. The neural plate of *Xenopus* embryos was imaged with confocal microscopy and subsequently analyzed using in situ hybridization, probing for calcium-related genes and neural markers. Calcium activity and gene expression were then correlated on an individual cell basis. Preliminary results have captured the dynamic patterns of calcium activity, including calcium spikes and waves.

Program/Abstract # 475

Loss of plasticity of neural precursor cells of the mesencephalon in culture: influence of fibroblast growth factor 2

Landgrave-Gómez, Jorge; Guerrero, Gilda; García, Celina; Pérez-Estrada, José Raúl (UNAM-Cuernavaca, Mexico); Maya-Espinoza, Guadalupe; Guerra-Crespo, Magdalena (UNAM- México city, Mexico); Covarrubias, Luis (UNAM-Cuernavaca, Mexico)

During development differentiating cells change their plasticity as a result of their interaction with environmental cues present in the embryo. How these cues are interpreted by cells such that differentiation potential restrictions contribute to define diverse lineages is a central question in developmental biology. Although cells with neurogenic potential can be generated and maintained in culture, their differentiation potential it has been difficult to evaluate. For example neural precursor cells (NPCs) cultured as neurospheres in the presence of EGF or as monolayer in the presence of Fibroblast growth factor (Fgf2) can generate neurons but a bias to differentiate specially into astrocytes. We have determined a marked reduction in neurogenic potential of putative NPCs in culture, grown as neurospheres or as monolayer, when were implanted into the neurogenic embryonic or adult brain tissue. Whether this change in plasticity is an intrinsic property of NPCs in culture (e.g., due to lack of conditions to retain their epigenetic status) or caused by

growth factors used to keep them alive and dividing is not known. We focus in determining the role of Fgf2 in this change in plasticity in culture. We found that even at the lowest dose required for survival and proliferation, change in the differentiation potential is noted. This effect was accelerated at higher doses. Interestingly, under inhibition of the PI3K signaling pathway, Fgf2 promoted neuronal differentiation of cultured NPCs, whereas astrocytes were obtained if, instead, MAPK pathway is blocked. Therefore, a single growth factor may activate conflicting signals causing the loss of NPCs original properties and unpredictable differentiation outcome. Supported by CONACyT 50956 and 131031

Program/Abstract # 476
Sensory diversity in the olfactory system: left-right neuronal asymmetry
Chuang, Chiou-Fen Chuang (Cincinnati Children's Research Foundation, USA)
Left-right asymmetry is an important aspect of normal brain development and function in organisms from worms to humans. Reduced and reversed brain asymmetry has been linked to a variety of neurodevelopmental disorders including autism and dyslexia. However, the mechanisms underlying lateralization of the developing nervous system are not completely understood. One way to generate brain asymmetry is to specify different fates and functions of individual cell types across the left-right axis. The *C. elegans* left and right AWC olfactory neurons differ in their expression of chemosensory receptor genes and in their functions. We previously showed that cell-cell communication between AWC neurons and non-AWC neurons through a transient, embryonic gap junction network (which consist of both AWCs and ~34 other neurons) establishes stochastic left-right AWC asymmetry, which is maintained throughout the life of an animal. I will present our recent findings that provide additional insights into the molecular mechanisms that specify AWC asymmetry. As mutations in the human homologs of the genes we study are associated with a variety of developmental disorders, understanding normal functions of these genes in mechanistic detail will inform novel therapeutic strategies.

Program/Abstract # 477
Mechanism of myelin basic protein mRNA localization
Meireles, Ana; Talbot, William (Stanford, USA)
Myelin is a specialized sheath that allows axons in the vertebrate nervous system to rapidly conduct action potentials. Myelin is disrupted in Multiple Sclerosis and other debilitating diseases, underscoring the importance of myelin in human health. In the brain and spinal cord, oligodendrocytes extend processes to multiple axons and form myelin around many axonal segments. In addition to specialized lipids and myelin proteins, oligodendrocyte myelin also contains mRNAs encoding Myelin Basic Protein (MBP) and a few other proteins. Although it is striking that these mRNAs are specifically localized in myelin, the mechanism and function of the RNA localization process are not well understood. We are working to test the hypothesis that mbp mRNA is localized to myelin to prevent MBP protein from accumulating in the oligodendrocyte cell body, where it might disrupt other cellular compartments by triggering ectopic membrane compaction. As a first step, we have developed a transgenic assay system to define sequences in the mbp mRNA that are sufficient to localize a heterologous RNA to myelin in oligodendroyctes in vivo. In preliminary studies, we have examined localization of 11 chimeric RNAs containing the coding sequence of mCherry and different segments of the mbp RNA. These experiments have defined a sequence of approximately 400 nt from the mbp 3' UTR that is sufficient for myelin localization. We are working to analyze TALEN-induced deletions in the mbp 3'UTR to identify essential localization sequences, and also to examine the effects of expression of mislocalized mbp mRNAs in vivo.

Program/Abstract # 478
Chemical and genetic screening for factors that regulate myelination in zebrafish
Petersen, Sarah C.; Monk, Kelly R. (Washington U, USA)
Myelin is a multilayered membrane that ensheathes axons and is crucial for neuronal function and survival. Although myelin is an essential component of vertebrate nervous systems, the mechanisms governing its development are not completely understood. A forward mutagenesis screen designed to reveal factors necessary for myelination uncovered *gpr126*. In these mutants, Schwann cells migrate and encompass peripheral axons but fail to wrap the axon (Monk *et al*. 2009, Monk *et al*., 2011). *gpr126* encodes an adhesion G protein-coupled receptor (ad-GPCR) which are purported to have dual roles in cell-cell or cell-matrix interactions and signal transduction. However, most ad-GPCRs, including Gpr126, are undercharacterized and orphaned. We are dissecting the role of Gpr126 in peripheral myelination with genetic and chemical screens utilizing a hypomorphic, point-mutant allele of *gpr126* that has reduced peripheral myelin in zebrafish larvae. With the aid of a transgenic fluorescent myelin marker, we are screening for mutations and small molecules that enhance or suppress the hypomorphic *gpr126* phenotype. Our pilot genetic screen has revealed two potential mutants that interact with *gpr126*: one, a candidate suppressor mutation that restores myelination, and two, a candidate enhancer mutation that results in hypomyelination in *gpr126* heterozygotes. Additionally, we have found multiple suppressor chemicals that promote myelination in *gpr126* hypomorphs. These represent potential exogenous ligands and could serve as therapeutic compounds for peripheral myelinopathies. Current studies are focused on identifying the genetic lesions that interact with *gpr126* and characterizing the small molecules that promote Gpr126 function.

Program/Abstract # 479
RPE specification is mediated by surface ectoderm-derived BMP and Wnt signalling in the chick
Steinfeld, Jörg, (Technische Universität Darmstadt, Germany); Steinfeld, Ichie (Nara Women's University, Japan); Coronato, Nicola;

Layer, Paul G. (Technische Universität Darmstadt, Germany); Araki, Masasuke (Nara Women's University, Japan); Vogel-Höpker, Astrid (Technische Universität Darmstad, Germany)
The retinal pigment epithelium (RPE) is a single-layered, pigmented tissue that is indespensable for vertebrate eye development and vision. According to a classical model of optic vesicle patterning, the surface ectoderm produces fibroblast growth factors (FGFs) that specify the neuroretina (NR) distally, while members of the TGF-β family released from the proximally located mesenchyme are involved in RPE specification. However, based on our expression study, we previously proposed that BMPs released from the surface ectoderm are essential for RPE specification in the chick. Here, we confirm and extent our previous study and show that the *Bmp*-expressing surface ectoderm is required for RPE specification. Furthermore, we show that, besides BMPs, Wnt signalling from the overlying surface ectoderm is involved in restricting RPE specification to the dorsal optic vesicle. Initially, *Wnt2b* is expressed in the dorsal surface ectoderm and subsequently in dorsal optic vesicle cells. Activation of the Wnt signalling pathway by either implanting Wnt3a-soaked beads or by inhibiting GSK3β at optic vesicle stages completely inhibits NR development and converts the entire optic vesicle into RPE. Surface ectoderm removal or inhibition of Wnt signalling at optic vesicle stages prevents pigmentation and results in downregulation of the RPE regulatory gene, *Microphthalmia-associated transcription factor (Mitf)*. Activation of the BMP or Wnt signalling pathway can replace the surface ectoderm to rescue MITF protein expression in optic vesicle cells. We provide further evidence that BMPs and Wnts cooperate via a β-catenin-independent pathway to ensure RPE specification in dorsal optic vesicle cells. Based on these results, we propose a dorso-ventral model of optic vesicle patterning, whereby initially surface ectoderm-derived Wnt signalling directs dorsal optic vesicle cells to develop into RPE through a stabilising effect of BMP signalling.

Program/Abstract # 480
The role of BMPs in chick Neural Retina development
Coronato, Nicola; Steinfeld, Jörg; Steinfeld, Ichie; Layer, Paul G.; Vogel-Höpker, Astrid (Technische Universität Darmstadt, Germany)
Little is known about the molecular mechanisms that lead to neuroretina (NR) specification in optic vesicle cells. During vertebrate eye development, FGFs appear to be released from the surface ectoderm to induce NR development in the underlying neuroepithelium of the optic vesicle. Previous studies also implicated a role of BMPs in NR specification. In those studies, inhibition of the BMP signalling pathway in mutant mouse embryos prevented the initiation of *Vsx2* expression, the earliest known marker of NR precursor cells. We have previously shown that BMPs are expressed in the surface ectoderm at the right time and place to be involved in NR induction in the chick. Consistent with this, we show that BMP application at optic cup stages results in the transdifferentiation of the retinal pigment epithelium into a multilayered NR. Currently, we are carrying out gain- and loss-of-function studies at optic vesicle stages to bring more insights into the molecular mechanisms that lead to NR specification in chick optic vesicle cells.

Program/Abstract # 481
Prdm1a directly activates foxd3 and tfap2a during zebrafish neural crest specification
Powell, Davalyn R.; Hernandez, Laura; Lamonica, Kristi; Artinger, Kristin (U CO-Denver, USA)
The neural crest comprises multipotent precursor cells that are induced at the neural plate border by a series of complex signaling and genetic interactions. Several transcription factors, termed 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 that the PR-SET domain containing transcription factor Prdm1a is co-expressed with two essential neural crest specifiers, *foxd3* and *tfap2a*, at the neural plate border. Through rescue experiments, chromatin-immunoprecipitation (ChIP), and reporter constructs, we have determined that Prdm1a directly binds to and transcriptionally activates enhancers for *foxd3* and *tfap2a* and that they are functional, direct targets of Prdm1a at the neural plate border. Additionally, dominant-activator and dominant-repressor Prdm1a constructs suggest that Prdm1a is required both as a transcriptional activator and transcriptional repressor for neural crest development to occur. These studies demonstrate that Prdm1a is a crucial transcriptional regulator of neural crest specification in zebrafish.

Program/Abstract # 482
***tfap2e* is required for Neural Crest Migration and Neuronal Differentiation**
Ruiz, Sofia; Lobanova, Anastasia; Eisen, Michael; Harland, Richard (UC Berkeley, USA)
The neural crest is a vertebrate embryonic structure that gives rise to sensory neurons, pigmented cells, bone and cranial cartilage, among other cell types. *tfap2e* is a member of the TFAP2 family of transcription factors that is exclusively expressed in the neural crest (NC) of *Xenopus* embryos. *tfap2e* is expressed during early neurulation in pre-migratory and migratory neural crest; once neurulation is completed its expression restricts to the trunk neural crest and to specific domains of the mid-, hind- and forebrain regions. Despite the low abundance of *tfap2e* transcripts, compared to the other four gene family members, its loss of function impairs cranial neural crest migration resulting in defects in craniofacial morphology in older tadpoles. *tfap2e* loss-of-function affects the ability of tadpoles to respond to touch; this phenotype is commonly associated with a decreased number of Rohon-Beard sensory neurons and a reduction of *tlx3* expression, a key determinant of this type of sensory neurons. Contrarily, *tfpa2e* gain-of-function exhibits ectopic *tlx3* expression and supernumerary RB neurons. Additionally, a conserved TFAP2 binding site is found in the enhancer region of *tlx3* gene, therefore *tfap2e* may mediate sensory neuron specification through direct transcriptional regulation of *tlx3*. We propose that during early stages of neural crest specification *tfpa2e* has redundant functions with other TFAP2 members but

it is still required to promote migration; however, later its functions is critical for the differentiation of the trunk neural crest derivatives, in particular Rohon-Beard neurons.

Program/Abstract # 483

Further evidence that Id-proteins act through E-proteins: the Id-protein Extramacrochaetae regulates R7 cell fate through the E-protein Daughterless in the *Drosophila* eye

Baker, Nicholas E.; Bhattacharya, Abhishek (Albert Einstein Coll Med, USA)

Our recent studies of Extramacrochaetae (Emc), an HLH protein that is the sole *Drosophila* representative of the Id gene family, showed that a major role of Emc was to prevent daughterless (da), the sole *Drosophila* representative of the E-protein family of bHLH genes, from autoregulating to high levels (Cell 147: 881-892, 2011). This seems to be a major part of preventing inappropriate differentiation in *Drosophila* tissues, and of promoting differentiation at the proper times and places. Aspects of the same regulation are seen in mammalian cells. The emc gene is also required for proper cell fate specification within the *Drosophila* eye. Without emc, R7 cells develop as R1/6-like cells, and there are delays and deficits in differentiation of non-neuronal cone cells. These cell fates also depend on Notch signaling, and previous studies indicated that emc was required for proper Notch signaling in these cells and for expression of Notch target genes in the Enhancer-of-split Complex. Despite the fact that none of these cell types normally depend on daughterless, we found that the effects of extramacrochaetae mutations were reverted in the absence of daughterless, so that R7 cells and cone cells differentiated with apparently normal frequency and timing within clones of cells lacking both daughterless and emc, and normal expression of the Notch target genes in the Enhancer-of-split Complex proteins was restored. These findings establish that unchecked daughterless expression interferes with Notch signaling and precludes proper fate specification, and that restraining daughterless expression and function is the crucial function of emc during R7 and cone cell development, just like the other tissues we have studied, supporting the model that modulating E protein expression and function is the major function of Id proteins.

Program/Abstract # 484

EYA1/SIX1 drive neuronal developmental program in cooperation with the SWI/SNF chromatin-remodeling complex and SOX2 in the mammalian inner ear

Xu, Pin-Xian; Ahmed, Mohi; Xu, Jinshu (Mount Sinai Sch Med, USA)

Inner ear neurogenesis depends upon the function of proneural basic helix-loop-helix (bHLH) transcription factors NEUROG1 and NEUROD1. However, the transcriptional regulation of these factors is unknown. Using loss- and gain-of-function models, we show that Eya1/Six1 are critical otic neuronal determination factors upstream of *Neurog1*/*Neurod1*. In mice lacking both *Eya1*/*Six1*, *Neurog1/Neurod1* are not expressed. Overexpression of both *Eya1/Six1* is sufficient to convert nonneuronal epithelial cells within the otocyst and cochlea as well as the 3T3 fibroblast cells into neurons. Strikingly, all the ectopic neurons express not only *Neurog1*/*Neurod1* but also mature neuronal markers such as neurofilament, indicating that *Eya1/Six1* function upstream of and in the same pathway as *Neurog1-Neurod1* to not only induce neuronal fate but also regulate their differentiation. We demonstrate that EYA1/SIX1 directly interact with the SWI/SNF chromatin-remodeling subunits BRG1 and BAF170 to cooperatively drive neurogenesis in 3T3 cells and cochlear nonsensory epithelial cells, and that SOX2 cooperates with these factors to mediate neuronal differentiation. Importantly, we show that the ATPase BRG1 activity is required for not only EYA1/SIX1-induced ectopic neurogenesis but also normal neurogenesis in the otocyst. These findings indicate that EYA1/SIX1 are key transcription factors in initiating the neuronal developmental program likely by recruiting and interacting with the SWI/SNF chromatin-remodeling complex to specifically mediate *Neurog1-Neurod1* transcription.

Program/Abstract # 485

Localization of Yap1 protein during blastocyst formation in the lab opossum

Spindler, Troy; Cruz, Yolanda (Oberlin College, USA)

Expression of transcription factor Cdx2 signals the specification of trophoblast in both eutherian (lab mouse) and metatherian (lab opossum) blastocysts. In the mouse, this event depends on the simultaneous nuclear presence of Yap1 and TEAD4, known co-activators of Cdx2. It has been shown that entry of Yap1 into the nucleus in the 8-16 cell stage is the proximate cause to the stabilization of Cdx2 expression, while TEAD4 is constitutively present in the nucleus in mice. This suggests that the trophoblast specification is ultimately guided by cell-cell contact through the Hippo signaling pathway (Niskioka et al., 2009). Using immunofluorescent staining and confocal microscopy, we explored the possibility that trophoblast differentiation is likewise dependent on the co-localization of Yap1 and Tead4 in the putative trophoblast nuclei of lab opossum embryos. We found that, in the opossums, Yap1 is constitutively expressed in the nuclei of all blastomeres, contrasting with its dynamic expression in mice. This indicates that the stabilization of Cdx2 expression and trophoblast differentiation is likely due to a different signaling pathway in opossums.

Program/Abstract # 486

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

Cockburn, Katie; Biechele, Steffen (Uof Toronto, Canada); Garner, Jodi (Sickkids Research Inst, Canada); Rossant, Janet (U of Toronto, Canada)

The first two lineages to segregate during mouse preimplantation development are the trophectoderm (TE) and the inner cell mass (ICM), which form from outside and inside cell populations of the embryo, respectively. Recent work has shown that the downstream Hippo signaling components Lats 1 and 2 (Lats1/2) and Yap play a role in the specification of these two lineages: in outside cells, Yap localizes to the nucleus and induces expression of the TE-specifying transcription factor Cdx2, while in inside cells it is phosphorylated by Lats1/2 and retained in the cytoplasm. The factors acting upstream of Lats1/2 and Yap in this context have not yet been identified. Here, we investigate the role of the upstream Hippo scaffold protein Nf2 in TE/ICM specification. Because Nf2 is maternally deposited, we have taken two approaches to entirely eliminate it from the early embryo: microinjection of a dominant negative form of Nf2 (dnNf2) and generation of maternal/zygotic *Nf2* (*Nf2*$^{m-/z-}$) mutants. In both cases, loss of function leads to increased Yap nuclear accumulation, decreased Yap phosphorylation and increased Cdx2 expression in inside cells, demonstrating a requirement for Nf2 upstream of Lats1/2 and Yap in the early embryo. Moreover, in addition to ectopic Cdx2, inside cells of *Nf2*$^{m-/z-}$ blastocysts are morphologically abnormal and express other TE markers while failing to express typical ICM markers. Consistent with a failure to establish an ICM, these mutants fail to generate embryonic stem cells *in vitro* and do not survive beyond implantation *in vivo*. Together, this data establishes a crucial role for Nf2 in preimplantation Hippo signaling and in segregating the ICM from the TE.

Program/Abstract # 487

The biology of avian macrophages and their function in development

Garcia Morales, Carla; Balic, Adam; Garceau, Valerie; Sang, Helen; Hume, David (Roslin Inst. UK)

Macrophages (Mφ) have been ascribed many roles during embryogenesis. They function during tissue remodelling, clearance of apoptotic cells, wound healing, tissue regeneration, inflammation and antigen presentation. In mammals and birds the first Mφ appear in the yolk sac, migrate into the embryo and infiltrate the head before the formation of vasculature. Subsequently monocytopoiesis is initiated in the aorta-gonad-mesonephros, and later in the foetal liver, before moving to the bone marrow (BM). The production of Mφ from BM progenitor cells in mammals is controlled by colony stimulator factor 1 (CSF1) which signals through the CSF1R. Expression of the csf1r mRNA is restricted to myeloid cells and their precursors. A recent study identified a second ligand for CSF1R, IL34. The importance of Mφ in development is highlighted by the abnormalities seen in CSF1 deficient mice and rats, such as low birth weight, growth retardation, osteopetrosis, sensory deficiencies and infertility. The chick provides a unique model in which to monitor Mφ roles in development in vivo . Both CSF1 and IL34 are present in chick and can promote Mφ differentiation from BM precursors as reported (Garceau et al., 2010). We have generated a Mφ-specific transgenic reporter line based upon CSF1R promoter elements and monoclonal antibodies against CSF1R. Spatio-temporal expression of CSF1R, IL34 and CSF1 by ISH in the chicken embryo revealed the appearance of CSF1R positive cells after the establishment of the circulation followed by rapid colonization of the whole embryo by stage H&H18. We are currently investigating the location of CSF1 receptor signalling in vivo using transgenic embryos.

Program/Abstract # 488

Fgf and Bmp signaling in the third pharyngeal pouch may inhibit Shh signals during development

O'Neil, John; Bain, Virginia; Manley, Nancy (UGA- Athens, USA)

The third pharyngeal pouch is an endoderm-derived epithelium that forms the thymus and parathyroid in the mouse. Little is currently known about the mechanism by which pouch epithelial cells are specified to thymus or parathyroid fate. Genetic manipulation of the Shh pathway suggests a necessary role for Shh signaling in parathyroid cell fate specification, as the Shh null mutation causes absence of parathyroid and expanded thymus fate. However, constitutive Shh signaling in the endoderm restricts thymus fate, but is not sufficient to expand the parathyroid domain. There is evidence that Bmp and Fgf signaling may promote thymus fate and proliferation. Additionally, in wild-type mice proliferation is increased within the thymus domain compared to the parathyroid domain early in pouch development. We postulate that Bmp and Fgf signaling may function to inhibit Shh signaling in the ventral pouch, either directly by inhibiting Shh or indirectly by reducing the period of time that the cells are receptive to Shh signal. We have developed a serum-free *ex vivo* tissue culture system in which tissue explants can be treated with signaling agonists and antagonists as well as CDK inhibitors. Early data suggests that cell fate is plastic within these cells and can be influenced by agonists and antagonists of the Shh and Bmp pathways as late as the 35 somite stage. We are using this system to elucidate the mechanism by which Shh, Bmp, and Fgf signals function to determine cell fate within the pouch, and may identify a novel mechanism by which Shh receptiveness can be modulated by cells during development.

Program/Abstract # 489

New structural characteristics and segregated cell components revealed in sections of sea urchin eggs and embryos by antibodies to density gradient fractions from fertilized sea urchin eggs and to sperm vesicles

Sparling, Mary L. (Cal State-Northridge, USA)

Tagging cytoplasmic components with antibodies locates them at fertilization and during cleavage and can reveal their redistribution and segregation regionally into an/veg positions during the development of cell polarity and embryo axes and into distinct cells developing from those regions. The antibodies were made against membranous components unspecified except for 4 different densities as isolated from fertilized eggs and for ethanol solubility from sperm vesicles. Each has unique polarized characteristic staining and localization of structures not previously seen in embryos during cleavage: fine granules which stain very darkly, lattices in the cortical region, vesicles stained in outline resembling popcorn in the cortical region, filaments with granules along them, stains

on the outside of small clear vesicles, granules along clear unstained tubules, donut shaped granules or lightly stained rippled cortex. The segregation of stain during development suggests a set of different density special vesicles laid down in the fertilized egg with special cohorts of signaling proteins and associated targeting machinery and adapters which each have different targets from the others in the group as they attach to different motors and cytoplasmic filaments in orderly steps of sorting with enough to last during cleavage. If the antibodies were made against the cytoskeleton or the motors they would stain every single cell along with antibodies to mitochondria, ribosomes, cilia, golgi, ER and the intrinsic membrane proteins. None of the antibodies stain the dorsal ectoderm but all stain some parts of the oral ectoderm and the gut at the pluteus stage. Thus are revealed the evolving cytoplasmic transport machinery with components from fertilized eggs participating as engines of change in early development.

Program/Abstract # 490

The hox gene lin-39 controls cell cycle progression during *C. elegans* vulval development

Roiz Lafuente, Daniel; Leu, Philipp; Hajnal, Alex (U of Zurich, Switzerland)

lin-39 hox plays crucial roles during vulval development. In L1 and L2 larvae, *lin-39* defines the vulval precursor cells (VPCs, P3.p through P8.p) by maintaining them as polarized epithelial cells. In this phase, LIN-39 represses the expression of the fusiogen *eff-1*. After the vulval cell fates have been specified by the inductive anchor cell and lateral NOTCH signals, LIN-39 is essential for the execution of the vulval cell fates during the L3 and L4 stages. Interestingly, in *lin-39(lf); eff-1(lf)* double mutants, the VPCs remain as polarized epithelial cells that start invaginating, but they do not proliferate. We are thus, investigating how LIN-39 promotes VPC proliferation. By inspecting the CHiP-seq data generated in the modENCODE project, we identified several cell cycle regulators as candidate LIN-39 targets and performed a targeted RNAi screen for genes inhibiting VPC proliferation in the *lin-39(lf); eff-1(lf)* background. Based on our results, we present the following model: LIN-39 promotes cell cycle progression by repressing *cdc-14* expression, which in turn leads to the degradation of the CDK/cyclin inhibitor CKI-1 and thus relieves the G1-arrest imposed by CKI-1. Taken together, LIN-39 HOX may directly control cell cycle progression in addition to its functions during VPC specification and fate execution.

Program/Abstract # 491

The alternative splicing regulator tra2b is required for proper somitogenesis in Xenopus, and regulates splicing of a novel wnt11b splice form

Dichmann, Darwin; Harland, Richard (UC-Berkeley, USA)

Alternative splicing is rare in simple animals but common in higher vertebrates where most genes have distinct spliceforms. It has been proposed that the resulting proteomic complexity is necessary for the development and function of higher vertebrates. Alternative splicing is regulated by splice regulatory proteins that interact with, and guide, the core spliceosome to generate different isoforms from individual genes. tra2b is a serine-arginine rich RNA-binding protein involved in splice site selection. We previously isolated tra2b and several other RNA-binding proteins in an expression-cloning screen for activities that perturb normal frog development. Here, we report that morpholino-mediated knockdown of *tra2b* results in pleiotropic defects in all germ layers, including mesoderm maturation, causing failure to form somites. We use RNA-Seq to detect changes in gene expression and isoform use. By this method we identify a striking increase in intron retention in *wnt11b* that results in a transcript encoding a truncated protein, which likely encodes a dominant-negative isoform. Using a splice blocking morpholino against the donor splice site flanking the retained intron in *wnt11b* , we mimic the somitogenesis defect, but not other defects in *tra2b* morphants, indicating that those are likely caused by other splice changes.

Program/Abstract # 492

Examination of Physiology and Gene Expression in Great Vessels of Differing Embryonic Origin

Pfaltzgraff, Elise R. (Vanderbilt, USA)

Vascular smooth muscle cells throughout the body are derived from different embryonic origins. Vessels originating from differing smooth muscle cell populations are known to have distinct vascular and pathological properties of calcification, atherosclerosis, and structural defects like aneurysms and coarctations. Thus, we hypothesized a link between embryonic origin of vascular smooth muscle cells and physiological characteristics of the vessels to which they give rise. Microarray analysis revealed that specific vessels of differing embryonic origin have distinct gene expression profiles in the adult. This was not the case in the ascending and descending aorta, which have different embryonic origins. Embryonically, these two regions of the aorta have distinct gene expression profiles; however, these differences are lost in adulthood. Myographic analyses demonstrated increased contractility of the embryonic descending aorta compared to the ascending aorta, but equivalent contractility in adults. Isolated embryonic aortic smooth muscle cells maintained characteristics indicative of their origin in the vessel while adult smooth muscle cells were homogeneous regardless of their position in the aorta. Our data describes both gene expression and physiological patterns in vasculature of distinct embryonic origin. These data suggest that while some vessels with smooth muscle cells from different embryonic origins are distinct in the adult mouse, the ascending and descending aorta lose embryonic differences during development and are homogenous in a healthy adult animal. These findings have implications for how we think of development and disease in this vessel.

Program/Abstract # 493

A network responsible for lineage segregation of lateral dermomyotome progenitors into myotomal and vascular fates

Applebaum, Mordechai (The Hebrew Univ of Jerusalem, Israel) Ben-Yair, Raz (Massachusetts General Hospital, USA); Chaya, Kalcheim (The Hebrew Univ of Jerusalem, Israel)

Lineage diversification from an apparently homogenous epithelium is a central event in development though the mechanisms underlying such processes are only slowly being discovered. To this end we implemented the avian dermomyotome (DM) as a model for studying lineage segregation. The DM is the dorsal remnant of the somite after the dissociation of its ventral aspect into the sclerotome. The DM primarily generates skeletal muscle and dermis and its lateral domain also gives rise to endothelium and vascular smooth muscle (vSM) of adjacent blood vessels. Focusing on the lateral DM domain, we uncovered a molecular network underlying myotomal (skeletal muscle) vs. vascular (endothelial and vSM) fates. We found that *Id2*, *Id3*, *FoxC2* and *Snail1* are enriched in the lateral DM and promote vascular at the expense of myotomal fates. These factors, together with *Pax7* and *Myf5*, constitute a regulatory network. Moreover, Notch signaling, previously shown to promote vascular fate, both regulates and is controlled by the network components. In this context, we found that Notch has a biphasic activity. Short exposure periods induce cells to enter the myotome, yet inhibit terminal differentiation into myofibers while maintaining Pax7 expression. Long exposure periods initiate a Snail1-dependent epithelial-to-mesenchyme transition thus promoting the vascular fate at target sites. Thus, our data illustrate a gene regulatory network through which the lateral DM segregates into its derivatives.

Program/Abstract # 494

The Notch pathway promotes vascular cell fates of multipotent Pax3+ progenitors, in the somite

Mayeuf, Alicia; Lagha, Mounia; Danckaert, Anne; Relaix, Frédéric (Inst Pasteur, France); Vincent, Stéphane (IGBMC, France); Buckingham, Margaret (Inst Pasteur, France)

Multipotent Pax3-positive cells in the somites give rise to skeletal muscle and to cells of the vasculature. We had proposed that this cell fate choice depends on the equilibrium between *Pax3* and *Foxc2*. We now focus on somite derivatives that migrate to the limb, which we characterise as Pax3-independent endothelial cells and Pax3-dependant myogenic progenitors. Using a conditional $Pax3^{NICD}$ allele, which leads to overactivation of Notch targets, we show a decrease in myogenic progenitors, with a corresponding increase in vascular endothelial cells. The resulting deficit in myogenesis is compensated by a Notch-induced delay in muscle differentiation, which is strikingly compensated during development when *Pax3* is down-regulated. Based on *in vivo* manipulation of the timing of *NICD* expression and on somite explant experiments, we show that Notch signalling affects the *Pax3/Foxc2* balance and promotes vascular cell fates, prior to migration to the limb, in multipotent Pax3-positive cells in the somite of the mouse embryo.

Program/Abstract # 495

Delineating the early molecular steps required for cardiac progenitor development in the zebrafish embryo.

Deshwar, Ashish R. (U of Toronto, Canada, Scott, Ian (Hosp for Sick Children, Canada)

The earliest molecular steps required for cardiac progenitor development remain unclear. Our lab has previously shown that in the zebrafish embryo Gata5 and Smarcd3b are key players in this process, able to direct cells to the anterior lateral plate mesoderm and drive development into all three major cardiac lineages. The signals that lie both up and downstream of these two genes remain relatively un-characterized. Through the use of a conditional version of Gata5, it was found that it is required early in gastrulation to drive cells to the heart. The study of several candidate genes at this same time point has revealed novel upstream regulators of Gata5, possibly revealing a genetic hierarchy required for its activation. To identify downstream targets transcriptional profiling of Gata5/Smarcd3b over-expressing embryos was performed and clusters of genes implicated in cell adhesion and migration were found to be up-regulated. Further study of these genes may reveal novel regulators of cardiac development.

Program/Abstract # 496

Crosstalk between cell adhesion and cell fate specification during zebrafish gastrulation

Barone, Vanessa; Heisenberg, Carl-Philipp (Institute of Science and Technology, Austria)

During gastrulation blastoderm cells, forming an initially homogeneous population, differentiate and segregate into the three germ layers: ectoderm, mesoderm and endoderm. Cell fate specification leads to differences in the adhesive properties between the progenitor cell types, driving their segregation into the germ layers. However, much less is known about potential feedbacks of cell adhesion on progenitor cell fate specification. To address such feedbacks, we study the crosstalk between progenitor cell fate specification and cell adhesion. We monitor cell fate using zebrafish transgenic lines expressing GFP in specific germ layer progenitor types, and characterize cell adhesion employing a Dual Pipette Assay. Specifically, we analyze the role of cell adhesion in the specification of mesendoderm cell fates. Our results show that cell adhesion does not affect the specification of early pan-mesendoderm progenitors, but instead is required for the maintenance of axial mesoderm (prechordal plate) progenitors. This could be due to signaling directly from cell-cell adhesion complexes, and/or paracrine Nodal/TGFbeta signaling between adhering cells, previously suggested to be essential for mesendoderm cell fate specification. To distinguish between these two possibilities, we interfered with progenitor cell-cell adhesion using a neutralizing E-cadherin antibody, and with Nodal/TGFbeta signaling using Nodal-receptor inhibitors. Our preliminary results show that both cell-cell adhesion and Nodal-signaling are required for prechordal plate cell fate maintenance, suggesting a cooperative effect of cell adhesion and Nodal-signaling. Our future work will elucidate the molecular and cellular mechanisms underlying this process.

Program/Abstract # 497

mef2ca controls the choice to make ligaments versus bones

Nichols, Jamie; Kimmel, Charles (U Oregon, United States)

Ligaments are fibrous tissues that bind bones together. Surprisingly, little is known about ligament biology in zebrafish. Moreover, the relatedness of bone cells (osteoblasts) and ligament cells is unknown in any system. Although, in the human disorder Eagle's syndrome ligaments become mineralized, suggesting these cell types may be closely related. We hypothesize bone cells and ligament cells are related in a lineage hierarchy and a transcription factor code governs the progenitor cell 'choice' to make bones versus ligaments. In our hypothesis the most closely related cell types are just 'one gene away' from each other. We propose that the transcription factor encoding gene *mef2ca* is one gene separating osteoblasts and ligament cells. Zebrafish mutant for the transcription factor encoding gene *mef2ca* develop ectopic bone. One dermal bone in particular, the opercle (op), is remarkably expanded in these mutants. Time lapse studies reveal ectopic bone cells arise at restricted locations or 'hotspots' along one edge of the op during early op growth. Our hypothesis predicts these hotspots of ectopic bone are sites where ligaments develop in wild type animals. We employed in situ, antibody, and transgene analysis to reveal the whereabouts and molecular identity of zebrafish larval ligaments. One ligament, which normally attaches to the op in wild types, is absent when ectopic bone develops in *mef2ca* mutants. These results indicate osteoblasts are gained at the expense of ligament cells in *mef2ca* mutants. Further, we discovered that three closely related cell types reside at the ligament-bone interface on the op: ligament cells, osteoblasts, and a ligament-bone hybrid cell type we refer to as connective osteoblasts. We propose that connective osteoblasts make a special region of bone to which the ligament attaches. Our findings suggest a model in which the wild-type *mef2ca* gene controls precursor cell's choice to differentiate into either a ligament- or bone-producing cell.

Program/Abstract # 498

Interplay between chemical and mechanical guidance during collective cell migration

Garcia, Simon (Univ de Barcelona, Spain); Theveneau, Eric; Mayor, Roberto (University College London, UK); Trepat, Xavier (Univ de Barcelona, Spain)

Collective cell migration is a biological process observed both during development and postnatal life. In physiological conditions, collective cell migration is central for proper morphogenesis (1), wound healing, and tissue regeneration (2). Moreover, collective cell migration is important in pathological states such as tumor mass invasion (3). Current understanding emphasizes that collective cell migration is guided by external signals, such as a soluble chemoattractant secreted by surrounding cells. The release of the chemoattractant signal generates a concentration gradient across the tissues, and the responsive cell cluster is able to sense the gradient and finally migrate in the direction of highest concentration (4). Recent findings show that this picture is incomplete, however. Indeed, it has been shown that collective chemotaxis requires intact cell-cell junction (5) but the mechanism remains unknown. Cohesiveness is the result of cell-cell interaction mediated by tight/adherent junction proteins and complexes, located at the membrane of cells in the motile cluster. Some of these proteins have been found to transmit forces and transduce these forces into biomechanical signals (mechanotransduction). Our hypothesis was that force transmission at cell-cell junction is required for sensing of a chemical gradient. To test this hypothesis we used *Xenopus leavis* Neural Crest chemotaxis model (5). Our aim was to study the interplay of cell-cell force transmission in this model during Stromal Derived Factor 1 (SDF1) gradient sensing using a reductionist in vitro system. In this system cell-cell mechanical interactions were measured with a technique called traction-force/stress microscopy that exploits cell compliant polyacrylamide substrates for force/stress mapping. Small (100-200 um) Neural Crest explants were obtained from *X. leavis* embryos and cultured on a compliant substrate in presence of a Placode explant, which is an embryonic tissue known to produce and secrete SDF1. Force transmission through adherent junctions (AJ) was studied by loss-of-function experiments targeting N-Cadherin (the main component of AJ), or acto-myosin contractility. Through these studies we observed a lack in cooperation of traction forces when N-cadherin was down-regulated, and a notable diminishment of intensity of forces when AJs were disrupted by lowering Ca2+ level in the medium. The evidences provided by these experiments strongly deposit in favor of the role of N-Cadherin not only in maintain physical connection but also in spatially coordinate and generally enhance traction force generation. 1. Rørth P. Whence directionality: guidance mechanisms in solitary and collective cell migration. (2011). Dev Cell. 18;20(1):9-18. 2. Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. (2010) Am J Physiol Lung Cell Mol Physiol.;298(6):L715-31. 3. Yilmaz M, Christofori G. Mechanisms of motility in metastasizing cells. (2010) Mol Cancer Res.;8(5):629-42. 4. Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. (2004) Physiol Rev. Jan;84(1):209-38. 5. Theveneau E, Marchant L, Kuriyama S, Gull M, Moepps B, Parsons M, Mayor R. Collective chemotaxis requires contact-dependent cell polarity. (2010) Dev Cell. 2010 Jul 20;19(1):39-53.

Program/Abstract # 499

Genetic and Biophysical Constraints on Collective Cell Motility

Starz-Gaiano, Michelle; Manning, Lathiena; Peercy, Bradford (U Maryland-Baltimore, USA)

For animal development to proceed normally, subsets of cells must acquire the ability to move away from their birthplace. The well-conserved Janus Kinase (JAK) and Signal Transducer and Activator of Transcription (STAT) pathway confers this ability on certain epithelial cells – called border cells - during *Drosophila* oogenesis. JAK/STAT activation is initiated via a diffusible protein, Unpaired (UPD), that is secreted from two cells in the anterior of the egg chamber. This signaling event must be precisely regulated, as too

much or too little activation results in defects in cell migration, revealed by mutant analysis. Cells with high activation levels amplify the signal and turn on the pro-migration transcription factor Slow border cells (SLBO), whereas cells with below-threshold signaling shut it off via another transcription factor, Apontic (APT). We are employing a variety of imaging techniques to observe the initial events in border cell specification and exit from the epithelium, in both wild-type and mutant flies. In addition, we are developing a computational approach to model the spatial constraints on activator diffusion that may contribute to optimizing the number of motile cells. We are also using mathematical models to investigate the minimal forces that contribute to cell rearrangements and coordinated cell-cluster movements. This research will improve our general understanding of collective cell migrations and epithelial-to-motile transitions.

Program/Abstract # 500

TIMP-2 interacts with MT-1 MMP to modulate migration and invasion of MCF-7 cells independent of MMP inhibition

Cepeda, Mario; Willson, Jessica; Nieuwesteeg, Michelle; Damjanovski, Sashko (Western Univ, Canada)

The matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) are the primary regulators of extracellular matrix (ECM) dynamics by allowing for degradation and remodeling of different ECM components. By virtue of their function, MMPs and TIMPS play an important role in the migration and invasion of cells both in vivo and in vitro. The N-terminal domain of TIMP-2 is an excellent inhibitor of MMP-2, -9 and MT-1 (Membrane Type-1) MMP, while the C-terminal domain has been shown to signal inside the cell through cell surface MT-1 MMP and alpha3beta1 integrin. The objective of this study was to examine the how MT-1 MMP alone or in conjunction with TIMP-2 affects the invasion of MCF-7 breast cancers cells and how essential the inhibitory activity of TIMP-2 is to elicit an effect through MT-1 MMP. To do this MCF-7 cells, which are deficient in MT-1 MMP, were transfected with full length or truncated MT-1 MMP, and then treated with serum free media, TIMP-2 or ALA+TIMP-2 conditioned media. ALA+TIMP-2 is a TIMP-2 mutant with a non-functional N-terminal domain, which allows for the isolation of the function of the C-terminal domain of TIMP-2. Overexpression of MT-1 MMP changed the migratory and invasive potential of MCF-7 cells, which was potentiated by addition of TIMP-2 and ALA+TIMP-2 conditioned media. Despite ALA+TIMP-2 not being able to inhibit MMPs, ALA+TIMP-2 is still able to interact with MT-1 MMP and affect cell behaviour independent of changes in MMP activity. This study further highlights that apart from being key regulators of ECM dynamics, MT-1 MMP and TIMP-2 can also regulate cellular functions that are independent of MMP activity.

Program/Abstract # 501

Analysis of the effects of TIMP-1 -2 and -3 N- and C-terminal domain overexpression during early *Xenopus laevis* development using immunohistochemistry

Nieuwesteeg, Michelle; Willson, Jessica; Cepeda, Mario; Damjovski, Sashko (Western Univ, Canada)

Extracellular matrix (ECM) remodeling is accomplished by matrix metalloproteinases (MMPs) which cleave components of the ECM needed for cell migration, while tissue inhibitors of metalloproteinases (TIMPs) are a small family of proteins that inhibit MMPs through their N-terminal domains. Regulation of MMP/TIMP activity is associated with MT1-MMP and RECK, and is essential for neurulation, organogenesis and angiogenesis. Interestingly TIMP C-terminal domains are structurally and functionally distinct from their N-terminal domains. *In vitro* studies demonstrate that the C-terminal domains play roles in regulating signaling pathways including apoptosis, proliferation and migration, however *in vivo* roles remain unclear. This study examined the individual roles of TIMP N- and C-terminal domains *in vivo* as they pertain to early *Xenopus laevis* development. Full-length TIMP-1, -2 or -3 or their individual N- or C-terminal domains were overexpressed in *X. laevis* embryos using RNA microinjection. Tissue sections of embryos at stage 15 (neurulation) or 30 (organogenesis) were examined and compared histologically and immunologically. TIMP N-terminal constructs all resulted in disrupted formation of neural structures. Immunostaining showed changes in localization of TIMPs and other regulators of ECM remodeling including MT1-MMP and RECK following overexpression of each full-length construct. N-terminal domains produced similar expression patterns to their full-length counterparts. In comparison, C-terminal domains resulted in distinct changes in the localization of MT1-MMP and RECK, indicating that this domain may play a role in development which is moderated in the full-length TIMP molecule.

Program/Abstract # 502

Analysis of RECK expression during Xenopus laevis development and its colocalization with MT1-MMP during neurulation

Willson, Jessica; Nieuwesteeg, Michelle; Cepeda, Mario; Damjanovski, Sashko (Western Univ, Canada)

Extracellular matrix (ECM) remodeling is important for the development and maintenance of multicellular organisms. Cleavage of ECM components occurs through the combined activity of matrix metalloproteinases (MMPs) and their inhibitors. Disturbances in the balance of MMPs and their inhibitors is associated with developmental defects, therefore, it is crucial that MMP activity be tightly regulated. Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is a membrane-anchored protein that has been shown to mediate ECM remodeling by inhibiting MMPs. To date, few studies exist examining RECK during embryonic development. The present study focuses on examining the expression pattern of RECK during early *Xenopus laevis* development. Semi-quantitative PCR and whole mount *in situ* hybridization were used to examine *RECK* RNA levels during development. *RECK* RNA levels were low during gastrulation but increased during neurulation and into organogenesis. Furthermore, *RECK* transcripts were localized to neural regions of the developing embryo. Since RECK has been found to inhibit MT1-MMP activity, immunohistochemistry was performed on sections of neurulating embryos to examine colocalization of RECK and MT1-MMP proteins. RECK and MT1-MMP proteins

were colocalized along the epidermis and in the dorsal regions of the neural tube, in agreement with the RECK *in situ* data. Overall, these results suggest that RECK is temporally and spatially restricted during early *Xenopus laevis* development and is colocalized with MT1-MMP during neurulation.

Program/Abstract # 503
Withdrawn

Program/Abstract # 504
The role of Retinoic acid signaling in tectal laminar formation
Kukreja, Shweta (Indian Institute of Technology Kanpur, India)
Retinoic acid (RA) is critical for morphogenesis and differentiation of many developing organs and tissues. In RA reporter mice high RA activity was detected in the developing visual system, i.e. the retina, optic tract, and the retino-recipient structure in the mid brain, superior colliculus(SC). SC is functionally homologous to the tectum in chick which comprises of 16 distinct cellular and fibrous layers, giving it a laminated structure. The development of these laminae occurs as a result of cell proliferation in the neuroepithelium, and three distinct waves of radial migration of differentiated neurons and glia. In accordance with the presence of RA activity in mouse SC, we found the expression of RA synthesizing enzyme ALDH1A2 in a transient lamina in developing chick tectum from embryonic day 6 onwards. Expression of RA degrading enzyme Cyp26B1 was found in the ventricular layer. The onset of expression of RA synthesizing and degrading enzymes coincides with the late migratory wave and with the innervation of tectum by retinal axons. The spatial and temporal expression of source and sink of RA in the tectum suggests that it may have a role in development of the laminar structure of the tectum. In our studies, we have observed that disruption of RA signalling in tectum by virus mediated misexpression of dominant negative receptor RAR alpha at early stages of the chicken embryo leads to thinning of the tectum. We found that this is primarily the result of severe lamination defects as characterized by expression of lamina specific genes. We are further investigating the mechanisms and intermediary players through which these defects occur, and the arborization defects of the RGC axons in the disrupted lamina.

Program/Abstract # 505
The Role of DCC for Mitral Cell Axon Guidance in Zebrafish
Horne, Jack; Sheth, Ruchi (Pace U, USA)
In vertebrates, the majority of olfactory sensory information is transferred to the brain through two basic relays: (i) first, from olfactory sensory neurons through the olfactory nerve to the glomeruli of the olfactory bulb; (ii) second, through the axon projections of mitral cells of the olfactory bulb to multiple higher brain targets in the telencephalon. While much is known about the guidance mechanisms of olfactory sensory axons, relatively little is known about the second relay, the axon guidance of mitral cells. This is an extremely important question as the molecular mechanisms that control this axon guidance process determine how olfactory sensory information is relayed and processed by the brain. Here we begin to characterize these mechanisms by assessing the role of DCC for mitral cell axon guidance through loss-of-function analysis in zebrafish. *In vivo* electroporation, in combination with a gal4 transgenic line, was used to temporally and spatially target DCC loss-of-function. Knockdown of DCC function at 24 hpf using anti-DCC siRNA oligonucletides led to significant alterations in the mitral cell axon projections at 5 to 7 dpf. The three-dimensional structure of the mitral cell axon projection was visualized by confocal imaging of a membrane-localized YFP, which was targeted to mitral cells by co-electroporation with the loss-of-function siRNA oligonucleotides. Embryos targeted with anti-DCC siRNA showed a significant decrease in the number of midline-crossing commissural axons. These results suggest that netrin-DCC plays a role in midline targeting of mitral cell commissural axons.

Program/Abstract # 506
The Role of Heparan Sulphotransferase Enzymes Hs2st and Hs6st1 in Corpus Callosum Development
Clegg, James; Pratt, Thomas (U of Edinburgh, UK)
Heparan sulphate proteoglycans (HSPGs) are complex macromolecules that are found at the cell surface and form part of the extracellular matrix. HSPGs are known to play a crucial role in the modulation of a number of different cell-cell signalling pathways during development; these include the signalling of axon guidance molecules such as Slit/Robo. HSPGs consist of a core protein to which heparan sulphate side chains are added, these side chains are extensively modified by the addition and removal of sulphate groups at specific positions on the carbohydrate ring. It has been proposed that the differential sulphation of these HS side chains may change the ability of cells or axons to respond to particular signalling molecules. This may help explain how a relatively small number of axon guidance molecules are able to guide the enormous number of axons found within the brain. The corpus callosum (CC) is a large axon tract which links the two cerebral hemispheres. Previously we have shown that in mice which lack the HS modifying enzymes Hs2st and Hs6st1 the CC fails to form, demonstrating that the HS sulphation provided by these two enzymes is required for the guidance of callosal axons. It remains unclear however whether this defect is primarily caused by a loss of HS sulphation at the growth cone of the callosal axons or whether the change in sulphation affects the midline environment through which these axons navigate. To answer this question we have used conditional mutagenesis to specifically ablate the expression of Hs2st or Hs6st1 in callosal projection neurons or populations of cells at the telencephalic midline and examined the effect this has on signalling and the development of the CC.

Program/Abstract # 507

Slit1a Promotes Axon-Glial Interations to Facilitate Post-Optic Commissure Formation in Zebrafish

Park, Jin Sook (Smith College, USA)

Bilaterally symmetrical organisms must be able to communicate between the two hemispheres of the brain and body. In order to achieve proper neural connections between the two halves of the central nervous system, axons must navigate across the midline, forming a commissure. It is known that the Slit-Roundabout signaling system plays a crucial guidance mechanism involved in commissure formation. Historically, Slit-Robo signaling has been known to function to repel path-finding axons; however, we propose a new function to this guidance system, in which *slit1a* may promote axon-astroglial interactions to facilitate post-optic commissure (POC) formation in the zebrafish forebrain. We show that the cells of the diencephalic glial bridge express *slit1a*, and global misexpression of *slit1a* causes ectopic wandering POC axons. We and others have shown that crossing by POC axons in the *you-too* (*Gli2DR*) mutant is greatly reduced, which is in part due to expanded midline expression of the known repellents *slit2* and *slit3*. We show here that global misexpression of *slit1a* in the *you-too* (*Gli2DR*) mutant can rescue POC midline crossing. Importantly, POC axons are always associated with astroglial cells even when either is displaced from their normal positions. This suggests POC axons and astroglial cells exhibit positive and potentially necessary interactions for commissure formation. We hypothesize that Slit1a-Robo signaling mediates this positive interaction to facilitate POC axons crossing over the prefigured diencephalic glial bridge. We have demonstrated that loss of *Robo1* function by morpholino knockdown in the context of *slit1a* misexpression is capable of eliminating the association of wandering POC axons with astroglial cells. These results suggest that *slit1a* functions distinctly from *slit2* or *slit3* to positively promote association of POC axons with astroglial cells in a Robo1-dependent manner during commissure formation in the zebrafish forebrain. We are currently testing the role of other Roundabout receptors in mediating axon-astroglial interactions during POC formation.

Program/Abstract # 508

The PCP factor Prickle1b and transcriptional repressor Rest function within facial branchiomotor neurons to regulate their migration during zebrafish development

Love, Crystal E. (U Chicago, USA); Sirotkin, Howard (Stony Brook, USA); Prince, Victoria (U Chicago, USA)

Facial branchiomotor neurons (FBMNs) migrate from rhombomere (r) 4 to r6 in the hindbrain during development. These neurons fail to migrate out of r4 in embryos lacking Prickle1b (Pk1b), a component of the planar cell polarity pathway. Localization of Pk1b to the nucleus of FBMNs is required for their proper migration, which suggests a PCP-independent role of Pk1b in this process. Pk1b has additionally been shown to interact with RE1-silencing transcription factor (Rest), a transcriptional repressor of neuronal maturation genes, and is required for the translocation of Rest to the nucleus in vitro. Rest is depleted from the nucleus of FBMNs in Pk1b-deficient embryos, suggesting Pk1b is similarly important for Rest nuclear localization in these neurons. Consistent with this hypothesis, we find FBMN migration is disrupted in mutant embryos expressing a nonfunctional Rest protein, and this phenotype is enhanced in maternal-zygotic mutant embryos. Using Tol2-mediated transgenesis to either derepress or activate Rest targets specifically within FBMNs, we show that Rest functions within the neurons, rather than in the surrounding neuroepithelium, to mediate migration. Our data also suggest that FBMNs mature too early when Rest function is disrupted in these neurons, including the inappropriate expression of Rest target genes. We propose a model in which Pk1b localizes Rest to the nucleus of FBMNs, with Rest in turn functioning to repress target genes in the FBMNs, and thus maintain their immature, migratory state.

Program/Abstract # 509

Migration of Cajal-Retzius cells in the olfactory region of the developing telencephalon

Frade, Daniela; Varela-Echavarria, Alfredo (UNAM-Queretaro, Mexico)

The mammalian cortex is organized into layers which depend for their organization on the glycoprotein Reelin secreted by Cajal-Retzius cells (CR) located in the outermost layer of the developing telencephalon. CR cells originate mainly in the cortical hem in the dorsal midline of the telencephalon, but are also known or proposed to be generated from other domains. One of the proposed domains is the lateral telencephalic region near the boundary between the pallium and subpallium in the ventral pallium (VP) and another one is located in the rostral region of the rostral cortex. Migration routes of cells in these regions have been little studied. It has been proposed that CR cells originating from the VP migrate to the dorsal cortex while the cells generated in the rostral region are positioned in the rostral cortex. We have analyzed the migration of cells in both regions during the stages where CR cells are being generated. By injecting a fluorescent tracer we observed that labeled cells along the anteroposterior extent of the VP migrate in ventral direction while cells from the rostral domain migrate caudally along the olfactory region and dorsally to the dorsal cortex. Moreover, we have observed that about one fourth of the migrating cells express Reelin. These results reveal novel routes of migration of uncharacterized cells including a Reelin-expressing population in the developing telencephalon.

Program/Abstract # 510

Neogenin/RGMa signaling may regulate polarized migration during neural convergent extension in *Danio rerio*

Olmo, Valerie; Jayachandran, Pradeepa; Brewster, Rachel (U Maryland-Baltimore County, USA)

The neural tube, the precursor of the CNS, is formed by morphogenetic changes that transform the neuroepithelium into a tubular structure during neurulation. Though aspects of neurulation vary by species, neural convergent extension (NCE), a very early stage of

neural tube morphogenesis, is conserved as PCP signaling is required for this process in all vertebrates analyzed thus far. During NCE, neuroepithelial cells elongate and extend medially-oriented protrusions to migrate towards the midline, however the mechanisms that direct this process are poorly understood. Our laboratory has identified the receptor *neogenin* (*neo*) and its ligand the repulsive guidance molecule *rgma* as candidates that regulate cell movement during NCE. In the absence of *neo*, cells extend randomly oriented protrusions that impede migration towards the midline. *neo*-depleted embryos also exhibit an aberrant microtubule network (MTs), implicating MTs in regulating the directionality of membrane protrusions during NCE. Studies using *Xenopus* suggest that polarized migration is regulated by a secreted signal emanating from the notochord that promotes migration towards the midline. Interestingly, rgma is strongly expressed in the notochord and may be cleaved from the plasma membrane raising the possibility that it functions as the postulated midline-derived signal that provides directional cues during NCE. To test this hypothesis, a transgenic line misexpressing *rgma* away from the notochord and exhibit delayed NCE has been generated. Current efforts aim to analyze the cell behaviors of *mRFP* labeled cells using time-lapse microscopy to determine if ectopic *rgma* expression influences the polarization of cell movement in the neural tissue during NCE.

Program/Abstract # 511
Candidate Modulators of Tubulin and Microtubule Dynamics in *C. elegans* Neural Development
Baran, Renee; Kim, Hyun Su; Shayler, Dominic (Occidental College, USA)
tba-1 encodes an alpha-tubulin widely expressed during *C. elegans* development. We showed previously that a dominant allele, *tba-1(ju89)*, causes defects in motor neuron development and uncoordinated movement. To identify TBA-1-interacting proteins and study how microtubule dynamics and function are regulated in developing neurons, suppressors of tba-1(ju89) were isolated in a genetic screen based on reversion to wildtype movement and synaptobrevin-GFP expression. SNP analysis was used to map two suppressors to separate regions of C. elegans chromosome III, and whole genome sequencing identified mutations in the *klp-7* and *zer-1* genes. *klp-7* encodes a member of the kinesin-13/MCAK family which activate microtubule depolymerization. Experiments are in progress to test if -KLP-7 expression and activity is altered in *tba-1(ju89)* mutants. Alternatively, altered KLP-7 activity in the suppressor mutant may compensate for another defect in microtubule dynamics. *zer-1* encodes a conserved substrate recognition subunit of an ubiquitin-mediated protein degradation complex. ZER-1 contains a VHL Box motif and interacts with *C. elegans* cullin-2 (Vasudevan, et al. 2007), but its substratres are unknown and it has not been implicated previously in neural development. ZER-1 may play a role in regulating tubulin stability directly or modulate expression of other regulators of microtubule dynamics.

Program/Abstract # 512
Live imaging of trunk neural crest cells in zebrafish reveals a role for Notch signalling
Richardson, Joanna; Linker, Claudia (King's College London, UK)
The vertebrate neural crest (NC) is a transient migratory cell population that forms early in development, at the border of the neural plate. After induction, NC cells migrate extensively and differentiate into a wide range of cell types (melanocytes, glia, neurons, etc.). NC migration shares numerous characteristics with metastasis: both involve the loss of epithelial polarity, changes in expression of adhesion molecules and activation of matrix metalloproteinases; and NC cells themselves are often the tissue of origin for several types of cancer, e.g. melanoma and neurofibromatosis. The Notch signalling pathway has been implicated in cell migration and cancer, and Notch has been shown to play a role in NC induction. Our work demonstrates a role for Notch signalling in trunk NC migration in zebrafish. Trunk NC cells express receptors and downstream targets of the Notch pathway, whereas adjacent cells express its ligands. Gain and loss of Notch function delays NC migration. Combining the use of a new transgenic line in which we can track NC nuclei and membranes in vivo with a new tracking algorithm for 3D movement we have been able to quantify the behaviour of NC cells. This analysis indicates that Notch is important to regulate the persistence and directionality of NC migration. In other developmental contexts, Notch has been shown to distinguish between leader and follower cells in collective cell migration. We are investigating the collective migratory behaviour of the NC population and the possible role of Notch signalling in this process.

Program/Abstract # 513
Poster Board # B81
Histone Deacetylase 9b is involved in neural crest development.
Espina, Jaime A.; Barriga, Elías H.; Reyes, Ariel E. (Univ Andrés Bello, Chile)
Histone deacetylases (HDACs) are proteins that remove acetyl groups from histones, leaving the chromatin on a compact state, which is usually associated with gene repression. HDACs also can interact with transcriptions factors in the nucleus to regulate gene expression. The function of several HDACs has been recently described during development of different organism. In our laboratory we are interested in the function of HDAC9b, in the development of zebrafish neural crest. By using morpholinos against Hdac9b we generate the knockdown of this protein. Our results show that the cranial cartilage (derived from neural crest) is not properly formed in morphant embryos. Using *in situ* hybridization against neural crest induction (*foxd3*, *tfap2*) and migration (*crestin*) markers, we determined that the neural crest induction is not affected but the migration of these cells was severely impaired. Using transgenic line *Tg*(*sox10:eGFP*) we have confirmed *in vivo* effect on cell migration on knockdown embryos. Our data suggest strongly a role of Hdac9b on neural crest cells migration, but not on NCC induction, and that Hdac9b is essential for the proper formation of cranial cartilage.

Program/Abstract # 514
Ric-8A is required for the neural crest cell migration
Toro-Tapia, Gabriela; Fuentealba, Jaime; Rodriguez, Marion; Hinrichs, Maria Victoria; Olate, Juan; Marcellini, Sylvain; Torrejon, Marcela (Univ de Concepcion, Chile)
Ric-8A is a highly conserved protein with GEF (Guanosine Exchange Factor) activity for different Gα subunits related with heterotrimeric G protein signaling. Ancestral Ric-8 is critical to asymmetric cell divisions in non-vertebrate organisms and recently a similar function was reported for Ric-8A in mammalian cells. In addition, Ric-8A is expressed in neural and neural crest cells during vertebrate development. In our laboratory we have observed that Ric-8A is necessary to the proper neural crest cells migration during *Xenopus tropicalis* development. Our goal is to study the mechanism how Ric-8A is involved in the migration of cranial neural crest (CNC) cells. First, we evaluated the effect of loss and gain of function of Ric-8A by injecting a specific morpholino or mRNA, respectively, and then we analyzed the effect over the cell morphology during cell migration on CNC explants. We observed that the overexpression of Ric-8A did not affect the migration of the CNC cells although the silencing of Ric-8A strongly abrogated the normal migration of these cells. Immunocytochemistry showed that Ric-8A is localized mainly at the cell cortex in explants of CNC cells. In addition, loss of function of Ric-8A altered the sub-cellular localization of aPKC, a protein involved in cell polarity. On the other hand, Ric-8A down regulation decreased the number of focal adhesion to fibronectin matrix. Finally, we proposed that Ric-8A acting through heterotrimeric G proteins plays essential roles during the migration of CNC cells, possible by regulating cell polarity and/or cell adhesion properties.

Program/Abstract # 515
Dynamic migratory behaviours of mouse sacral neural crest cells
Chan, Wood Yee; Chen, Jie-Lin; Wang, Xia (The Chinese Univ of Hong Kong, China); Enomoto, Hideki (RIKEN Center for Developmental Biology, Japan)
The neurons and glial cells of the distal hindgut are derived from two sources of the neuraxis: vagal and sacral neural crest. Vagal neural crest cells (NCCs) enter the rostral foregut at E9.5 and migrate rostro-caudally to reach the distal hindgut at E14.5, while sacral NCCs enter the caudal hindgut via pelvic ganglia at E13.5 and migrate caudo-rostrally toward the proximal hindgut. These two populations of NCCs together form a complete neuronal network in the hindgut. The aim of the present study was to examine with time-lapse live cell confocal imaging the dynamic migratory behaviours of sacral NCCs both within hindgut explants *ex vivo* and when they were cultured *in vitro*. Within the hindgut explants, sacral NCCs migrated along nerve fibres extending from the pelvic ganglia. When a sacral NCC met a vagal NCC on a nerve fibre, they collided, pushed against each other and then intermingled to form a cellular network with more vagal than sacral neural crest-derived cells. *In vitro*, sacral NCCs also migrated along nerve fibres from the pelvic ganglia. When the fibre extension from the pelvic ganglia was perturbed by wheat germ agglutinin, sacral NCCs stayed on the nerve fibres but their distribution was altered. In a proliferation medium where the fibre extension was delayed, many sacral NCCs were found outside the fibres, indicating that nerve fibres were permissive but not necessary for sacral NCC migration under this *in vitro* condition. In conclusion, our results demonstrate that sacral NCCs exhibit migratory behaviours which are distinct from those of vagal NCCs. This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project no. CUHK461808).

Program/Abstract # 516
Rabconnectin-3a Regulates Vesicle Endocytosis and Canonical Wnt Signaling in Zebrafish Neural Crest Migration
Tuttle, Adam M.; Hoffman, Trevor; Schilling, Tom (UC-Irvine, USA)
The neural crest (NC) is a population of cells in vertebrates characterized by an epithelial-to-mesenchymal transition (EMT) followed by multiple waves of migration to many parts of the developing embryo and gives rise to several distinct cell lineages. The initiation of NC cell EMT, migration, and path-finding requires a variety of signals, including canonical and non-canonical Wnts, and dynamic regulation of the expression and subcellular localization of cell-cell adhesion molecules, such as Cadherins. Both of these processes can be regulated by controlled endocytosis, lysosomal degradation, and recycling of ligand-receptor complexes and cell-cell adhesion molecules from the plasma membrane of migrating cells. We discovered a gene, *rabconnectin-3a* (*rbcn3a*), with novel early NC expression in zebrafish whose knockdown disrupts migration of a subset of NC cells. Our data suggest *rbcn3a* is required for proper endosomal maturation independent of acidification in NC cells. Maturation and fusion of endosomes is known to regulate signaling pathways, such as Wnt, by aiding in the formation of multivesicular bodies (signalosomes) or contributing to lysosomal degradation of receptors and/or ligands. Knockdown of *rbcn3a* downregulates direct canonical Wnt targets necessary for NC EMT and migration during a critical time period in NC development. Furthermore, *rbcn3a*-deficient NC cells that fail to migrate differentiate into pigment progenitors and display aberrantly high levels of Wnt receptor at the membrane with corresponding high levels of canonical Wnt signaling at later developmental stages. We propose a novel developmental role for Rbcn3a in EMT of the NC, in which it acts, at least in part, through its regulation of Wnt signaling in both pre- and post-migratory NC cells and its requirement in early endocytosis.

Program/Abstract # 517
Hypoxia regulates neural crest migration by promoting Chemotaxis and Epithelial-to-Mesenchymal-Transition
Barriga, Elias (Univ Andres Bello, Chile); Maxwell, Patrick H. (University College of London, UK); Reyes, Ariel E (Univ Andrés, Santiago, Chile); Mayor, Roberto (University College of London, UK)

Hypoxia-inducible factors (HIFs) are essential in response to hypoxia. *in vitro* assays have show that HIF-1 directly regulates key regulators of cancer cell progression and migration, as these factors are also expressed during normal embryonic development we decided to test whether Hif-1α was required for these process. We focused our attention on the development of the neural crest, a highly migratory embryonic cell population. Inhibition of Hif-1α by antisense morpholinos in *Xenopus* or zebrafish embryos led to complete inhibition of neural crest migration. Our data show that Hif-1α controls the expression of Twist, which in turn represses E-cadherin during epithelial-to-mesenchymal transition (EMT) of neural crest cells. Thus, Hif-1α allows cells to initiate migration by promoting the release of cell-cell adhesions. Additionally, Hif-1α controls chemotaxis towards the chemokine SDF-1 by regulating expression of its receptor Cxcr4. Our results point to Hif-1α as a novel and key regulator that integrates EMT and chemotaxis during migration of neural crest cells.

Program/Abstract # 518

Hox genes control cell migration in the lateral line primordium and regulate expression of chemokine receptors downstream of Wnt signalling

Xu, Qiling; Breau, Marie A; Wilkinson, David G (MRC Nat Inst for Med Res, UK)

Collective cell migration, in which cells retain contacts with each other and move as a coherent assembly, is a crucial aspect of embryo development, tissue repair and cancer metastasis. An amenable model to study this process is the development of the posterior lateral line in zebrafish, which is formed by a cohesive primordium that migrates from head to tail and deposits clusters of cells at intervals that later differentiate to form the mechnosensory neuromasts. The directional migration is guided by the chemokine, Sdf1a, expressed along the path and two chemokine receptors Cxcr4b and Cxcr7b, expressed in different regions within the lateral line primordium. Localized Wnt signaling in the leading part of the primordium controls migration by regulating spatial expression of the chemokine receptors, but the downstream effector(s) mediating this process is not known. We report the identification of Hoxb8a as a critical component of the network required for correct migration of the primordium. We show that Wnt pathway activity is required for Hoxb8a expression in the leading zone, which in turn controls the spatially restricted distribution of Cxcr4b and Cxcr7b. Our work has unraveled new functional links between Wnt, Cxcr4/Cxcr7 and Hox transcription factors, which are involved in many processes during embryo development and in cancers such as myeloid leukemia.

Program/Abstract # 519

Fgf signaling is required for proper cell convergence during pectoral limb bud formation

Stinnett, Haley K; Mao, Qiyan; Ho, Robert K (U Chicago, USA)

The zebrafish limb bud provides an excellent system to explore the genetic basis of coordinated cell behaviors underlying organ morphogenesis. Previous work in our laboratory has demonstrated that zebrafish limb progenitors undergo spatio-topic convergence along the AP axis during pectoral limb bud initiation. Fgf24, a zebrafish fibroblast growth factor, is required for limb progenitor convergence, as limb progenitors fail to converge in Fgf24 morphants. Based on the function and expression pattern of Fgf24 in the limb field, we hypothesize that Fgf24 supplies a convergence cue for oriented limb progenitor migration. However, as Fgf24 is expressed at several stages throughout the limb progenitor lineage (gastrulation, limb field progenitor convergence and subsequent limb outgrowth), it is not clear whether the Fgf24 morphant phenotype reflects the critical function of Fgf24 during limb field progenitor convergence. In this study, we sought to determine if Fgf signaling is required specifically during the period of limb field convergence. Using the FGF receptor inhibitor SU5402, we inhibited Fgf signaling immediately prior to limb field migration and used 4D live imaging to extensively quantify the resulting cell movements. Our results show that Fgf signaling is required during the limb field convergence period for proper cell migration, and suggest that Fgf24 is a candidate for a convergence cue in the lateral plate mesoderm.

Program/Abstract # 520

Long-distance cell migrations during larval development in the appendicularian, *Oikopleura dioica*.

Nishida, Hiroki; Kishi, Kanae; Onuma, Takeshi (Osaka U, Japan)

The appendicularian, *Oikopleura dioica*, is a planktonic tunicate (urochodate). Its simple and transparent body, invariant cell lineages, and short life cycle of five days make it as a promising new model system of chordates development. Cleavage pattern, cell lineages and morphogenesis during embryogenesis of three hours are well described. While little information is available on larval development of seven hours after hatching. In this study, morphogenetic movements during larval development of *O. dioica* were investigated. Organogenesis is completed in 10 hours post fertilization (hpf) and fully functional adult-type body is formed. Time-lapse microscopy of developing larvae with aid of fluorescent labeling of nuclei visualized an orchestrated organogenesis. We also traced eventual fates of migrating cells using photoconvertible fluorescent protein, Kaede. There are three cell populations that exhibit directional and long-distance migration: (i) Four oral gland cell precursors at the posterior trunk region migrate to vicinity of the mouth by 7 hpf; (ii) Endodermal strand cells in the tail are retracted into the trunk by 5.5 hpf and give rise to digestive organs; (iii) Two subchordal cell precursors in the trunk migrate posteriorly to tip of the tail. The migration of subchordal cells starts when all of the endodermal strand cells enter into the trunk, and follow the same way but in the opposite direction. These well-defined movements of precursor cells would provide us with opportunities to analyze mechanisms underlying cell migration in this transparent larva of the model system of chordates.

Program/Abstract # 521

Mechanisms of primordial germ cell migration in the sea urchin, *Lytechinus variegatus*

Megan Martik, David McClay (Duke, USA)

The sea urchin small micromeres arise at the vegetal pole from an unequal 5th cleavage, and their progeny are specified to become the primordial germ cells of the embryo. We show, by high-resolution time-lapse microscopy, that the small micromeres reach the coelomic pouches via a directed homing mechanism. Throughout gastrulation, small micromeres adhere to one another by LvG-cadherin-mediated adherens junctions. Once gastrulation nears completion, the tip of the gut undergoes basement membrane remodeling that allows the small micromeres to undergo an epithelial-mesenchymal transition (EMT), and migrate over the archenteron and to the posterior half of the forming coelomic pouch. Small micromere progeny that will become the primordial germ cells preferentially migrate to the left coelomic pouch while a smaller number reach the right coelomic pouch and are apoptosed with the larval support system during metamorphosis. Ectopically placed small micromeres also home to the coelomic pouches. When placed at the equator of the 16-cell embryo, the small micromeres undergo a precocious EMT at the mesenchyme blastula stage and actively migrate to the tip of the early archenteron during its invagination. Ectopic insertion of 32-cell-stage small micromeres into the blastocoel of an early gastrula host embryo is followed by attachment of the small micromeres to the archenteron tip as soon as they become motile, independent of LvG-cadherin adherens. Current aims are to understand the signaling and chemoattractant mechanisms by which the small micromeres undergo such a dramatic feat of finding their way home.

Program/Abstract # 522

Identifying the link between Nodal signaling and cell migration within the cardiac cone

Rowland, Jessica R. (Princeton, USA)

Asymmetries in the zebrafish heart are established through a series of dynamic cell migrations. The first migration event, known as cardiac jogging, consists of the conversion of the cardiac cone into the linear heart tube. Recent work from our lab has shown that the laterality of cardiac jog is directed by Nodal expression through increasing cell migration rates of the left atrial cells. My work focuses on gaining a better understanding of how Nodal signaling influences cardiac cell movements during the establishment of asymmetries. We recently conducted a microarray designed to identify novel downstream genetic targets of Nodal signaling within the heart. Our results suggest that Nodal may influence several cell biological events including additional TGFbeta signaling pathways, interactions with the extracellular matrix, and regulation of the actin cytoskeleton. Our preliminary results suggest that Nodal-mediated changes in cell migratory behavior are due to changes in small GTPase activity. Our transcriptional analysis identifies a set of small GTPases that may regulate actin dynamics and endocytosis during this event. We will present our efforts to characterize the function of these small GTPases and our attempts to correlate changes in actin dynamics during jogging.

Program/Abstract # 523

LifeMap Discovery™: The embryonic development, stem cells, and regenerative medicine research compendium

Edgar, Ron; Mazor, Yaron; Rinon, Ariel; Blumenthal, Jacob; Golan, Yaron; Buzhor, Ella; Livnat, Idit; Ben-Ari, Shani; Lieder, Iris; Shitrit, Alina; Gilboa, Yaron; Edri, Osnat; Shraga, Netta; Bogoch, Yoel; Leshansky, Lucy; Aharoni, Shlomi (LifeMap Sciences, Israel); D. West, Michael (BioTime Inc., USA); Warshawsky, David; Shtrichman, Ronit (LifeMap Sciences, Israel)

In-depth understanding of the differentiation processes occurring during embryonic development is instrumental toward derivation of functional stem cells in vitro. Profiling the genes and signals regulating mammalian cell differentiation is essential for identification and classification of stem cells, and to foster design of differentiation protocols and therapeutic products. LifeMap Discovery^{TM}, http://discovery.lifemapsc.com, maps the ontology of cellular development and stem cell differentiation. The database is based on systematic assimilation of scientific data detailing distinct developmental paths, from the progenitor cells until determination of their terminal fates. The database encompasses cellular and anatomical development, supplemented with qualitative gene expression patterns, signaling pathways, *in-situ* hybridization and high throughout experimental data, related diseases, images and relevant references.

The database is divided into the following: 1. *In-vivo* development - cell lineages arising in the embryo. 2. Stem cell differentiation - cultured cells and differentiation protocols. 3. Gene expression and signaling – gene expression and signaling cascades related to development and differentiation. 4. Regenerative medicine – application of stem cells in therapeutics. These four segments are connected and interlaced by computational and hand-curated methods. Most noteworthy, are the *in-vivo* entities which are linked to their closest *in-vitro* entities, based on gene expression analysis. LifeMap Discovery's value lies in the combined power of the presented data, which enables identification and prediction of differentiation paths and potential regenerative medicine applications.

Program/Abstract # 524

Induction of osteo-chondroprogenitors formation by transcription-factor mediated reprogramming process

Cheung, Martin; Wang, Yinxiang; Lu, Lorraine; Wu, Ming-Hoi; Sham, Mai-Har; Chan, Danny; Cheah, Kathryn (The University of Hong Kong, China)

Stem-cell based skeletal tissue engineering has been limited by its heterogenous and uncontrolled differentiation. Osteo-chondroprogenitors co-expressing *Sox9* and *Runx2* are lineage restricted skeletal precurors to differentiate into chondrocytes and osteoblasts without generating other cell types favourable for skeletal regeneration. Therefore, developing tactics to generate osteo-chondroprogenitors are essential.

To address this issue, we have taken advantage of a lineage reprogramming approach in which fetal fibroblasts can be converted into murine chondrocytes by retroviral-mediated expression of transcription factors *Sox9*, *Klf4* and *c-Myc* and examined whether osteo-chondroprogenitors could be formed during 14 days of the reprogramming using Sox9-EGFP knock-in mice as a reporter. We obtained significant number of Sox9-EGFP/Runx2 positive nodules from day 10 to 14 upon culturing the transduced cells in mTeSR medium. Consistent with this, real-time PCR analysis also revealed these transduced nodules express osteo-chondroprogenitors-associated genes such as *Sox9*, *Runx2*, *Col2a1*, *Sox5* and *Col1a1*. In addition, the Sox9-EGFP/Runx2+ve cells can be differentiated into chondrogenic and osteogenic lineages both in vivo and in vitro. Taken together, these findings suggest that *Sox9*, *Klf4* and *c-Myc* are sufficient to convert mouse fibroblasts into cells that closely resemble osteo-chondroprogenitors.

Program/Abstract # 525

(-)-epicatechin-induced differentiation of human bone marrow mesenchymal stem cells to osteoblasts

Diaz, Hector; Parra, Alberto; Mera, Elvia; Salas, Jose L; Acevedo, Leonardo F; Benitez, Gamaliel; Caceres, Julio R; Najera, Nallely; Rubio, Angel I O; Palma, Icela; Ceballos, Guillermo M; Gutierrez, Gisela (Escuela Superior de Medicina, Mexico)

Introduction: Osteoporosis is a skeletal disease where bone mass decreases inducing bone fragility and susceptibility to fractures. In Mexico (2006), the prevalence of osteoporosis in old women, without social security, was of 4.16% costs about 97 million US dollars. The FDA has approved several drugs for its treatment, but almost all have important side effects. Some clinical studies have showed that the intake of tea improve bone mineral density in older women, probably due to antioxidants, called flavonoids. It has been proposed the use of Mesenchymal Stem Cell for elucidate the mechanisms of flavonoides effects, demonstrating that (-)-catechin, epigallocatechin and (-)-epigallocatechin-3-gallate induced cell differentiation. In this work we study the effect of (-)-epicatechin in cell differentiation of human Bone Marrow Mesenchymal Stem Cells (hBM-MSCs). Results: Flow cytometry; Positive markers of hBM-MSCs were: CD105: 10.81%, CD73: 77.08% and CD90: 61.53% and negative markers were: CD34: 0.67%, CD45: 0.15%, HLA-DR: 0.16%. RT-PCR: Expression of bmp2 was negative in hBM-MSCs without stimuli. All concentrations of OSTEO alone induced the expression of bmp2. OSTEO concentrate with EPI at 1uM, 10uM and 100uM did not induce the expression of bmp2. The combinations of OSTEO 50%/100uM EPI, OSTEO 50%/10uM EPI, OSTEO 50%/1uM EPI, OSTEO 30%/100uM EPI, OSTEO 30%/10uM EPI and OSTEO 30%/1uM EPI were positive to bmp2 in different ratios. All samples expressed ostenonectin and runx2. Discussion: For study the osteogenic effects of EPI we chose hBM-MSCs as a model, because they are able to differentiate into bone, heart or adipose tissue and other tissues. On the other hand, it was shown that bmp2 has the highest osteogenic activity among the bmp family members, for this reason we decided to evaluate the effect of EPI on expression of bmp2. Our results demonstrated what BM-MSCh treated with OSTEO in different concentrations induced the expression of bmp2, the same expression was observed when were added EPI (1uM, 10uM and 100uM) in presence of OSTEO at 50% or 30%, coinciding with previous reports demonstrating the osteogenic activity of some polifenols. We were unable of defect the expression of bmp2 in control negative, neither in the samples treated with OSTEO concentrate in presence of EPI. We think that EPI has an antagonist effect with any compound associated to differentiation effect of OSTEO. osteonectin was observed in all samples, but its expression was highest in hBM-MSCs treated with concentrate OSTEO and less in OSTEO concentrations, in agreement with reports related about osteogenic differentiation in hBM-MSCs, however they do not explain the basal expression of osteonectin in negative control (NC). We suggest that osteonectin is involved in different pathways because osteonectin also plays important roles in development, wound healing, adipogenesis, angiogenesis and others, not only in osteogenic induction of hBM-MSCs. We also evaluated the expression of runX2 on osteogenic differentiation and we observed its expression in all samples. runX2 has been associated to cell cycle exit, this can explain the expression of runX2 in our NC. Due to osteoblastogenesis is regulated by multiple signaling pathways, we think that canonical and non-canonical Wnt signaling is the way through which (-)-epicatechin exerts its effect, this because we did not observed marked difference in the expression of osteonectin and RunX2 but if in bmp2. Conclusions: We propose hBM-MSCs as a suitable model of osteogenic differentiation. (-)-epicatechin alone at 1uM, 10uM and 100uM is able to induce the expression of bmp2. Although must be investigate the pathways of osteogenic differentiation induced by EPI of hBM-MSCs and its inhibitory effect in presence of OSTEO. These results support that (-)-epicatechin can be used as adjuvant in the treatment on osteogenic disease not only in Osteoporosis.

Program/Abstract # 526

PLZF: a master regulator of mesenchymal stem cell lineage commitment

Djouad, Farida; Tejedor, Gautier; Toupet, Karine; Maumus, Marie; Chuchana, Paul; Jorgensen, Christian; Noël, Danièle (INSERM, France)

Objectives: Mesenchymal stem cells (MSC) are an attractive cell source for cartilage and bone tissue engineering given their ability to differentiate into chondrocytes and osteoblasts. However, the common origin of these two specialized cell types raised the question about the identification of an essential regulatory factor determining whether MSC will differentiate into chondrocyte or osteoblast. Methods: Using affymetrix gene chips we performed a transcriptomic analysis of MSC differentiated into chondrocytes, osteoblasts and adipocytes to identify a regulatory factor that signs MSC commitment: PLZF. We examined, in vitro, the multi-differentiation potential of MSC overexpressing PLZF and, in vivo, in a mouse osteochondral defect model we investigated their reparative potential. Results: We found out that PLZF induced expression in MSC is the mark of their commitment toward the 3 main lineages. PLZF acts as an upstream regulator of both Sox9 and Runx2 and its overexpression in MSC improved chondrogenesis and osteogenesis while inhibiting adipogenesis. In vivo, C3-PLZF implantation in mice full thickness osteochondral defects resulted in the formation of a reparative tissue cartilage- and bone-like. Conclusions: Our findings demonstrate that PLZF is a key factor for MSC

differentiation potential acting as a molecular switch between osteoblast and chondrocyte cell fates according the culture or environmental conditions. This study paves the way for developing specific therapeutic approaches for cartilage and bone repair.

Program/Abstract # 527

Evaluating of hematopoietic and mesenchymal stem cell markers during limb bud development

Camargo Sosa, Karen; Marin Llera, Jessica Cristina; Soldevila Melgarejo, Gloria; Chimal Monroy, Jesús (UNAM-Mexico City, Mexico)

As it is proposed, "undifferentiated zone" of developing limb is the source of skeletal structures, tendons and dermis. Also, interdigital mesenchymal cells are competent to cartilage differentiation, proven by exogenous implantation of TGF-β soaked beads. Therefore, these undifferentiated cells have potential to generate several cell types. Another undifferentiated and multipotent population has been described in adult bone marrow, these are MSC (Mesenchymal Stem Cells) and HSC (Hematopoietic Stem Cells). How and when do these cells population appear within limbs during embryo development remain unknown. Mouse HSC has been described as Sca-1 and c-Kit positives, and considered negatives for lineage specific markers (Lin-). By contrast, mouse MSC have not been that well characterized, but the most accepted immunophenotype in humans, is $CD73^{+}$, $CD90^{+}$ and $CD105^{+}$, as well as Lin^{-}. In this work, we evaluate if described adult HSC and MSC markers are expressed and can be detected by flow cytometry during early limb bud development. Results showed MSC markers CD73 and CD105 are present in a low percentage of limb bud cells but not CD90 in the evaluated stage, it will be interesting to determinate if the same cells are expressing both markers at the time they are negative for hematopoietic lineage specific markers like CD45 also expressed in a low percentage of limb bud cells. In the evaluated stages, CD117 was not detected but Sca-1 did. Since both markers expressions are necessary for HSC identification, next, we will be looking for their expressions at later stages. This study was partially supported by CONACyT grants 53484 and 168642, DGAPA-UNAM grants IN214511 and IN220808.

Program/Abstract # 528

Defining the origins of the hemogenic endothelium, the source of hematopoietic stem cells

Naiche, L.A. (National Cancer Institute, USA); Klarmann, Kimberly; Keller, Jonathan (SAIC-Frederick National Lab,USA); Lewandoski, Mark (National Cancer Institute, USA)

Differentiation generally proceeds linearly from less to more differentiated cell fates, and exceptions are usually pathological (*i.e.* cancer) or artificial (*i.e.* iPS cells). However, during normal development, hematopoietic stem cells (HSCs) arise from apparently differentiated, functional endothelial cells in the endothelial walls of specific embryonic vessels, called the hemogenic endothelium. An unanswered question is the difference between hemogenic and non-hemogenic endothelium, which both express markers of differentiated endothelium. The vasculature of the placenta and umbilical cord, which are hemogenic tissues, originates in an embryonic organ called the allantois. We have observed that the bulk of the allantois is formed from cells that express *Tbx4* or their descendants (the *Tbx4* lineage), but the allantois-derived endothelium arises from two different lineages. The endothelium of the umbilical cord and proximal placenta are derived from a distinct population of cells that are not part of the *Tbx4* lineage, while the endothelium of the distal placenta is derived from the *Tbx4* lineage. This spatial distribution correlates with observed hemogenic fate and non-hemogenic fate, respectively. Despite abundant *Tbx4* lineage endothelium in the placenta, the *Tbx4* lineage does not populate circulating blood or HSC niches. These results indicate that endothelium can be sorted into two populations: a hemogenic lineage that has never expressed *Tbx4*, and the non-hemogenic *Tbx4* lineage. Because the *Tbx4* lineage is defined several days prior to hematopoietic activity, isolation of these lineages allows us to investigate the underlying factors that determine the potential of endothelium for future stem cell differentiation.

Program/Abstract # 529

SOX2 is Required for Correct Pituitary Morphogenesis

Goldsmith, Sam; Rizzoti, Karine; Lovell-Badge, Robin (MRC NIMR, UK)

Pituitary hormonal deficiencies, or hypopituitarism, can be caused in humans and mice by haploinsufficiency of the HMG box transcription factor SOX2 (Kelberman et al, 2006). During embryogenesis, *Sox2* is expressed ubiquitously in the pituitary anlagen or Rathke's Pouch, (RP) and is then down-regulated, as endocrine cells differentiate. In the adult gland, a small population of $SOX2^{+ve}$ progenitors remains (Fauquier et al, 2008). We, and others, have shown that SOX2 is required for proper pituitary morphogenesis. Conditional deletion of *Sox2* in RP leads to a hypoplastic pituitary, likely caused by a reduction in progenitor proliferation (Jayakody et al 2012, S. G. unpublished). Using different Cre strains, we show that *Sox2* deletion efficiency correlates with further reduction in pituitary size. The *Sine Oculis* homeodomain protein SIX6, required for progenitor proliferation in particular in the eye and the pituitary (Li et al 2002, Tetreault et al 2009), is downregulated upon *Sox2* deletion while expression of LHX3, a LIM homeodomain transcription factor also required for RP progenitor proliferation is maintained. This suggests that SOX2 may directly up-regulate *Six6* expression, as shown in other tissues, promoting RP progenitor proliferation. Later on, as endocrine cells differentiate, lineage tracing experiments show that the endocrine differentiation potential of *Sox2* deleted progenitors is impaired. These data suggest that during normal development SOX2 drives early progenitor proliferation through the upregulation of *Six6*. Later on, *Sox2* deleted progenitors are largely unable to give rise to endocrine cells. Ongoing experiments address a direct role of SOX2 for *Six6* transcriptional regulation in RP.

Program/Abstract # 530

The MADS protein XAANTAL2 (XAL2/AGL14) is required to control auxin transport via direct PIN regulation during *Arabidopsis* root development

Garay, Adriana; Ortiz-Moreno, Enrique; Sánchez, María de la Paz (UNAM, Mexico); Murphy, Angus S. (Purdue, USA); Marsch-Martínez, Nayelli; de Folter, Stefan (CINVESTAV-Langebio, Mexico); García-Ponce, Berenice; Corvera-Poiré, Adriana; Jaimes-Miranda, Fabiola; Pacheco-Escobedo, Mario A. (UNAM, Mexico); Dubrovsky, Joseph G. (UNAM-Cuernavaca, Mexico); Pelaz, Soraya (Barcelona, Spain); Álvarez-Buylla, Elena R. (UNAM, Mexico)

Molecular links between cell-fate regulatory networks and dynamic patterning modules are key for understanding development. Auxin is important for plant patterning and particulary for root development, where it provides important positional information for cell-fate decisions. *PIN* genes codify for plasma membrane proteins that are important auxin efflux transporters and their mutants have altered root meristems and stem-cell patterning. However, *PIN* direct regulators have remained elusive. Here, we show that a MADS-box gene (*XAANTAL2, XAL2/AGL14*) whose mutants also have altered stem-cell patterning, root meristems and growth, control auxin transport via *PIN* transcriptional regulation during Arabidopsis root development. Concordantly, *XAL2* is necessary for normal shootward and rootward auxin transport, as well as for maintaining normal auxin distribution along the root. Furthermore, this MADS-domain transcription factor up-regulates *PIN1* and *PIN4* by direct binding to their regulatory regions and it is required for *PIN4* dependent auxin response. Interestingly, *XAL2* responds to auxin levels thus establishing a positive feedback loop between auxin levels and PIN regulation that is likely important for robustness of root patterning. This work is supported by CONACyT (180098; 180380; 167705; 152649; 105678) and DGAPA, UNAM (IN204011-3; IN203113-3; IN226510-3; IB201212-2) grants.

Program/Abstract # 531

A protein network controls protective quiescence in the *Arabidopsis* root stem cell organizer

Cruz Ramírez, Luis Alfredo (Univ de La salle Bajío, Mexico); Diaz Trivino, Sara; Wachsman, Guy; Du, YuJuan (Wageningen Univ, Netherlands); Arteaga-Vazquez, Mario (Universidad Veracruzana, Mexico); Zhang, Hongtao; Blilou, Ikram (Wageningen Univ, Netherlands); Chandler, Vicky (U AZ, USA); Scheres, Ben (Wageningen Univ, Netherlands)

Quiescent long-term somatic stem cells reside in plant and animal stem cell niches. Within the *Arabidopsis* root stem cell population, the Quiescent Centre (QC), which contains slowly dividing cells, maintains surrounding short-term stem cells and may act as a long-term reservoir for stem cells. The RETINOBLASTOMA-RELATED (RBR) protein cell-autonomously reinforces mitotic quiescence in the QC. RBR interacts with the stem cell transcription factor SCR through an LxCxE motif. Disruption of this interaction by point mutation in SCR or RBR promotes asymmetric divisions in the QC that renew short-term stem cells. Analysis of the *in vivo* role of quiescence in the root stem cell niche reveals that slow cycling within the QC is not needed for structural integrity of the niche but allows the growing root to cope with DNA damage.

Program/Abstract # 532

Intestinal stem cell dynamics in induced human intestinal organoids

Finkbeiner, Stacy; Rockich, Briana (U Michigan-Ann Arbor, USA); Vallance, Jeff; Shroyer, Noah (Cincinnati Children's Hospital, USA); Spence, Jason (U Michigan-Ann Arbor, USA)

Our understanding of human intestine development and regulation has largely been inferred from animal models and limited cell culture systems due to a lack of suitable, realistic 3-dimensional models for studying human intestine. However, we have recently reported the use of a directed differentiation scheme, which mimics stages of intestinal organogenesis, to generate 3-dimensional intestinal tissue from pluripotent stem cells, called “induced human intestinal organoids” (iHIOs). iHIOs contain a pseudo-lumen and an epithelial layer containing differentiated cell types surrounded by a layer of mesenchymal cells. They also contain LGR5+/ASCL2+ cells suggesting the presence of intestinal stem cells (ISCs). However, it is still unclear if the LGR5+/ASCL2+ cells are bona fide ISCs. In our current studies we have begun to analyze the presence and function of putative ISCs in iHIOs. We demonstrate expression of ISC markers at the RNA and protein levels and show responsiveness of the ISC populations to Wnt and Notch signaling. This work is an important step in further understanding the promising iHIO model so that it can be used to study mechanisms of stem cell maintenance, intestinal development, tissue regeneration, intestinal physiology, and enteric microbiology among many other avenues of research.

Program/Abstract # 533

Totipotent embryonic stem cells arise in ground state culture conditions

Morgani, Sophie (Danstem Ctr, Denmark); Canham, Maurice (MRC Ctr Regen Med, UK); Nichols, Jennifer (Wellcome Trust Ctr for Stem Cell Res, UK); Sharov, Alexei (NIA/NIH, USA); Migueles, Rosa (MRC Ctr Regen Med, UK); Ko, Minoru (Keio U, Japan); Brickman, Joshua (Danstem Ctr, Denmark)

Embryonic stem cells (ESCs) are derived from mammalian embryos during the transition from totipotency, when individual blastomeres can make all lineages, to pluripotency, when they are competent only to make embryonic lineages. ESCs maintained with signalling inhibitors (2i), are thought to represent a homogeneous pluripotent, embryonic ground state. Using a mouse line containing a sensitive reporter for the endoderm marker *Hex* we observed that embryos cultured in 2i exhibited endoderm precursor specification, but no lineage segregation. Similarly 2i ESC cultures contained a *Hex* positive fraction primed to differentiate into trophoblast and extraembryonic endoderm. Single *Hex* positive ESCs coexpressed epiblast and extraembryonic genes and contributed to all lineages in

chimaeras. The cytokine LIF, necessary for ESC self-renewal, supported the expansion of this population, but did not directly support *Nanog* positive epiblast-like ESCs. Thus 2i and LIF support a totipotent state comparable to early embryonic cells that coexpress embryonic and extraembryonic determinants.

Program/Abstract # 534

Cell fate decisions regulating stem cell origins during preimplantation mouse development

Ralston, Amy (UC-Santa Cruz, USA)

Using the mouse embryo, we aim to understand the origins and regulation of stem cells during development. Our approach is to examine the roles of stem cell factors in cell fate decisions in the mouse early embryo. Oct4 and Sox2 are essential regulators of pluripotency in embryonic stem cells, where they regulate each other's expression. However, we show that in the mouse blastocyst, neither zygotic nor maternal Oct4 is required for expression of Sox2, nor is maternal/zygotic Sox2 required for Oct4. Rather, Oct4 and Sox2 both regulate formation of the primitive endoderm, an extraembryonic tissue. Primitive endoderm cell fate is known to depend on FGF signals from the epiblast, suggesting that the requirement for Oct4 and Sox2 in primitive endoderm development could be non cell-autonomous. We show that while Sox2 regulates primitive endoderm development non cell-autonomously upstream of FGF signaling, Oct4 regulates primitive endoderm cell-autonomously downstream of FGF signaling. Finally, we examine stem cell factors thought to be required for trophectoderm, another extraembryonic lineage of the blastocyst. Maternal Cdx2 and Sox2 are each thought to be required for trophectoderm development. However, deletion of Cdx2 in the female germline does not disrupt trophectoderm cell fate nor mouse development. We also show that maternal Sox2 is not required for trophectoderm development. Rather, we show that the trophectoderm-specifying pathway upstream of Cdx2 also regulates expression of Sox2. Ultimately, our data support regulative, rather than deterministic, models of early mammalian development.

Program/Abstract # 535

Cell competition in the mammalian epiblast selects cells with higher Myc levels

Clavería, Cristina; Giovinazzo, Giovanna; Sierra, Rocío; Torres, Miguel (Spanish National Centre for Cardiovascular Research (CNIC), Spain)

The epiblast is the mammalian embryonic tissue that contains the pluripotent stem cells that generate the whole embryo. We have established a method for inducing functional genetic mosaics in the mouse. Using this system we found that induction of a mosaic imbalance in c-Myc expression provokes the expansion of cells with higher c-Myc levels through the apoptotic elimination of cells with lower levels, without disrupting development. In contrast, a homogeneous shift in c-Myc levels did not affect epiblast cell viability, indicating that the observed competition results from comparison of relative c-Myc levels between epiblast cells. During normal development we found that c-Myc levels are intrinsically heterogeneous among epiblast cells, and that endogenous cell competition refines the epiblast cell population through the elimination of cells with low relative c- Myc levels. These results show that natural cell competition in the early mammalian embryo contributes to the selection of the epiblast stem-cell pool.

Program/Abstract # 536

The study of the first heart field cardiac progenitor cells via ES cell derivation

Kokkinopoulos, Ioannis; Saba, Rie; Ishida, Hidekazu (Queen Mary Univ of London, UK); Hamada, Hiroshi (Osaka Univ, Japan); Suzuki, Ken; Yashiro, Kenta (Queen Mary Univ of London, UK)

The heart is the first visible organ during vertebrate embryogenesis. It originates from the most anterior part of lateral plate mesoderm. Cardiac progenitor cells (CPCs) are divided into two major populations, occupying the first and second heart fields during development. First heart field CPCs are labelled with *Nkx2-5* and/or *Tbx5* and are mainly destined to from the left ventricle and atria of the heart, while the second heart field CPCs, labelled with *Isl1*, will give rise to the right ventricle and the outflow tract. To date, it has been shown that *Nkx2-5*$^{+}$ CPCs are able to give rise to smooth muscle cells and cardiomyocytes, while *Isl1*$^{+}$ CPCs are multipotent able to differentiate to cardiomyocytes, smooth muscle and endothelial cells. There has been no report examining the potential of *Tbx5*$^{+}$ cells as CPCs, to date. To better understand CPCs onset, we have performed single cell cDNA analysis on cells from the early allantoic bud (EB) stage to early headfold (EHF) stage on embryonic day (E) 7.5. This study showed that *Tbx5*$^{+}$, *Nkx2.5*$^{+}$ and *Tbx5*$^{+}$*/Nkx2.5*$^{+}$ cells are the three major CPC subpopulations. As heart development proceeds, the *Tbx5*$^{+}$ cell population begins to diminish, with the *Tbx5*$^{+}$*/Nkx2.5*$^{+}$ to steadily increase while approaching the late allantoic bud (LB) stage. By the early somite stage, no *Tbx5*$^{+}$ cells are present. To test the hypothesis of whether *Tbx5*$^{+}$*/Nkx2.5*$^{+}$ CPCs descend from *Tbx5*$^{+}$ CPCs, we generated a tamoxifen-inducible BAC *Tbx5*$^{(CreERT2/+)}$*;Rosa26R*$^{YFP/-}$ transgenic mouse. With this system, we are able to trace *Tbx5*$^{+}$ CPCs *in vivo* as well as deriving ES cell lines from these transgenic mice *Tbx5*$^{+}$ CPCs for tracing analysis *in vitro*. We will present our findings with this newly established experimental system.

Program/Abstract # 537

Single-Cell cDNA Analyses of embryonic cardiac progenitor cells

Yashiro, Kenta; Kokkinopoulos, Ioannis; Saba, Rie; Ishida, Hidekazu (Queen Mary University of London, UK); Saga, Yumiko (Natl Inst of Genetics, Japan); Azuma-Kanai, Masami (Tokyo Med and Dental Univ, Japan); Kitajima, Keiko; Meno, Chikara (Kyushu Univ, Japan); Kanai, Yoshiakira (The Univof Tokyo, Japan); Koopman, Peter (The Univ of Queensland, Australia); Hamada, Hiroshi (Osaka Univ, Japan); Suzuki, Ken (Queen Mary Univ of London, UK)

Better understanding of the underlying mechanism of differentiation of cardiac progenitor cells (CPCs) is vital for the production of the cells from pluripotent stem cells or from directly reprogrammed somatic cells to use in regeneration therapy to treat the heart failure patients as well as congenital heart diseases. However, our knowledge is still insufficient for generating a sufficient amount of pure, high quality cardiomyocytes or other cell types of the heart for the clinical arena. Therefore further study is required.
Currently, CPCs are classified into two major groups, the first heart field cells and the second heart field cells. The first heart field cells are destined mainly to the atria and the left ventricle, represented by T-box transcription factor Tbx5 as well as homeodomain transcription factor Nkx2-5 expression. The second subgroup is destined to the right ventricle and the outflow tract, represented by LIM-homeodomain transcription factor *Isl1* expression. Thus far, *Nkx2-5*-expressing CPCs and *Isl1*-expressing CPCs have been shown to be multipotent to differentiate into cardiomyocytes, smooth muscle cells and endothelium/endocardium. On the other hand, *Tbx5*-expressing CPCs have not been elucidated in detail.
To understand embryonic CPCs in detail, we have performed single cell cDNA analyses of mouse embryonic CPCs. Whole mount in situ hybridization revealed that both of *Nkx2-5* and *Tbx5* expression begins from the early allantoic bud stage to early head fold stage. We have constructed single cell cDNAs from this initial stage of embryonic CPCs in mouse embryos. We will discuss the issues which this study has raised; (1) Detailed information of subpopulations of the initial CPCs and (2) a novel hypothetical model of a lineage tree of CPCs' progenies.

Program/Abstract # 538
A novel somatic role of Piwi in the central nervous system of the ascidian *Ciona intestinalis*
Shimai, Kotaro (Konan U, Japan); Horie, Takeo (Univ of Tsukuba, Japan); Nishitsuji, Koki (Okayama Univ, Japan); Shirae-Kurabayashi, Maki; Nakamura, Akira (RIKEN CDB, Japan); Kusakabe, Rie (Kobe Univ, Japan); Nakai, Kenta (The Univ of Tokyo, Japan); Inoue, Kunio (Kobe Univ, Japan); Kusakabe, Takehiro G. (Konan Univ, Japan)
Piwi proteins and the small non-cording RNA, called Piwi-interacting RNAs (piRNAs) are required for the maintenance and formation of germline cells, the repression of transposable elements, and the epigenetic gene expression control. Recent reports showed that the Piwi/piRNA pathway also has roles in cell differentiation. For example, the Piwi expression is detected in the somatic or multipotent stem cells in sponges, ctenophores, planarians, and colonial ascidians. Several cancer cell lines have the elevated Piwi expression. Moreover, the epigenetic control by Piwi is involved in the synaptic plasticity of *Aplysia* sensory neurons. In this study, we explored a somatic role of Piwi in chordates by looking at a *piwi* orthologue of the ascidian *Ciona intestinalis*. The *Ciona* genome contains three piwi/argonaute family genes, *Ci-piwi-like1*, *Ci-piwi-like2*, and *Ci-Argonaute*. Maternal Ci-piwi-like1 proteins are localized during early embryogenesis, and later they are co-localized with the vasa-homolog, CiVH in a few cells in the endodermal strand of the larva. Interestingly, *Ci-piwi-like1* transcripts are also expressed conspicuously in the larval brain, including some differentiated sensory organs and a part of ependymal cells. Brain ependymal cells in the *Ciona* larva are known to be stem-cell-like cells for the adult nervous system. Using a photo-convertible fluorescent protein Kaede, we examined developmental fate of the larval cells expressing *Ci-piwi-like1* during metamorphosis, and found that some of these cells were kept and exhibited a neuron-like feature in the central nervous system of juveniles. Our results suggest that cells expressing Piwi contribute to the formation of the adult nervous system.

Program/Abstract # 539
Investigating the Role of SOX9 in Human Neural Stem Cells
Hui, Man Ning; Wu, Ming Hoi; Chan, Ken Kwok-Keung; Cheung, Martin (The Univ of Hong Kong, China)
Neural stem cells (NSCs) exist in both embryonic and adult nervous tissues and are characterized by their self-renewal capacity and multipotency that contribute to the formation of three major cell types in the central nervous system (CNS): neurons, oligodendrocytes and astrocytes. The tremendous therapeutic potential of NSCs to treat the neurodegenerative diseases and repair brain injuries has provoked intensive study in the molecular regulation of their induction, maintenance and differentiation. Previous study reported that Sox9, a member of SoxE transcription factors family, plays important roles in directing the formation and the maintenance of NSCs in both mouse and chick CNS [1], as well as the cell fate switch between neural and glial [2]. Whether it plays similar roles in human NSCs (hNSCs) is still unknown. Our RT-qPCR analysis showed that SOX9 is expressed at a basal level in human embryonic stem cells (hESCs) and upregulated upon commitment into neural lineage and maintained at a high level in hESCs-derived hNSCs. We therefore hypothesized that SOX9 might also involve in the induction, maintenance and differentiation of hNSCs. To test this, we have generated a hESC line (HES2) stably expressing short hairpin RNA (shRNA) against SOX9 . Upon neural induction, commitment of SOX9-knockdown hESCs to neural fate still occurs suggesting that SOX9 is not required for the neural induction. Characterization of the impact of SOX9 knockdown on self-renewal capacity and differentiation of hESCs-derived NSCs are ongoing. References: 1. Scott, C.E., et al., Nature neuroscience, 2010, 13(10): p. 1181-9. 2. Stolt, C.C., et al., Genes & development, 2003, 17(13): p. 1677-89.

Program/Abstract # 540
Gene expression and functional analysis indicate that taurine affects the proliferation and survival pathways of neural precursor cells
Ramos-Mandujano, Gerardo; Hernández-Benítez, Reyna; López-Guzmán, Karla; Pasantes, Herminia (UNAM, Mexico)
We recently reported a positive influence of taurine on growth and neuronal formation increasing markedly the number of neural precursor cells (NPCs) as well as the number of neurons formed upon differentiation. This was found in NPCs cells from mice adult

brain and also in NPCs from fetal human brains. The mechanism responsible for these effects of taurine remains undefined. In the present study we examined possible mechanisms and signaling pathways which may account for the observed taurine actions. NPCs obtained from the subventricular zone of adult mice were cultured in the absence or presence of taurine (10 mM) and cell proliferation and viability were assessed. Results showed that taurine enhanced by 120% the number of cells in culture and also increased the number of BrdU+ cells. The cell cycle analyzed by flow cytometry showed that taurine was increasing the percentage of cells in S-phase by 40% over controls. Taurine reduced apoptosis (assayed by flow cytometry with annexin V/PI), from 11% in controls to 8%, decreased necrosis (assayed by calcein-AM) from 13% to 7%, and increased viable cells from 72% to 81%. The partial transcriptome analysis (BioCarta & KEGG pathway maps) showed that cells grown in the presence of taurine exhibit an increase in the expression of genes from the synthesis of elements of pathways such as sonic hedgehog, WNT and p53, involved in survival and proliferation. By improving the operation of these pathways, taurine is presumably enhancing the survival and proliferation of NPCs. These results provide a rationale for considering the use of taurine as a factor to enrich niches of neurogenesis when the cell replacement therapy is the strategy of choice for brain injuries and diseases.

Program/Abstract # 541

Spatial and temporal heterogeneity in the formation of adult pallial neural stem cells in the zebrafish telencephalon

Dirian, Lar; Galant, Sonya; Coolen, Marion (CNRS, Gif-sur-Yvette, France); Chen, Wenbiao (Nashville, USA); Mosimann, Christian (Harvard, USA); Houart, Corinne (King's College London, UK); Bally-Cuif, Laure; Foucher, Isabelle (CNRS, Gif-sur-Yvette, France)

Important aspects of neural stem cells (NSCs) biology are to determine from which embryonic cell populations they derive, and when and how it occurs during development. Do they arise from embryonic neural precursors already actively involved in embryonic neurogenesis and brain formation, or alternatively, the non-exclusive possibility would be that NSCs-fated quiescent neural precursors are set aside as an early reserved pool?

In the zebrafish adult telencephalon, NSCs are self renewing and multipotent radial glia. They are located along the ventricular zone and have been best characterized in the dorsal telencephalon (pallium). Here, we investigated the contribution to this adult NSCs population of a large group of embryonic progenitors expressing the E(spl) transcription factor-encoding gene *her4*, (ortholog to mouse Hes5), which highlights active neurogenic zones in the early embryo. Using a conditional Cre/lox genetic strategy driven by the *her4* promoter, we could specifically and permanently fate map the embryonic cell population expressing *her4* at chosen developmental stages. Surprisingly, our results show that the *her4*-positive population at embryonic stages generates only a sub-population of pallial adult NSCs located in the medial pallium. On the contrary, we show for the first time that lateral adult NSCs derive from progenitors that express *her4* de novo later on during development, from larvae stage. *her4* expression is comcomitant with progenitors amplification and the onset of neurogenesis, thus driving the late construction of the lateral domain. Together, these results demonstrate the dual origin of the pallial aNSCs deriving from spatially distinct and strongly heterochonous long- lasting progenitors.

Program/Abstract # 542

Investigating the role of Plzf in neural progenitors

Constable, Sean; Wilkinson, David (National Institute for Medical Research, UK)

Neurogenesis must be tightly regulated both spatially and temporally to give rise to the full spectrum of neuronal cell types. The promyelocytic leukaemia zinc finger (Plzf) transcription factor is required for the maintenance of stem cells of the spermatogonial and hematopoietic systems. Our previous work has identified Plzfa as a widely-expressed inhibitor of primary neurogenesis in zebrafish, but suggests that it acts redundantly with other mechanisms, including Notch-mediated lateral inhibition. Here, we have investigated whether Plzfa and its paralogue, Plzfb, act in parallel to regulate neurogenesis. Both *plzfa* and *plzfb* are expressed throughout the developing nervous system and are down-regulated in post-mitotic differentiated neurons. Whereas single gene knockdowns have no effect, knockdown of both *plzfa* and *plzfb* lead to a neurogenic phenotype associated with a loss of neural progenitors. These findings provide support for a role of Plzfa and Plzfb in the maintenance of neural progenitors.

Program/Abstract # 543

Transcription Factor Sox11 Is Essential for both Embryonic and Adult Neurogenesis

Lei, Lei (Univ of New England, USA)

Background: Neurogenesis requires neural progenitor cell (NPC) proliferation, neuronal migration and differentiation. During embryonic development, neurons are generated in specific areas of the developing neuroepithelium and migrate to their appropriate positions. In the adult brain, neurogenesis continues in the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricle. Although neurogenesis is fundamental to brain development and function, our understanding of the molecules that regulate neurogenesis is still limited. Results: In this study, we generated a Sox11 floxed allele and a Sox11 null allele using the Cre-loxP technology. We analyzed the role of the transcription factor Sox11 in embryonic neurogenesis using Sox11 null embryos. We also examined the role of Sox11 in adult hippocampal neurogenesis using Sox11 conditional knockout mice in which Sox11 is specifically deleted in adult NPCs. Sox11 null embryos developed small and disorganized brains, accompanied by transient proliferation deficits in neural progenitor cells. Deletion of Sox11 specifically in adult

NPCs blunted proliferation in the SGZ. Using functional genomics, we identified potential downstream target genes of Sox11. Conclusions: Taken together, our work provides evidence that Sox11 is required for both embryonic and adult neurogenesis.

Program/Abstract # 544

Expression of histamine receptors during midbrain development of rat embryos

Vargas Romero, Fernanda; Escobedo Avila, Itzel; Velasco Velazquez, Ivan (UNAM, Mexico)

During formation of the midbrain (Mb) one key event is differentiation of neurons, which takes place between embryonic days 12 to 16 (E12-E16). Histamine (HA) is one of the first neurotransmitters synthesized in the developing brain. Particularly, in the Mb region, the highest concentration of HA is found at E14-16, which coincides with neurogenesis in this cerebral region. Our group has shown that HA induces neuronal differentiation of cortical neural progenitors into FOXP2 neurons, an effect that is due to activation of HA receptor type 1 (HR_1). In the central nervous system, HA acts through activation of G-coupled protein receptors (HR_1-HR_3). Hybridization studies have shown that these HA receptors are expressed in the developing brain. However, the identity of the cells that express such receptors is unknown. In this work, we aimed to characterize the expression HR_1 and HR_2 during de first stages of Mb neurogenesis. We found that expression of HR_1 is homogeneous at E12 on Nestin-positive neural progenitors, and as development proceeds this expression is limited to the ventral-most region of the Mb. No co-expression of this receptor with Tuj1+ differentiated neurons was found. On the other hand, although the expression of HR_2 at E12 is found throughout the Mb in Nestin+ cells, as development proceeds, HR_2 is just expressed on the dorsal Mb. These results constitute a starting point to explore HA actions during Mb formation.

Program/Abstract # 545

Characterization of Medulloblastoma and Glioblastoma Variants with Molecular Markers of Neural Stem Cells

Toledo Hernández, Diana; Ponce Regalado, María Dolores; Lira Díaz, Eduardo; Stephenson Gussinyé, Tania; Esquivel Estudillo, Joel; Jaimes Jiménez, Venus Deyanira; Tenorio Mina, Andrea (UNAM-Cuernavaca, Mexico); Rembao Bojórquez, Jesús Daniel (UNAM, Mexico); Ocampo Roosens, Valeria; Ontiveros Nevares, Patricia (UNAM-Cuernavaca, Mexico); Pérez González, Oscar A. (UNAM, Mexico); Galvez Molina, Yolanda; Contreras Florencia, Armando; Santa Olalla Tapia, Jesús (UNAM-Cuernavaca, Mexico)

Medulloblastoma (MB) and Glioblastoma (GB) are the most aggressive and common of the Central Nervous System (CNS) tumors in children and adults, respectively. At present, there are some difficulties in establishing an accurate diagnosis of these neoplasms, due to their heterogeneous cell morphology and distinct histological patterns. On the other hand, tumors recurrences have shown conventional therapies to be ineffective, because they are not able to remove infiltrating or quiescent cells. There is evidence that has helped to explain therapeutic failures and recurrences of tumors. It demonstrates the existence of Brain Tumor Stem Cells (BTSCs), which were identified by Neural Stem Cell (NSC) markers. These BTSCs are capable of generating MB and GB, when transplanted into immunodeficient mice. We propose that BTSCs acquire features from NSCs that have been described during nervous system development. It is known these NSCs possess different proliferation rates and differentiation potentials that may be recreated by BTSCs, and have been identified by molecular markers that include growth factor receptors. Therefore, identifying these cells will allow determining the aggressiveness of MB and GB variants. This knowledge will be useful to establish a more accurate diagnosis; it will also help to propose novel therapeutic targets. We have already standardized and established immunohistochemical procedures to detect NSCs markers, such as Sox1, Sox2, LIFR, FGFR1, EGFR1 and BLBP. We have detected LIFR, FGFR1, EGFR1 and BLBP on three MB variants, and found different expression patterns between LIFR and FGFR1, on the most aggressive histological variants, which may correlate with poor clinical outcome.

Program/Abstract # 546

Transcriptomes of Proliferating Neural Stem Cells, Differentiating Progenitors and Newborn Neurons Identify Long Non-Coding RNAs as Novel Players in Corticogenesis

Perez, Julieta Aprea; Prenninger, Silvia; Wessendorf, Elke (CRT-Dresden, Germany); Ghosh, Tanay (Paris, France); Alexopoulou, Dimitra; Lesche, Mathias; Dahl, Andreas (CRT-Dresden, Germany); Groszer, Matthias (École des Neurosciences Paris, France); Hiller, Michael; Calegari, Federico (CRT-Dresden, Germany)

Transcriptome analysis of somatic stem cells and their progeny is fundamental to identify the molecular mechanisms regulating the transition from proliferation to differentiation. However, analysing transcriptomes of individual cell types in complex tissues remains a challenge. We generated a RFP/GFP double-reporter mouse line to isolate proliferating neural stem cells, differentiating progenitors and newborn neurons that coexist as intermingled cell populations in the developing cortex. Transcriptome sequencing of these three cell types revealed numerous uncharacterized protein-coding genes and several long non-coding (lnc)RNAs with highly specific and transient expression patterns. Most identified lncRNAs overlapped genes implicated in neurogenesis and shared with them a nearly identical expression pattern suggesting that lncRNAs control neural stem cell differentiation by regulating the expression of cell fate determinants. Finally, we investigated the function of one lncRNA during cortical development and found that it is involved in neurogenic commitment of neural progenitors as well as survival of newborn neurons. Our study provides the most comprehensive and quantitative transcriptome resource during the switch of neural stem from proliferation to differentiation to date and identifies crucial roles of lncRNAs during mammalian corticogenesis.

Program/Abstract # 547

Control of daughter cell proliferation in the embryonic CNS by Temporal, Hox and Notch cues

Bivik, Caroline; Baumgardt, Magnus; Karlsson, Daniel; Yaghmaeian, Behzad; MacDonald, Ryan; Gunnar, Erika; Thor, Stefan (Linkoping Univ, Sweden)

Substantial progress has been made with respect to cell fate specification in the nervous system. In contrast, less is known regarding the control of proliferation, such that proper numbers of each neural cell type is generated. In the embryonic *Drosophila* nerve cord, neuroblasts (NBs) generate the CNS by dividing asymmetrically, renewing themselves while budding off daughter cells, the ganglion mother cells (GMC). Each GMC in turn divides asymmetrically to produce two different neurons and/or glia. This is denoted a Type I division mode, because daughters divide once. Recent studies have identified an alternate division mode, where NBs bud off daughters that directly differentiate. We propose that this division mode should be denoted Type 0, since daughter cells do not divide. However, the extent of Type I and Type 0 proliferation in the CNS, and the extent to which NBs display switches in the proliferation modes were hitherto unknown. By mapping several specific NB lineages, and conducting a global analysis of division mode, we find that some half of all NB lineages in the nerve cord undergo a Type I to Type 0 switch. While Prospero plays a key role in controlling daughter cell proliferation in Type I, Pros does not direct Type 0 mode. The switch from Type I to Type 0 is combinatorially controlled by the temporal genes castor and grainyhead, the Hox gene Antennapedia and the Notch pathway. These regulatory cues are activated in the latter part of many lineages, thus ensuring proper temporal control. Analysis of 22 key cell cycle genes showed that the dacapo gene (p21CIP/p27KIP) is the key player triggering this switch. These findings reveal a novel global principle for proliferation control in the *Drosophila* CNS.

Program/Abstract # 548

Regulation of neural stem cell transition from symmetric to asymmetric cell division

Contreras Sepúlveda, Esteban; Brand, Andrea (Cambridge, UK)

Neural stem cells (NSCs) have the ability to divide symmetrically to amplify their pool and asymmetrically to self-renew and differentiate into neurons or glial cells. Precise control of NSC proliferation is essential for the proper development of the nervous system. Disrupting the balance between symmetric and asymmetric division can cause NSC overproliferation, triggering tumour formation, or premature differentiation, resulting in fewer neurons or glial cells. To understand how NSCs control the balance between amplification and differentiation we use the developing visual system of *Drosophila melanogaster*, the optic lobe, as a model. Neurogenesis in the optic lobe resembles the development of mammalian cerebral cortex. The optic lobe is composed of two types of NSCs: symmetrically dividing neuroepithelial (NE) cells and asymmetrically dividing neuroblasts (NBs). NE cells transform into NBs to generate optic lobe neurons in a similar manner than NE cells and radial glia in the cerebral cortex. Several signalling pathways (JAK/STAT, Notch, EGFR) have been described to regulate the transition from symmetric to asymmetric division, however, interactions between these pathways remain unclear suggesting that further regulatory components may be involved. To identify new genes controlling the switch from symmetric to asymmetric division, we took advantage of a transcriptome analysis of NE cells and NBs previously performed in the lab. We knocked down 35 genes by transgenic RNAi. Eight of these genes showed a phenotype, including two defects in adult optic lobe morphology and one impairment in NE cell to NB transformation, suggesting a role in the transition from symmetric to asymmetric cell division.

Program/Abstract # 549

Kif11 dependent cell cycle progression in radial glial cells is required for proper neurogenesis in the zebrafish neural tube.

Johnson, Kimberly A. (U Mass Amherst, USA); Moriarty, Chelsea; Tania, Nessy; Ortman, Alissa; DiPietrantonio, Kristina; Edens, Brittany; Eisenman, Jean; Ok Deborah; Krikorian, Sarah; Gole, Christophe; Barresi, Michael (Smith College, USA)

Radial glia serve as the resident neural stem cells in the embryonic vertebrate nervous system, and their proliferation must be tightly regulated to generate the correct number of neuronal and glial cell progeny in the neural tube. We recently identified the *kif11* zebrafish mutant during a forward genetic screen that displayed a significant increase in radial glial cell bodies at the ventricular zone throughout the developing spinal cord. Kif11, also known as Eg5, is a plus-end directed motor protein responsible for stabilizing and separating the bipolar mitotic spindle. We show here that Gfap+ radial glia express *kif11* throughout the ventricular zone and floor plate. Loss of Kif11 by mutation or pharmacological inhibition with S-trityl-L-cysteine (STLC) results in monoastral spindle formation in radial glial cells characteristic of mitotic arrest and accumulation of M-phase radial glia over time at the ventricular zone. Mathematical modeling of the radial glial accumulation predicted a delayed entry into the cell cycle and increased cell death in *kif11* mutants, which was supported by BrdU pulse-fix analysis and anti-activated Caspase 3 labeling, respectively. Lastly, we show that secondary interneurons, motorneurons, and oligodendroglia were significantly reduced following the loss of Kif11 function. We propose a model that Kif11 functions during mitotic spindle formation to facilitate the progression of radial glia through mitosis, which leads to the maturation of progeny into specific secondary neuronal and glial lineages in the developing neural tube. Currently we are testing this model with a genetic cell ablation line to determine whether radial glia are required for the formation of these cell lineages.

Program/Abstract 550

Withdrawn

Program/Abstract # 551

Evolutionarily Repurposed Networks Reveal the Well-Known Antifungal Drug Thiabendazole to Be a Novel Vascular Disrupting Agent and It Acts Through Microtubule-associated Proteins

Cha, Hye Ji; Byrom, Michelle (UT-Austin, USA); Mead, Paul (St Jude Chidren's Res Hosp, USA); Ellington, Andrew; Wallingford, John; Marcotte, Edward (UT- Austin, USA)

In the course of systematically identifying associations between genes and traits, we found gene modules that were shared between evolutionary distant organisms. In particular, genes responding to stress in yeast, which have no blood vessels, regulate angiogenesis in vertebrates. By analyzing such repurposed networks, we reasoned that small molecule inhibitors targeting the pathway in yeast might act as angiogenesis inhibitors suitable for chemotherapy in vertebrates. We computationally prioritized candidates based upon measured synthetic genetic interaction of known drugs. This strategy led to the finding that thiabendazole (TBZ), an orally available FDA-approved antifungal drug, also potently inhibits angiogenesis in animal models and in human cells. Moreover, TBZ disassembles pre-existing vessels, marking it as a Vascular Disrupting Agent and thus as a potential complementary therapeutic for use in combination with current anti-angiogenic therapies. *In vivo* imaging and a quantitative in vitro assay suggest that defects in vascular morphogenesis may stem from impairment of junctional integrity and endothelial cell behavior. Cellular phenotypes implied increased Rho signaling, and indeed, pharmacological disruption of Rho kinase elicited rescue of the TBZ-induced motility defect. TBZ had a very slight effect on the organization of the microtubule, but significantly reduced the abundance of tubulin and impaired microtubule-associated proteins. We also show that TBZ slows tumor growth and decreases vascular density in fibrosarcoma xenografts. Thus, an exploration of the evolutionary repurposed networks identified a potential new angiogenesis drug, and the organismal and cellular level analysis revealed its mode of action.

Program/Abstract # 552

Hyperexcitability and exaggerated activation of hypoglossal motorneurons in 22q11DS neonatal mice

Wang, Xin; Popratiloff, Anastas; Maynard, Thomas; Moody, Sally; LaMantia, Anthony; Mendelowitz, David Mendelowit (George Washington U, USA)

Approximately 80% of infants with developmental disorders, including DiGeorge/22q11Delection Syndrome (22q11DS), possess feeding/swallowing disorders that compromise nutritional status, impede mental and physical development and increased risk of respiratory infection due to aspiration. Feeding is composed of a complex series of oro-facial events including the tongue moving food from the mouth to the oro-pharynx cavity. Normal feeding and swallowing depends upon an initial sequential activation of hypoglossal motor neurons (XII MNs) that innervate the tongue, followed by inhibition to ensure appropriately timed, uni-directional swallowing. In this study we tested if activation of XII MNs in the brainstem upon electrical stimulation of afferent fibers to initiate fictive swallowing is altered in neonatal 22q11DS (Lgdel) mice. Electrical stimulation of afferent fibers in wild-type control animals evoked an excitatory post-synaptic current (EPSC) in XII MNs that was sufficient to increase the firing activity in these neurons, that was followed by an inhibition of firing. In contrast, in LgDel pups, activation of the swallowing reflex elicited a larger than normal EPSC in XII MNs, and a prolonged and exaggerated increase in firing in XII MNs, that was followed by a sustained increase in firing in XII MNs devoid of a post-stimulus inhibition. These preliminary results suggest activation of the swallowing reflex causes an exaggerated hyperexcitability that is not followed by a normal inhibitory phase in XII MNs from LgDel pups. This hyperexcitability may be a potential cause of the swallowing dysfunction that can occur in children and infants with 22q11DS.

Program/Abstract # 553

Modeling and computation of tissue growth driven by stem cell niches

Figueroa, Seth Amin; Ovadia, Jeremy; Nie, Qing (UC-Irvine, USA)

Formation and sustenance of a stem cell niche in stratified epithelia is key in controlling the tissue's growth, morphology and regenerative capabilities. Often, stratified epithelia develop advantageous finger-like structures, such as rete ridges (or rete pegs) in the epidermis and the palisades of Vogt in the limbal corneal epithelium, along which the stem cell niche forms. These structures provide the basal layer of the epithelia with better protection and allow the tissue a more efficient wound response. However, how these undulating structures are formed, and the role of the spatial aspects of the niche on its local environment, is not yet fully understood. Interesting questions that arise from this are: (i) How do extracellular cues and the tissue's underlying genetic system affect niche formation and tissue morphology? (ii) How does the tissue's morphology, in return, affect the dynamics of the cell lineage and the stem cell system's regenerative capabilities? Here we present a two dimensional multiscale model of stratified epithelial growth. The tissue growth model consists of stem cells, cell lineages and regulatory diffusive molecules. We have shown that stem cell niche development triggers distorted epithelial morphologies, similar to rete ridges, with stem cells accumulating along the tips, agreeing with experimental observations. Furthermore, we explore factors affecting niche formation and size, as well as potential biochemical regulations that can prompt formation and stabilization of an advantageous tissue architecture.

Program/Abstract # 554

A new landmark-independent tool for assessing and quantifying morphologic change and phenotypic variation.

Rolfe, Sara; Cox, Liza; Camci, Esra; Fu, Tina; Shapiro, Linda (U Washington, USA)

Embryologic and developmental biology studies have traditionally relied on subjective assessments of gross phenotypic changes, whether for description of normal changes during embryogenesis or how mutants differ from controls. However, as advances in multi-

dimensional imaging are made, there will be a growing need for tools that enable quantitative assessment of both embryonic and postnatal structure and form. To this end, we have developed a landmark-independent deformation-based registration algorithm that can utilize 3D surface images generated by any multi-dimensional imaging modality. Here, using different example tomographic datasets, we will demonstrate the utility and sensitivity of this tool: 1) to quantify morphologic change over a normal developmental time series of embryonic facial development acquired using optical projection tomography; 2) to determine the impact of both a transient localized genetic manipulation and an environmental (or epigenetic) change on facial morphogenesis; and 3) to detect subtle morphological phenotypes in postnatal specimens, in this case mandibles from control and mutant mice at a single postnatal time-point (acquired using micro-computed tomography). For all datasets, shape differences - between developmental stages, treatment groups, or wildtype and mutants - can be assessed using different means of regional clustering of the dense vector field. Because of the micron/sub-micron resolution of the imaging modalities and the voxel sensitivity of the computational algorithm, this method permits quantitative assessment of potentially biologically relevant changes in 3D shape whether natural, caused by mutation, or influenced by non-genetic factors. A web-based graphical user interface (GUI) has been developed such that the algorithms are openly available to the research community.

Program/Abstract # 555

The inverted cistern: a model for dorsal-ventral specification in the developing mouse limb

Arques, Carlos G; Torres, Miguel (Spanish National Centre for Cardiovascular Research, Spain)

An example of a gene expression domain whose emergence during development has lacked a satisfactory explanation is that of Lmx1b in the dorsal limb bud of the mouse embryo. This domain occupies the dorsal half of the limb bud mesenchyme, and its ventral limit matches reasonably well the central plane of the organ. The only molecule identified so far as being responsible for the induction of Lmx1b expression in the distal limb bud is Wnt7a, which diffuses from the dorsal ectoderm and is known to be a short-range signal. However, it has been suggested that such a signal cannot, on its own, account for the three-dimensional shape of the Lmx1b domain. In recent years, light has been shed on this question with the discovery of dorsal-ventral compartmentalization of the limb bud mesenchyme and key evidence has been presented showing that the Lmx1b expression domain is coincident with the dorsal compartment. To further expand our understanding of how dorsal-ventral patterning of the vertebrate limb bud takes place, we have devised a model that we call *inverted cistern*. In this model, dorsal-ventral compartmentalization is central to explain how a short-range dorsal ectodermal signal is able to generate a solid three-dimensional domain comprising the dorsal half of the limb bud mesenchyme. We have computer-simulated this model and the result satisfactorily matches what is known for the wild-type organ.

Program/Abstract # 556

Modeling somitogenesis with and without a clock

Belmonte, Julio M. (Indiana U-Bloomington, USA); Dias, Ana (University College London, UK); Susan, Hester (U of Arizona, USA); Clendenon, Sherry; Gens, Scott (Indiana U-Bloomington, USA); Stern, Claudio (Univ Coll London, UK); Glazier, James (Indiana U-Bloomington, USA)

During somitogenesis, the presomitic mesoderm (PSM) lying on either side of the central notochord divides into a series of roughly spherical epithelial segments called somites, which later will form the vertebrae, ribs and many muscles of the adult organism. The spatio-temporal periodicity and dynamic morphology of somitogenesis is believed to be regulated by the interaction of an oscillating regulatory network (the clock) with a molecular gradient (the wavefront). Identification of several oscillating proteins in the PSM leaded to the wide acceptance of the clock-and-wavefront model (CAW). Recent experimental results, however, demonstrated that normal somites, with correct size, shape, somite-specific gene expression and ability to form musculoskeletal derivatives, can be generated simultaneously from chick mesodermal cells with no cycling clock. We developed and compared two somitogenesis models. The first is a multi-cell, composite model that combines the current prevailing hypothesis of the CAW model, including ODEs submodels of the clock and Delta/Notch signaling, PDEs models for the Wnt3a and FGF8 determination front and a Boolean read-out mechanism. We identified inconsistencies between existing hypothesis and gaps in the current understanding of somitogenesis, most strikingly the lack of experimental evidence to connect clock components to the somite-formation process. In the second, we built a pure mechanistic model of mesenchymal-to-epithelial transition capable of forming somite-like structures. This model addresses many of the inconsistencies found in the current CAW model and is capable of reproducing the recent experiments where somites are formed without the presence an oscillating clock.

Program/Abstract # 557

A Boolean network model for Human sex determination

Rios Vargas, Osiris Yuriko; Torres Maldonado, Leda Carolina (Ins Nac de Pediatría, Mexico); Mendoza Sierra, Luis Antonio (UNAM, Mexico); Rodríguez Gómez, Alfredo; Frias Vázquez, Sara (Inst Nac de Pediatría, Mexico)

Human sex determination (HSD) is a complex biological process that encompasses sex chromosomes, differential gene expression of genes on autosomes and sex chromosomes, as well as interaction among their products during the embryonic development. These functional interactions conduct to normal development of testis and ovaries. Current understanding of the genetic control of HSD has arisen mostly from mutational and functional analyses of patients with disorders of sex development (DSDs). This process involves a large number of genes acting as a gene regulatory network (GRN), the alteration of the delicate interactions between these genes and their products may generate a DSD. However, the components and the dynamical behavior of this GRN are not fully known. Our

group is interested in inferring and studying the dynamics of the HSD-GRN. To this end we developed a discrete Boolean network, which describes the functional state of a gene or protein with as being as either "ON" (1) or "OFF" (0). For each node there is an associated logic function that describes the conditions for the node to be ON/OFF, depending on the regulatory inputs upon the node. The preliminary model consists of 23 nodes that integrate consecutive developmental stages that lead to male and female pathways of sex determination. Simulations of the GRN describe the commitment to male and female sex determination. We also simulated mutations on each component of the proposed GRN; many of those mutations coincide with DSD phenotypes such as 46, XX (SRY+) female-to-male sex reversal.

Program/Abstract # 558

The Cellular Potts Model for the spatio-temporal modelling of the root stem cell niche of *Arabidopsis thaliana*

Garcia Gomez, Monica Lisette; Azpeitia, Eugenio; Martinez, Juan Carlos; R. Alvarez-Buylla, Elena (UNAM, Mexico)

The root stem cell niche(RSCN) of *Arabidopsis* is the microenvironment that contains the organizer cells surrounded by stem cells, which self regenerate and give rise to the different cell types found in the root. Its organization is in part the result of genetic interactions and hormone action. Auxin has a gradient pattern and a maximum located at the RSCN organizer and it affects the gene regulatory network(GRN) underlying RSCN patterning. WOX5 is a key gene within this GRN and is expressed only in the RSCN organizer cells that signal stem cells, and its expression is indispensable to maintain the distal stem cells undifferentiated. Previous models described the dynamics of intracellular GRN or the transport dynamics of auxin and their independent critical role in RSCN cellular patterning. However, the coupling of these two processes during RSCN cellular patterning had not been addressed in previous models. Hence, previous models could not account for the dynamic positioning and size determination of the RSCN. In this study, we aim to understanding how the RSCN is located and maintained through the cooperative action of auxin signaling and WOX5 as a minimal model. To achieve this goal we are developing a multiscale model using the Cellular Potts Model (CPM) formalism, that allows the simulation of cells with intercellular transport dynamics of molecules that couple the dynamics of intracellular GRN. We build a model of the RSCN with the dynamics of auxin transport and the auxin signalling network with WOX5 as an auxin responding gene. With the model we aim at understanding how WOX5 is restricted to the RSCN organizer and we are able to put forward several novel predictions that could eventually be tested experimentally.

Program/Abstract # 559

Interactions between physical and molecular aspects during Arabidopsis thaliana root patterning

Hernández-Hernández, Valeria (UNAM-Veracruz, , Mexico); Barrio, Rafael; Garay, Adriana; Benitez, Mariana (UNAM, Mexico)

Among the key processes in plant morphogenesis are the spatio temporal regulation of cell proliferation, the formation of gradient morphogens (e.g., gradient concentration of auxin hormone) and physical fields (e.g., arising from tension and compression forces). Recent studies suggest that the polarization of auxin efflux transporters PIN-FORMED (PIN) at the plasma membrane, which are in turn largely responsible for the formation of auxin gradients, respond to physical forces that result from cell wall mechanical properties changes or the dynamics of cellular division. R Barrio and collaborators (accepted in Plos Comp Biol) postulated a mathematical model that aims at studying the organization of the root meristem of *Arabidopsis thaliana* by integrating polar auxin transport, cell cycle division, and a physical field that results from cellular elongation and division behaviors. This model reproduces the different zones of functional organization along the apical-basal axis as well as the reported longitudinal profile of cell reproduction rates. Because of the tight interactions between the different dynamics, changes in any of the processes modeled will affect the dynamics of the others and result, for example, in different meristem sizes. The present work explores the effects of changes in the parameters used in the mentioned model. Moreover, we test how changes in the physical field affect the polarization of PIN proteins and the resultant auxin gradients and apical-basal functional organization of the root. This will lay the foundations to generate novel predictions to be tested experimentally and to understand some of the observed variations in *A Thaliana* root as a result of changes in any of the modeled dynamics.

Program/Abstract # 560

Dynamic Network Model of Cell Cycle Control in *Arabidopsis thaliana*

Ortiz-Gutiérrez, Elizabeth; García Cruz, Karla Verónica; Castillo Jiménez, Aarón; Sánchez Jiménez, Ma de la Paz; Álvarez-Buylla, Elena (UNAM, Mexico)

Key to plant development is the modulation of cell division and cell differentiation during morphogenesis. In plants a complex regulatory network integrates extracellular and intracellular signals to modulate cell division, cell cycle arrest and endoreduplication. We propose a discrete model of cell cycle (CC) regulation using logical rules as a means to formally integrate data from plant, animal and fungal cells, to reproduce the dynamical behavior and properties of plant CC regulation in wild-type and mutant cells. Our model recovers gene and protein configurations that have been described during the proliferative cellular state as a cyclic attractor, and the endoreduplication entry as a fixed-point attractor. Given the high conservation of CC components and interactions among all eukaryotes, we suggest a minimal regulatory core that could underlie CC regulation in plants. Several components and interactions suggested in such core have not been documented yet in plants, but our model provides a formal framework to substantiate novel predictions concerning plant CC regulation. Our study further supports the overall conservation of the CC control mechanisms among eukaryotes and could be integrated with other gene regulatory models underlying cell differentiation to explore how cell differentiation/proliferation balance is achieved during organ development and growth.

Program/Abstract # 561

Rab23 is a novel regulator of epithelial morphogenesis, polarity and lumen formation

Gual Soler, Maria Margarita; Luo, Lin; Taguchi, Tomohiko; Venturato, Juliana (Inst for Molecular Bioscience, Australia); Bryant, David M.; Mostov, Keith E. (Univ of California, San Francisco, USA); Martin Belmonte, Fernando (Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Spain); Wicking, Carol; Stow, Jennifer L. (Inst for Molecular Bioscience, Australia)

The small GTPase Rab23 has been linked to developmental defects, vertebrate ciliogenesis and cancer, but its intracellular functions remain largely unknown. Here we show that GFP-Rab23 localizes on recycling endosome (RE) membranes and on the plasma membrane of epithelial Madin-Darby Canine Kidney (MDCK) cells. We explored the role of Rab23 in epithelial polarity and morphogenesis using loss and gain-of-function approaches in MDCK monolayers and cysts. In monolayer cultures, overexpression of GFP-Rab23 altered monolayer morphology by increasing cell height and packing, whereas siRNA-mediated Rab23 depletion resulted in mislocalization of adherens junction markers E-cadherin and ß-catenin, irregular monolayer morphology and altered tight junction integrity. However, the most profound defects were found in three-dimensional cyst cultures, in which GFP-Rab23 overexpression produced aberrant cysts with loss of cell polarity, absence of lumen and increased cell proliferation. Furthermore, *de novo* lumen formation was disrupted by overexpression of wild-type and a dominant-negative version of Rab23 (S23N), as well as by shRNA-mediated Rab23 knockdown. Both overexpression and depletion of Rab23 impaired the delivery of podocalyxin to the cyst interior and the generation of an apical surface to initiate the lumen. Together, our findings reveal a key role for Rab23 in polarity and lumen formation, suggesting that precise control of Rab23 dosage is critical for epithelial morphogenesis.

Program/Abstract # 562

Characterization of Combover/CG10732, a Novel *Drosophila* Rho Kinase Substrate and its Potential Role in Planar Cell Polarity Signaling

Fagan, Jeremy K. (Albert Einstein Coll Med, USA); Lu, Qiuheng; Adler, Paul (U Virginia, USA); Jenny, Andreas (Albert Einstein Coll Med, USA)

Cellular polarization is essential for nutrient transport, cell-cell communication and other cellular processes including spindle orientation during cell division, cell migration and cell differentiation. In addition to apical-basal polarity, polarity across the plane of an epithelium is a fundamental phenomenon required for the formation of complex tissues. This phenomenon is known as Planar Cell Polarity (PCP) and is controlled by the non-canonical Wnt/Fz-PCP signaling pathway. While some are known, most downstream effectors of the PCP pathway remain elusive. Particularly, it is not understood how Rho kinase, a known PCP effector also implicated in tumor cell migration, exerts its function. In a genome-wide Rho kinase substrate screen we identified Combover/CG10732 as a novel Rho Kinase substrate *in vitro*. RNAi knockdown of Combover in the *Drosophila* wing yielded a multiple wing hair phenotype, indicative of a defect in planar cell polarity signaling. Using mass spectrometry, we have identified several sites of Rho Kinase phosphorylation and site-directed mutagenesis has confirmed that these are bona-fide sites of Drok phosphorylation. Using a Yeast-2-Hyrbid approach, we have identified the planar cell polarity effector Multiple wing hair (Mwh) as a Combover interacting protein. We are currently performing GST pull-down and co-immunoprecipation experiments to support the hypothesis of a physical interaction between Combover and Mwh. A *combover* mutant allele generated by homologous recombination was homozygous viable that failed to phenocopy the initial multiple wing hair RNAi phenotype. Nevertheless, overexpression of both isoforms of Combover (RA and RB) causes a strong multiple wing hair phenotype observed when combined to wing specific Gal4 drivers. Importantly, this *mwh* phenotype can be enhanced by removal of certain members of the *mwh* group of PCP effector genes. Future work will further investigate the role of Combover as a novel Rho Kinase substrate and potential wing-specific planar cell polarity effector gene.

Program/Abstract # 563

Lens placode apical actin network: the role of PAR3 and ROCK

Melo, Maraysa de Oliveir; Borges, Ricardo; Yan, Chao (Univ de São Paulo, Brazil)

Vertebrate lens originates from pre-lens ectoderm, a simple cuboidal epithelium that overlies the optic vesicle. Thereafter, the pre-lens ectoderm cells elongate become columnar and form the lens placode.We showed previously that actomyosin filaments are distributed along the apico-basal cell sides of the chick pre-lens ectoderm and become enriched apically in the lens placode. Here, we investigate the role of the PAR complex in the apical localization of actin filaments. PAR3 is a scaffold protein that forms a complex with PAR6/aPKC and establishes and maintains epithelial cell polarity in a diverse set of models. PAR3, PAR6 and aPKC are already at the apical domain in the pre-lens ectoderm and remain apical during lens placode elongation. We overexpressed aPKCΔN and different deletion mutants of PAR6 in pre-lens ectoderm and these did not alter the polarization of the actin filaments. In contrast, PAR3(T833A) caused a slight increase in apical actin filaments in lens placode. PAR3(T833A) is not phosphorylated by ROCK and increases the stability of the PAR complex. Surprisingly, this same mutant significantly increased apical actin in the adjacent ectoderm as well. This phenotype is unexpected because the placode´s surrounding ectoderm does not polarize actin. Inhibition of ROCK by Y27632, disrupted the polarization of actin and myosin II but not of PAR3. Taken together, these data suggest that: 1) Phosphorylation of Thr833 in PAR3 inhibits actin polarization in non-placodal ectoderm; 2) ROCK activity is required for maintenance of actin polarization in the lens placode.

Program/Abstract # 564
Withdrawn

Program/Abstract # 565
ADAM10 and ADAM19 proteolytically process Cadherin6B during epithelial-to-mesenchymal transitions of the cranial neural crest
Schiffmacher, Andrew T.; Taneyhill, Lisa (U Maryland, USA)
Epithelial-to-mesenchymal transitions (EMTs) occurring in chick cranial neural crest cells permit these cells to delaminate from the neuroepithelium *en masse*, acquire motility, and emigrate from the dorsal neural tube. Epithelial adherens junction disassembly is an essential component of this EMT process. Within the premigratory neural crest domain, N-cadherin and Cadherin6B (Cad6B) are downregulated prior to completion of EMT, and this loss of protein is necessary for proper cranial neural crest emigration. While Cad6B downregulation is controlled in part by Snail2-mediated transcriptional repression, we hypothesize that post-translational mechanisms are also utilized to promote the observed rapid turnover of Cad6B protein due to its usual long half-life. For the first time *in vivo*, we demonstrate the presence of a putative N-terminal fragment (NTF) generated by Cad6B proteolysis that correlates with a reduction in full-length Cad6B and the onset of EMT. Importantly, we note the concomitant appearance of two C-terminal cleavage fragments (CTFs) that are absent in embryos treated with a broad-spectrum metalloproteinase inhibitor (GM6001). By co-expressing relevant proteases with Cad6B *in vitro*, we identified that A Disintegrin and Metalloproteinases (ADAM) ADAM10 and ADAM19, along with γ-secretase, cleave Cad6B to give rise to the NTF and CTFs previously observed *in vivo*. As both ADAMs are expressed in the appropriate spatio-temporal pattern to process Cad6B *in vivo*, they were assessed for Cad6B proteolytic activity *in vivo*. Overexpression of either ADAM within premigratory cranial neural crest cells prior to EMT results in premature loss of Cad6B protein. Together, these results suggest a two-part mechanism for Cad6B proteolysis involving ADAM10, ADAM19, and γ-secretase during cranial neural crest cell EMT.

Program/Abstract # 566
Ephrin signaling maintains apical adhesion of neural progenitors
Davy, Alice; Arvanitis, Dina (CBD, France); Behar, Annie (IPBS, France); Tryoen-Toth, Petra (INCI, France); Bush, Jeff (UCSF, USA); Jungas, Thomas (CBD, France); Vitale, Nicolas (INCI, France)
Apical neural progenitors are polarized cells whose apical membrane is the site of cell-cell and cell-extracellular matrix adhesion events that are essential to maintain the integrity of the developing neuroepithelium. Apical adhesion is important for several aspects of the nervous system development including morphogenesis and neurogenesis, yet the mechanisms underlying its regulation remain poorly understood. Herein, we show that ephrin-B1, a cell surface protein that engages in cell signaling upon binding cognate Eph receptors, controls normal morphogenesis of the developing cortex. Efnb1 deficient embryos exhibit morphological alterations of the neuroepithelium which correlate with neural tube closure defects. Using loss-of-function experiments by ex vivo electroporation we demonstrate that ephrin-B1 is required in APs to maintain their apical adhesion. Mechanistically, we show that ephrin-B1 controls cell/ECM adhesion by promoting apical localization of integrin-beta1 and we identify ADP-ribosylation factor 6 (ARF6) as an important effector of ephrin-B1 reverse signaling in apical adhesion of APs. Our results provide evidence for an important role for ephrin-B1 in maintaining the structural integrity of the developing cortex and highlight the importance of tightly controlling apical cell/ECM adhesion for neuroepithelial development.

Program/Abstract # 567
The regulation of epithelial cell adhesive forces by the MID1/Alpha4/PP2Ac complex and its implications for cleft lip susceptibility
Cox, Timothy C. (U Washington, USA), Huang, Yongzhao; Koto, Cathy (Seattle Children's Res Inst, USA)
The embryonic orofacial epithelium facilitates the fusion of converging facial prominences that is essential for proper oronasal form and function. Failure to properly fuse these prominences results in cleft lip/palate (CLP), one of the most common birth defects, yet little is known about the role of causative genes. MID1 is a microtubule-associated E3 ubiquitin ligase, the loss of which results in a syndromic form of CLP. MID1 interacts strongly with Alpha4, the mammalian homolog of yeast Tap42. As in yeast, Alpha4 binds the catalytic subunit of protein phosphatase 2A (PP2Ac) to uniquely regulate its activity and that of the TOR pathway. We report here that perturbation of MID1 function in polarized MDCK cells results in reduced cell-cell adhesive strength and altered cell-ECM interactions, with concomitant changes in mTOR phosphorylation, Rac1 activation, and phospho-FAK levels. In non-polarized cells, disruption of MID1 reduces adhesion to laminin but not collagen, and perturbs collective migration. Taken together, our data suggest that mTOR/Rac1 signaling is the downstream pathway affected by the MID1/Alpha4 axis in the regulation of epithelial cell adhesion. In support of the importance of this epithelial function of MID1 during formation of the upper lip, in ovo electroporation of the same dominant-negative form of MID1 into chick pre-fusion orofacial epithelia was found to result in cleft lip in this species. Ongoing studies using fabricated micropost technology are aimed at determining the relative contributions of different downstream effectors of MID1 and quantifying their impact on intercellular adhesive forces. These studies provide the first clues as to the cellular mechanisms responsible for CLP.

Program/Abstract # 568

A novel RIPK4 - IRF6 connection is required to prevent cadherin-mediated epithelial fusions characteristic for popliteal pterygium syndromes

Vleminckx, Kris; De Groote, Philippe; Tran, Hong Thi; Fransen, Mathias; Rosselet, Corinne; Tanghe, Giel; Vandenabeele, Peter; Lippens, Saskia; Declercq, Wim (Ghent University, Belgium)

Genetic studies have linked the genes encoding the kinase RIPK4 and the transcription factor IRF6 to the popliteal pterygium syndrome (PPS). RIPK4- or IRF6-deficient mice have epidermal defects and fusion of epithelia similar to PPS in humans. So far, there is no mechanistic explanation for the RIPK4 deficiency phenotype and it is unclear whether IRF6 and RIPK4 function in the same pathway. We found that epithelial fusions in RIPK4 knockouts are associated with abnormal periderm development and ectopic localization of E-cadherin on the apical membrane of the outer peridermal cell layer. In *Xenopus*, morpholino-mediated depletion of zygotic derived RIPK4 resulted in cloacal fusion, which reflects the anal fusion observed in RIPK4-/- mice. Interestingly, similar to the phenotype described upon IRF-6 depletion, we observed a severe gastrulation block and abnormal cadherin localization when depleting both maternally- and zygotic-derived RIPK4. This gastrulation defect was rescued by ectopic expression of wild type human RIPK4, but not by RIPK4 mutants identified in PPS or kinase-dead mutants. In addition, we found that RIPK4 expression levels are controlled by IRF6 and ectopic RIPK4 expression can rescue the gastrulation defect in *Xenopus* caused by dominant negative IRF-6. In conclusion, we show that RIPK4 is implicated in the regulation of E-cadherin membrane expression, which can explain epithelial fusions characteristic for PPS. We also provide a novel molecular link between IRF-6 and RIPK4 that unifies the different PPS to a common molecular pathway.

Program/Abstract # 569

Quantitative analysis of cell arrangement indicates the early differentiation of neural region during *Xenopus* gastrulation

Yamashita, Satoshi; Michiue, Tatsuo (University of Tokyo, Japan)

Cell adhesion property changes along its differentiation, causing specific rearrangement of cells within the tissue under morphogenesis. Any such different cell arrangement must be a result of change of the adhesion property. Thus the cell arrangement is expected to be an indication of differentiation and phenotype. However, there is no established system, neither theoretical nor experimental, to describe general cell arrangement. In order to evaluate cell arrangement quantitatively, we used a mathematical graph model in which cell arrangement is simplified to the adjacency relationship. We introduce a new index of cell arrangement defined in term of how much of different particular small graphs are included in the graph of cells. The index, represented by vectors, enables the comparison of areal cell arrangements. A border between areas with different cell arrangement is expected to correspond to a border between differentiating regions. In an analysis of *Xenopus* ectoderm, the border was detected in an area between dorsal and ventral region at late gastrula, and moved toward dorsal midline at early neurula, similar to the behavior of a prospective neural region. The graph model of cell arrangement was shown to be able to distinguish difference in cell arrangements which are hard to tell by intuitive analysis of microscopic images. The behavior of cell arrangement border suggests that it corresponds to a neural-epithelial border, and the neural differentiation at late gastrula.

Program/Abstract # 570

Withdrawn

Program/Abstract # 571

The lack of catalase is not essential for mouse development but alters glucose and lipid metabolism in the adult

Perez Estrada, Jose Raul; Cuevas-Benítez, Osiris; Hernández-García, David; Covarrubias, Luis (UNAM-Cuernavaca, Mexico)

Reactive oxygen species (ROS) can influence cell physiology by two general mechanisms: damaging macromolecules or playing a role as second messengers. Both can cause cell death, but the second may be relevant in the control of developmental cellular processes. We have shown that during development high ROS levels correlate with areas of massive cell death. In particular, during digit individualization the expression of genes encoding several antioxidants enzymes (e.g. Gpx4, Sod1-3, catalase) are down-regulated in the interdigital regions, which could be the cause of the increase in ROS. To study in more detail the role of ROS in cell death during development, we generated mice carrying a null mutation in the catalase gene (Cas-/-). Although the catalase gene is abundantly expressed during development, Cas-/- mice are viable and do not show any apparent developmental defect, and increase in oxidative damage is not evident in adult tissues. Nonetheless the above and in contrast with wild type (WT) mice, Cas-/- mice fed with a high fat diet (HFD) do not become hyperglycemic and do not accumulate lipids in the liver. Consistently, the expression of many lipogenic genes (e.g. Acc-1, Fas and Pparγ) decreased in Cas-/- mice fed with HFD, while the expression of the gene encoding Aco1, an enzyme required for beta-oxidation, increased compared with obese WT mice. These data suggest that at least H_2O_2 can influence metabolic pathways that may be relevant during development. Despite a limb phenotype is not evident in Cas-/- mice, it will be interesting to determine whether the time and space pattern of interdigital cell death and digit individualization are altered in the absence of catalase. Supported by PAPIIT IN225910/IN209813

Program/Abstract # 572

Withdrawn

Program/Abstract # 573
Fluoride induces apoptosis in Sertoli cells in vitro
Erkan, Melike (Istanbul Univ, Turkey)
Fluorine is potent toxicant, widely distributed through drinking water and food. Fluorine is not freely found in nature, it combines with almost all metals and nonmetals, except oxygen and the noble gases. Fluoride is found in drinking water and foods threat to the environment and human health. Although several hypotheses have been proposed, the exact mechanism of fluoride has not been clearly defined. Fluoride accumulation leads to a large number of hematological, hepatic, renal and neurological disorders. Although research has been carried out on the effects of fluoride on the reproductive organs and reproductive system impairment in animal models, the results are controversial. Increasing exposure of fluorides on daily basis might have a potential negative impact on male fertility, including spermatogenesis and sperm fertilizing ability. However, the effects of fluoride on reproductive organs are not fully understood. The present study was aimed at determining the direct effects of sodium fluoride on Sertoli cell viability, proliferation, cytotoxicity and apoptosis/necrosis rate *in vitro*. In this study, sodium fluoride was exposed to TM4 Sertoli cells for 24, 48 and 72 hours at 2 ppm and 16 ppm doses. After incubation, the treated cells were used for measurement of cell viability, proliferation, lactate dehydrogenase assay and propidium iodide and Hoechst stain for apoptosis/necrosis rate. The results indicated that cell viability and cell proliferation are decreased, while lactate dehydrogenase levels and apoptosis/necrosis rate are increased. These findings suggest that direct exposure to fluoride could induce cytotoxicity and resulting in the apoptosis of Sertoli cells.

Program/Abstract # 574
Telomere biology in the switching of reproductive modes in planarian Dugesia ryukyuensis
Nodono, Hanae (Keio Univ, Japan); Aboobaker, Aziz (Oxford, UK); Matsumoto, Midori (Keio Univ, Japan)
Eukaryotic chromosomes get shorter as cells divide and cells senesce when telomeres shorten to a critical length, which is hypothesized to be a contributory factor to ageing and mortality in sexually reproducing organisms. However, some eukaryotes can reproduce both sexually and asexually. It is unclear whether reproductive mode and ageing correlates or not. To ask this question, we focus on freshwater planarians. They reproduce either asexually, by fission, or sexually, by cross-fertilization. Several planarians use exclusively one mode of reproduction; others might alternate between them. *Schmidtea mediterranea* is one of the former species, comprised of obligate asexuals and sexuals. Recently, it was showed that asexuals recover age-related telomere shortening through fission, whereas sexual worms only achieve telomere elongation through sexual reproduction. This difference correlated with the expression level of an active spliceform of the telomere reverse transcriptase subunit (*Smed-tert*). Our model species *Dugesia ryukyuensis* is comprised of populations with different reproductive strategies: exclusively innately asexual, exclusively innately sexual, and seasonally switching. Moreover asexuals can be sexualized by feeding them with minced sexuals. In this report, we compared telomere biology of these strains. In contrast to *S. mediterranea*, *D. ryukyuensis* sexual worms showed a slight telomere shortening. Then a *tert* homolog (*Dr-tert*) was identified and characterized. Though two alternate splice sites were identified like *Smed-tert*, all strains expressed high-level of active isoform, which correlated with their telomere length dynamics and telomerase activity. These results suggest the existence of immortal sexually reproducing animals that can maintain their telomere length.

Program/Abstract # 575
Effect of maternal glucocorticoid exposure on mouse embryonic development
Lee, Ji-Yeon Lee; Yun, Hyo Jung; Kim, Jongsoo; Kim, Myoung Hee (Yonsei Univ Coll of Med, Rep of Korea)
Prenatal stress is known to cause intrauterine growth retardation and physiological dysfunctions in various aspects. In addition, many of the diseases associated with prenatal stress exhibit a sex bias. Perturbations and vulnerability to prenatal stress are often more profound for the male fetus, however, the mechanisms responsible for this relationship are not clear. We previously have shown that administration of dexamethasone at gestational day 7.5~9.5, a critical time point on early placenta development, induces placental defects as well as embryonic growth restriction. In the present study, we further examined the dexamethasone-induced morphological changes during fetal growth and investigated the influence of prenatal glucocorticoid exposure on sex-specific gene expression and male gonad development. Compared with the control group, 30% of the experimental group exhibited homeotic transformation and other alterations of the axial skeleton. Moreover, embryos exposed to dexamethasone showed smaller rib cages and varying degrees of xiphoid process protrusion. Here we will present these skeletal malformations and morphological development of the testes in male mouse fetuses treated in utero with dexamethasone.

Program/Abstract # 576
The Effect of Methylmercury on Neural Gene Expression in Zebra Finch Development
Murray, Jessica R.; Ramos, Claire; Cristol, Dan; Saha, Margaret (College of William and Mary, USA)
Mercury is a highly toxic pollutant that adversely affects neural development. Preliminary data reveal that male juvenile zebra finch (Taeniopygia guttata) learn a less complex and lower pitched song after developmental exposure to methylmercury. Zebra Finches are the most commonly used laboratory songbird species, yet their embryological development has been poorly characterized. Most studies to date apply Hamburger and Hamilton stages derived from chicken development, however significant differences exist between development of the two species. Here we provide the first detailed description of embryological development in zebra finch under standard artificial incubation. Once the embryology was characterized, expression of key song learning genes (e.g., FoxP2) in Zebra Finch embryos whose parents had been raised on a diet containing low, biologically relevant levels of mercury was

characterized. We used in situ hybridization to compare spatiotemporal gene expression patterns between unexposed control embryos and embryos developmentally exposed to 1.2ppm and 2.4ppm methylmercury. Levels of gene expression of FoxP2 were quantified using qRT-PCR. Preliminary results suggest no significant differences between treatment groups, however we also note that embryos had a great deal of biological variation in FoxP2 levels at the stages analyzed. To further examine the effects of prenatal methylmercury exposure, we quantified the levels of FoxP2 expressed in juvenile male brains and show that a subset of juvenile males prenatally exposed to 2.4ppm MeHg has significantly lower FoxP2 levels. We have also conducted microarray analysis to identify additional genes and pathways that could potentially affect song learning.

Program/Abstract # 577

Adaptation to hydrogen sulfide induces a reversible developmental plasticity in *C. elegans*

Fawcett, Emily; Miller, Dana (U Washington, USA)

Developmental plasticity, a phenomenon where early environmental conditions dictate adult phenotypes, is generally believed to increase the likelihood of survival. However, there may be negative consequences if the future environment is different than predicted. Understanding the molecular underpinnings of reversible plastic phenotypes will provide insight into how individuals respond to a changing environment. We discovered that transient exposure to low levels of the gas hydrogen sulfide (H_2S) allows for survival of otherwise lethal H_2S concentrations in *C. elegans*. We have found that memory of H_2S requires the SWI/SNF chromatin-remodeling complex, suggesting that the response to H_2S stimulates epigenetic adaptations with long-lasting consequences. Remarkably, we have shown that H_2S memory is reversible. The memory of H_2S adaptation is erased by brief periods of fasting, but not by exposure to other environmental stresses, including hypoxia and heat shock. These results suggest that there is a specific interaction between the fasting response and H_2S memory. Consistent with this model, we show that the insulin/IGF1-like signaling (IIS) pathway modulates the persistence of H_2S memory in fasting. While loss-of-function mutations in the FOXO transcription factor DAF-16 enhances the effects of fasting on H_2S memory, constitutive activation of DAF-16 protects against fasting-induced memory loss. Taken together, our results demonstrate that the cellular response to nutrient availability through the IIS pathway is required for the persistence of H_2S memory upon nutritional stress. We are currently working to understand the molecular underpinnings that integrate IIS signaling with the epigenetic memory of adaptation to H_2S.

Program/Abstract # 578

The Effects of Cadmium and Temperature on Zebrafish Development

Warren, Kerri S.; Subramaniam, Janani; Stevenson, Laura (Roger Williams Univ., USA)

Climate change and chemically-contaminated water increasingly impact coastal aquatic ecosystems and the effects of small temperature change fluctuations on developmental metal sensitivity are not well understood. This project utilized a cadmium toxicity bioassay in zebrafish embryo-larvae to examine the combined effect of low-level cadmium exposure with otherwise-tolerable temperature shifts. Zebrafish embryos were exposed to 0, 0.5 and 5um of cadmium chloride ($CdCl_2$) at 25 and 32C and examined from day 2- day 5 of development. General morphology and developmental progress was assessed as were specifics of cardiovascular development. Vasculogenesis and angiogenesis were characterized using transgenic endothelial cell green fluorescent protein (GFP) zebrafish, vessel maturation and patterning with alkaline phosphatase assays, and vessel integrity and patency with microscopic observation. Temperature-specific outputs included increased rate of developmental progression and elevated heart rate and metabolism. Cadmium-specific, temperature insensitive parameters included cadmium-induced cardiac arrhythmia and cranial hemorrhage. The combined effect of cadmium and temperature, however, reduced cadmium-tolerance more universally, with increased rates of edema, necrosis and disaggregation. These results suggest very low levels of metal contamination of coastal waters pose a significant future environmental threat.

Program/Abstract # 579

Macondo crude oil from the Deepwater Horizon oil spill disrupts specific developmental processes during zebrafish embryogenesis

Chen, Diane; Kesich, Lydia-Rose; de Soysa, Yvanka; Ulrich, Allison (Smith College, USA); Friedrich, Timo (UMass Amherst, USA); Pite, Danielle (Smith College, USA); Compton, Shannon (UMass Amherst, USA); Ok, Deborah; Bernardos, Rebecca (Smith College, USA); Downes, Gerald (UMass Amherst, USA); Hsieh, Shizuka; Stein, Rachel; Lagdameo, Maria Caterina; Halvorsen, Katharine; Barresi, Michael (Smith College, USA)

On April 20th 2010, the Deepwater Horizon oil platform sank, spilling approximately 4.93 million barrels of oil and making it the largest accidental marine spill in history. Even though the oil has, for the most part, stopped flowing, concerns have been raised about the effects of crude oil on the marine flora and fauna in the Gulf. Since utilizing native species is difficult due to issues of availability, genetic variation, and domestic husbandry, a model organism must be used to assess the effects of these compounds on embryonic development. Since development is conserved across species, the Zebrafish (*Danio rerio),* with its ease of maintenance, large clutch size, and completely sequenced genome, is well suited suited to model the teratogenic effects of toxins. We investigated the effects of the water accommodated fraction (WAF) of crude oil on the development of the zebrafish embryo, and found several defects in treated embryos which had been previously seen, such as cardiovascular and craniofacial deformities, which we postulate could be the result of impaired cranial neural crest cell development. In addition, we also discovered a novel locomotor phenotype, perhaps as a result of observed deformities in slow muscle, as well as a reduction in the sensory axons of the peripheral nervous system and

oligodendrocytes of the central nervous system. We have also observed possible estrogenic effects, which we are currently investigating further. Thus, our research supports and expands on what is known about the effect of crude oil on the developing embryo, and allows us to model what might have happened and will happen to native fish species in the Gulf.

Program/Abstract # 580

Isolation and Characterization of an ethanol sensitive zebrafish mutant

Lovely, Charles B. (UT- Austin, USA); Ackerman, Matt (U Indiana-Bloomington, USA); Henegar, Taylor (St. Edwards Univ, U SA); Eberhart, Johann (UT-Austin, USA)

Fetal ethanol exposure causes a wide range of developmental defects that can include lower jaw hypoplasia. Cranial neural crest cells generate much of the craniofacial skeleton, including the lower jaw. They migrate from the dorsal neural tube and condense in the pharyngeal arches, where complex interactions between the neural crest cells and the adjacent epithelia are crucial in proper jaw formation. While there is a greater understanding of effects of ethanol dosage and timing, little is known of the genetic predisposition to ethanol-induced developmental defects. Using zebrafish, we performed a forward genetic screen to identify ethanol-sensitive mutants, from which we recovered *uta-15*. Under control conditions, *uta-15* embryos have no apparent craniofacial defects. Upon exposure to 1% ethanol, 25% of embryos exhibit lower jaw hypoplasia. *uta-15* embryos are most sensitive to ethanol between 24-48 hours post fertilization (hpf). By 32 hpf, the neural crest cells that generate the lower jaw have properly condensed into the pharyngeal arches and no defects in endoderm morphology were apparent. At 36 hpf, expression of oral ectoderm markers was normal, as was that of the ventral neural crest markers, *hand2* and *msxe*. However, two neural crest markers necessary for proper lower jaw formation, *barx1* and *nkx3.2*, were misexpressed. In ethanol treated *uta-15* embryos expression of *barx1*, which labels the prechondrogenic condensations in the arches, was lost in the first pharyngeal arch and reduced in the subsequent arches. Additionally, *nkx3.2*, which labels the jaw joint, appears reduced. Whole genome sequencing has identified a 20 Mbp interval on chromosome 14 that contains the lesion and we are currently narrowing this interval. These data suggest a model where ethanol interacts with *uta-15* altering the expression of the specific neural crest markers necessary for proper lower jaw development. Overall, this work will provide greater insight into gene-ethanol interactions and will allow for better diagnosis and treatment.

Program/Abstract # 581

High sucrose ingestion during the critical window of the pancreas modifies vascular contractility leading to metabolic syndrome and hypertension in adult rats

Guarner, Veronica; Rubio-Ruiz, Maria Esther; Perez-Torres, Israel (Inst Nac de Cardiologia "Ignacio Chávez", Mexico); Diaz-Diaz, Eulises (Inst Nac de Ciencias Medicas y Nutricion "Salvador Zubiran", Mexico)

Adverse conditions during early stages may permanently modify the function of the organism having consequences that are adaptive in early life but may lead to metabolic syndrome (MS) in adult life. The effects of modified diets during early stages upon susceptibility to hypertension in adults haven't been studied. There is a critical period of pancreatic development (CPPD) in the rat (postnatal days 12 to 28) with changes in plasma insulin and glucose. We study aortic vasoreactivity in rats during CPPD and compare it to vasoreactivity in control and MS rats. We also study if high sucrose ingestion during CPPD modifies vascular contractility leading to hypertension. Newborn male Wistar rats were divided into four groups: a) rats receiving sucrose 30% in drinking water during CPPD, b) sucrose after CPPD until 6 months, c) sucrose during and after CPPD (MS rats), d) without sucrose. Glucose and insulin were higher during suckling and decreased after weaning. NE induced contraction increased from day 12 to 21 but stabilized form day 21 to day 28. Vaso-relaxation to acetylcholine didn't change during the neonatal period. Changes were smaller than in MS rats. Adult rats that had received sucrose during the critical window and after it had increased blood pressure as adults. NE-contraction was similar to controls and relaxation was diminished. NO sinthase expression was increased while responses to endothelin receptor blockers were not modified. In conclusion, there is a postnatal critical window in vaso-reactivity that accompanies that of the pancreas and variations are smaller than in MS rats. A high sucrose diet during the critical window of the pancreas predisposes to the development of hypertension in the adult.

Program/Abstract # 582

Pharmacological doses of biotin administered during the post-weaning period accelerate morphological and functional development of pancreatic islet

Flores-Aguilar, Maura; Díaz-Martínez, Emmanuel; Fernández-Mejía, Cristina (UNAM, Mexico)

Biotin is a water-soluble vitamin that acts as a coenzyme of carboxylases. Unrelated to this role, pharmacological concentrations of biotin has a wide repertoire of effects on syste mic processes such as development and carbohydrate metabolism. In previous studies we found that after eight weeks of biotin supplementation in the diet increases the expression of genes regulating insulin synthesis, also increases insulin secretion and augment the proportion of β cells. Furthermore in our studies the administration of the biotin began in the weaning stage, a critical peri od in the islet development, we try to demonstrate if it is possible that the changes produced by biotin supplementation observed at eight weeks of diet administration are carried out during post-weaning period. To test this hypothesis, male BALB/cAnN Hsd mice were fed with control or a biotin-supplemented diet over seven days post-weaning (0.8 or 100 mg biotin/kg). At the end of treatment we found that the isolated islets from vitamin-supplemented mice presents glucose-stimulated insulin secretion due to an enhancement expression of the protein regulating glucose sensing (Glut2), whereas the control group did not respond to stimulation as usual in immature neonatal islet. Consistent with these effects, glucose and insulin tolerance

was improved, and post-prandial glucose was lower. We observed that in biotin supplemented animals, the islet was closer to achieve the adult typical morphology due to a faster cell remodeling as well a major expansion rate of β cell mass. The augmented proportion of β cell mass is caused by the increase of proliferation and neogenesis. This remarks finding in our data suggest that biotin accelerates postnatal maturation of islet.

Program/Abstract # 583

***Drosophila* survival can be altered by protein diet**

Pena Rangel, Maria Teresa; Riesgo, Juan (UNAM-Querétaro, Mexico)

Regulation of metabolism is a central feature of life. This encompasses growth, development, sexual maturation, fertility, health, survival, and overall lifespan. Dietary composition is critical in the conformation of the ultimate organismal energy balance. In many organisms like *C. elegans*, *D. melanogaster*, mice, and primates feeding under caloric restriction that do not lead to overt malnutrition has been found to extend lifespan. Reduced insulin signaling can also increase the longevity in flies and rodents. Despite this, little has been done to test the effect on survival of diets with different amounts of proteins. To test the effects of dietary protein restriction on survival in wild type flies, we evaluated isocaloric normal and low protein diets with or without the addition of methionine using both molasses-yeast based standard food recipe, and a chemically defined fly diet formulation. Our results show increased survival with the addition of methionine in the low protein molasses-yeast diet. We are currently extending the effects of the same dietary conditions on mutant genotypes of the insulin-signaling pathway.

Program/Abstract # 584

Influence of Dietary Minerals on Sex Determination of Mice Embryos

Faqih, Reham; Alhimaidi, Ahmad (King Saud Univ, Saudi Arabia)

The embryo sex preselection before pregnancy received considerable attention at the level of human and animal with a controversy over the years. The application of the embryo sex preselection production systems have become essential at the level of human and animal and increasingly for medical and economical interest. There are many ways to preselect the sex of the embryos, including specific meals and minerals which it is perfect because of its simplicity, low cost and ethical popular acceptance. The aim of this study is investigate of the influence of dietary minerals on sex determination of mice embryos. Mice food formula was prepared by ARASC animal food company, for male preselection (Na and K) was increased about 20% more than the regular or control mice diet, while for female preselection (Ca, Mg and P) were increased about 20%. The three experimental animal SWR/J mice (Male, female preselection and the control group) were contain 11 females, each 2or 3 females were housed with one male. The offspring data were collected, liver samples and blood samples were collected from each group. Data were satirically analyzed by GraphPad InStat Program soft wear. The result show a significant differences ($P < 0.05$) in the male preselection diet group (Na, K) they produce (53 males 62.6%) compared to the female offspring (30 female 37.2%). While the data show no significant differences in the female or control groups. In the blood parameters the results also showed the existence of significant differences at ($P<0.05$) in the average measurements of GLU between the control group (mean= 5.75) and of females group average (8.33) and average male group (m = 6.50).While there was no significant differences in all other blood parameters and the liver enzymes (GOT and GPT) between the three groups. So the method for male diet of sex preselection increase, with less side effect in the mothers. *This project were supported by King Saud University/ College of Science.

Program/Abstract # 585

Study of behavior in a Herpes simplex virus UV radiation environment

Arriaga Garza, Jesús; Torres López, Ernesto (UANL, Mexico); Castaño Meneses, Victor Manuel (UNAM-Queretaro, Mexico); Belmares Perales, Sergio; Elizondo Villarreal, Nora (UANL, Mexico)

The objective of this study is to analyze the behavior of herpes simplex virus 1 and 2 in an atmosphere of ultraviolet radiation in the band of wavelengths (185-230) nm and at various exposure times, all this in order to inhibit replication of said virus.

Program/Abstract # 586

Ultraviolet B radiation induces DNA damage and cell cycle impairments in embryos of freshwater prawn *Macrobrachium olfersi*

Zeni, Eliane; Silva, Heloisa; Maia, Guilherme; Müller, Yara; Ammar, Dib; Nazari, Evelise (Univ Fedl de Santa Catarina, Brazil)

Aquatic environments are highly vulnerable to the impact of ultraviolet B (UVB) radiation that reaches the surface of the Earth. There is evidence that high levels of UVB radiation have harmful effects on the DNA. *Macrobrachium olfersi* is a freshwater prawn that lives and reproduces in clear shallow waters. During the breeding season, the females of this species carry yolky eggs in an external brood pouch until they hatch. Therefore, the eggs are exposed to environmental conditions during all embryonic stages. The aim of this study was to investigate whether UVB radiation induces DNA damage and impairments on cell cycle in embryos of *M. olfersi*. Thus, we simulated the natural UVB irradiance (310 $mW.cm^{-2}$) that embryos receive during the breeding season. Embryos at early post-naupliar stage (E5) were irradiated using a UVB (312 nm) lamp 6W for 30 min. After, the embryos were kept for 2 days under natural photoperiod, and analyzed at E7. Non-irradiated embryos were used as controls. After irradiation procedures we observed, using flow cytometry and immunohistochemistry, DNA damage, specifically the formation of cyclobutane pyrimidine dimer. This damage was accompanied by a decrease of proliferation on embryonic cells. When we analyzed the proteins involved on the cell

cycle, no changes were observed in expression of p21 and proliferating cell nuclear antigen (PCNA) after irradiation. Since that DNA lesion may be caused by different damaging agents, we investigate if lesions caused by UVB radiation activate the p53 pathway. UVB irradiated embryos showed an overexpression of p53. Our data indicates that after irradiation, embryos at E7 were not able to repair DNA damage, but may activate a possible apoptosis pathway.

Program/Abstract # 587

Spectral confocal imagining with aberration correction as a tool for 3D rendering from whole mouse embryos

Moody, Sally A.; Popratiloff, Anastas; Oakley, Beverly; Maynard, Thomas; Meechan, Daniel; Wang, Xin; Mendelowitz, David; LaMantia, Anthony (George Washington U, USA)

Although confocal microscopy has been broadly used for acquiring 3D data sets from whole embryos, a number of barriers limit the utility of this approach. Tissue transparency, auto-fluorescence and spherical aberration are among the leading restrictive factors. In an effort to refine existing approaches, we combined: use of clearing agents; proper aberration correction; and use of spectral confocal imaging with linear unmixing. Using this combination of refined approaches we investigated the utility of 3D registration of confocal data sets from whole mouse embryos. E10-11 day mouse embryos double immunolabeled (ICC) for neurofilament and PCAM or embryos expressing endogenous GFP were used. ICC embryos were cleared with BABB (benzyl alcohol, benzyl benzoate, 1:2) and imaged from a glass depression chamber in BABB with coverslip. Fixed embryos expressing GFP were cleared with Scaleview A2 solution. GFP fluorescence was imaged from whole embryos with or without a coverslip. Imaging was performed with multi-immersion 25x/0.8 objective lens using Zeiss 710. Using spectral linear unmixing the auto-fluorescence was readily separated from Alexa Fluor 488 and 647, thus allowing for unobstructed visualization of the blood vessels with PCAM and cranial nerves with neurofilament antibodies. Using a matching refractive index between the point source and the objective lens minimized the spherical aberration and provided reliable 3D registration. A similar approach was taken to produce 3D sets of scale-cleared embryos. A combination of tile- and z-scanning allowed for generation of 3D data sets from large areas of the embryo, while also preserving the resolution at a fine scale including individual axons and growth cones.

Program/Abstract # 588

Doxycycline-controlled and Recombinase-Activated Gene OverexpressioN (DRAGON): an intersectional strategy for targeting precise and reversible gene expression in mice

Zeni, Eliane; Silva, Heloisa; Maia, Guilherme; Müller, Yara; Ammar, Dib; Nazari, Evelise (Univ Fedl de Santa Catarina, Brazil)

The combination of tissue-specific Cre drivers and Cre-responsive mouse lines has allowed the development of sophisticated genetic approaches for studies of gene function in the mouse. However, it is very common that the cell subpopulation of interest is not defined by the expression of a single marker, but by a combination of at least two. Here we present the initial characterization of a strategy that utilizes a *Rosa26* knock-in allele to express a gene only in cells defined by the combination of tissue-specific Cre activity and regulated (r)tTA activity. We name this system Doxycycline-controlled and Recombinase-Activated Gene OverexpressioN (DRAGON). This intersectional strategy achieves two important goals: 1) expression of a gene is restricted to the subpopulation of cells that expresses Cre and (r)tTA; and 2) temporal control of gene expression is provided via doxycycline administration, allowing a gene to be turned on or off at will. The DRAGON allele is also designed with fluorescent markers to identify cells that have undergone Cre recombination and in which (r)tTA is active, while a different fluorescent protein is expressed in the remaining (Cre–) cells in which (r)tTA is active, enabling the different cell populations to be characterized or isolated for further molecular analysis. With great utility, the DRAGON system takes advantage of the growing number of Cre(ER) and (r)tTA alleles available, with the potential for almost unlimited cell-type specific targeting. In conclusion, this approach should provide the opportunity to address biological questions in vivo that were previously hampered by technical limitations.

Program/Abstract # 589

Chemical control of Wnt/b catenin signalling during development

Gonzalez Malagon, Sandra Guadalupe; Liu, Karen (King's College London, UK)

The ability to manipulate protein function is very important when studying biological events. A classic approach is to genetically eliminate the protein of interest. Although "knockout" models have been most successful in biological research, there are drawbacks. For example, if the protein has a significant role at different stages of development, the knockout model may not survive to the stage of interest. To overcome this problem, alternative conditional strategies have been developed; but again, they are limited. Small molecules would be ideal, as these have the advantage of being reversible and can be administered at specific time points. Nevertheless, the huge challenge is to find small molecules that are specific, with minimal secondary effects. We are testing a novel approach that makes use of engineered chemically regulated destabilizing domains. These domains confer drug-dependence to a protein of interest. In the absence of drug, the protein is rapidly degraded. We are assessing the advantages of this technology by targeting the key players of the Wnt signalling pathway, including b-catenin and GSK3b, which are both essential during development. In this way we aim to develop a highly useful tool for biologists in diverse research fields that will provide the advantages of specific and reversible control of protein function.

Program/Abstract # 590

In vivo imaging of *Xenopus laevis* development using an Ultra-Compact MRI

Huebner, Kelli R. (Knox College, USA); McDowell, Andrew (ABQMR, Inc., USA); Thorn, Judith (Knox College, USA)

Magnetic resonance imaging (MRI) is a noninvasive technique used to reconstruct a three dimensional image of an intact subject. MRI is necessary for *in vivo* imaging of internal development of *Xenopus laevis* embryos because they are opaque. An Ultra-Compact MRI (UC-MRI) was created specifically for imaging of *X. laevis* development in place of a superconducting MRI system which is both large and expensive for a research setting. The UC-MRI has successfully imaged various events in *X. laevis* development without affecting embryo viability. Germinal vesicle breakdown in a progesterone treated oocyte has been imaged over 13 hours. Early cell cleavage and gastrulation have been imaged in a time series both with and without a T1-weighted contrast agent— gadolinium chelated with diethylenetriaminepentaacetic acid (Gd-DTPA; MAGNEVIST). This identifies morphological changes to the blastocoel and epibolic movement of the ectodermal sheet. Furthermore, a chart of internal images throughout various stages of development which correlates to the external pictures of the Nieuwkoop and Faber series (1994) has been assembled. Current and future experiments involve manipulating the dorsal/ventral axis patterning to see if failure to gastrulate can be detected internally before phenotypic changes occur and also *in vivo* fate mapping of tissues using magnetic nanoparticles.

Program/Abstract # 591

Highly effective ex vivo gene manipulation to study kidney development using self-complementary adeno-associated viruses (scAAV)

Zhou, Qin; Chen, Tielin; Wang, Honglian (West China Hospital, China); Gao, Guangping (U Mass Med Sch, USA)

Ex vivo cultures of intact embryonic kidneys have become a powerful system for studying renal development. However, few methods are available for gene manipulation in those ex vivo intact tissue cultures and have impeded identification and studies of genes in this developmental process. Here we demonstrate that self-complementary adeno-associated virus（scAAV）is highly effective in delivering and expressing genes into the deep tissues of cultured kidney and that the effectiveness varies with different scAAV serotypes. These findings are expected to expedite and expand use of the ex vivo embryonic kidney cultures for kidney development research and our understanding of Kidney development.

Program/Abstract # 592

Withdrawn

Program/Abstract # 593

Development and evolution of vertebrate external genitalia

Martin Cohn (U Florida, USA)

Few morphological characters in the animal kingdom rival the diversity, complexity and evolvability of the external genitalia. Among animals with internal fertilization, genital morphology evolves rapidly, and the size, shape and anatomical details of the external genitalia can show dramatic variation, even between closely related species. External genital development begins with the initiation of paired genital swellings that merge to form the genital tubercle, the precursor of the penis and clitoris. While some aspects of an ancient appendage development network, including Shh, Fgf10-FgfR2, Wnt5a, Hoxa/d13 and Bmps, are involved in outgrowth and patterning of the genital tubercle, other genes known to play central roles in limb development, such as Fgf8, play no role in genital development. In addition to proximodistal outgrowth and dorsoventral patterning, the genital tubercle of mammals undergoes tubular morphogenesis of the endodermal epithelium to form the urethral tube. Formation of a closed urethral tube goes awry at a high frequency in humans, with incomplete closure (hypospadias) affecting ~1:250 live births. Urethral tube closure is a relatively recent evolutionary innovation that occurs only in mammals; in non-mammalian amniotes, the phallus forms an open groove (sulcus) rather than a tube. Turtles form such a phallus with a sulcus whereas squamate reptiles (snakes and lizards) have paired phalluses, known as hemipenes, situated on either side of the cloaca. In birds, the phallus was reduced or lost in ~97% of species. The developmental mechanisms responsible for the evolution of external genitalia are unknown. By integrating mouse developmental genetics with comparative studies of external genital development across a range of taxa, we are gaining new insights into the molecular mechanisms of external genital defects in mammals and the diversification of genital form during vertebrate evolution.

Program/Abstract # 594

Cardiac gene regulatory networks in health and disease

Romaric Bouveret, Mirana Ramialison, Reena Singh, Nicole Schonrock (Victor Chang Cardiac Res. Inst., Australia); Antoine Bondu (U Libre de Bruxelles, Belgium); Li Xin, Gavin Chapman, Sally L. Dunwoodie (Victor Chang Cardiac Res. Inst., Australia); Cedric Blanpain (U Libre de Bruxelles, Belgium); Richard P. Harvey (Victor Chang Cardiac Res. Inst., Australia)

Our previous studies have identified a number of highly conserved transcriptional factors (TFs), signalling molecules and non-coding RNAs that are the controlling elements of the gene regulatory networks essential for heart specification, morphogenesis and function. However, the architecture of the cardiac gene regulatory networks as they evolve in time and space remains ill-defined. Progress in this area is essential for understanding metazoan biology and for realising the promise of new therapies for a host of diseases. The cis-regulatory interactions between TFs and their target gene promoters/enhancers is a major determinant of network architecture. We

have begun to document the targets of a large number of developmentally-active and tissue-restricted cardiac TFs, and mutant versions, using the DamID technique and the HL-1 cardiomyocyte cell line, which beats homogeneously in culture. Our findings suggest that ubiquitous TFs are embedded within the cardiomyocyte network at an executive level, and that mutant TFs bind a surprising number of targets through co-factor interaction, including to hundreds of "off-targets" never seen by the wildtype factor and which may play a role in congenital heart disease pathology.

Program/Abstract # 595

Defining mechanisms of imprinted expression at Igf2r/Airn during mouse gastrulation

Marcho, Chelsea; Bevilacqua, Ariana; Veeramani, Swarna; Mager, Jesse (Univ of Massachusetts-Amherst, USA)

Genomic imprinting is an epigenetic mechanism resulting in differential gene expression in a parent-of-origin manner. In the mouse genome, there are approximately 100 imprinted genes, many of which occur in imprinted gene clusters. DNA methylation and histone modifications have been shown to be allele specific at imprinted loci, corresponding with imprinted (allele specific) expression. At the Igf2r/Airn cluster (which contains 3 maternally expressed genes Igf2r, Slc22a2, and Slc22a3), the paternally expressed non-coding RNA Airn is thought to be responsible for silencing the paternal alleles of Igf2r, Slc22a2, and Slc22a3, resulting in reciprocal imprinting at the locus. Here we examined the changes in imprinted gene expression in a tissue-specific and time-dependent manner during gastrulation. We found that in embryonic tissue prior to gastrulation, Igf2r is biallelic and Airn is not expressed. Once gastrulation commences, Igf2r and Airn become reciprocally expressed and imprinted. To examine the epigenetic mechanisms involved in the establishment of Igf2r/Airn imprinting, we characterized changes in DNA methylation by bisulfite sequencing of two differentially methylated regions (DMR) at the locus. Consistent with ES cell models, we found spreading of DNA methylation at DMR2 during the start of gastrulation, corresponding to the changes in expression. Additionally, expression of CTCF, a factor involved in imprinting regulation at the H19/Igf2 locus, is only present once gastrulation begins, suggesting CTCF may also play a role in imprinting at Igf2r/Airn. Our data suggest a model similar to H19/Igf2 in which DNA methylation changes at onset of gastrulation, blocking the binding of the methylation sensitive factor CTCF, which allows paternal Airn expression and silencing of paternal Igf2r.

Program/Abstract # 596

Vascular plastic response in the primary somatosensory cortex of birth-enucleated rats

Silvia Zenteno De León, Raquel Martínez Méndez, Gabriel Gutiérrez Ospina (UNAM, México)

Blind individuals display an expansion of the primary somatosensory cortex (S1) and increments of its vascular density. Even though both S1 expansion and increased vascular density in birth enucleated rats are thought to result from increased sensory experience, we have recently published evidence that sensory experience is not increased in birth-enucleated rodents (Fetter-Pruneda et al., 2013). The mechanisms leading to S1 vascular plasticity must then be carefully revised. We then evaluated vascular density in 7 days old (PD7) and adult rats enucleated at birth and in those having intact sight. Although we confirmed previous results showing increments in vascular density in the adult S1 of birth-enucelated rats, we were able to see such increments only in 20% of the sampled birth enucleated rats at PD7. Interestingly, this change occurs when all birth enucleated rats had already displayed an expanded S1. Our results thus support that increased vascular density is a consequence of S1 expansion in birth enucleated rats and that is not necessarily related with increments in the inflow of sensory experience. Financial aid came from CONACYT 82879.

Program/Abstract # 597

Morphogenesis in the sea urchin: linking dynamically remodeling network states to protease function in development of skeletogenic and non-skeletogenic mesoderm

Lyons, Deirdre C.; Dougherty, Mark; Saunders, Lindsay; McClay, David (Duke University, USA)

In the sea urchin embryo 64 Primary Mesenchyme Cells (PMCs) synthesize a calcium carbonate skeleton that defines the shape of the larva. The PMC lineage comprises 4 cells at the vegetal pole in the early embryo. These cells then divide, invade the blastocoel via an epithelial-to-mesenchymal transition (EMT), and undergo directed migration in response to ectodermal cues. Next, PMCs fuse forming a syncytium within which the skeletal rudiment is secreted and subsequently elaborated by branching and elongation. Previous research established a gene regulatory network (GRN) for PMC specification. We focus on uncovering subroutines controlling morphogenetic processes. We and others identified subsets of transcription factors in the PMC GRN that are necessary for behaviors such as cell invasion, migration, cell fusion and skeletal patterning. Using GRN analysis and time-lapse imaging we connect these subnetworks to cell biological machinery proteins that carry out specific cellular processes. For example, metalloproteases are necessary for invasion, migration and cell fusion in many cell types in other systems. We find that several families of metalloproteases are dynamically expressed in the PMCs during EMT, migration and fusion, and are even expressed by certain PMC nuclei at the growing rod tips. These patterns suggest a highly regulated spatiotemporal program of extra cellular matrix (ECM) modification by the PMCs during successive step of differentiation. This work establishes the PMCs as a model for studying how a dynamically remodeling GRN launches successive cellular behaviors within a single cell type. Furthermore, protease expression is also observed in non-skeletogenic mesodermal cell types that are known to undergo later EMTs and remodel the ECM presumably to enable cell migration and tissue fusion. Treating embryos with MMP inhibitors causes the PMCs to fail to make skeleton, and prevents proper mouth formation and pigment cell differentiation, which involve non-skeletogenic mesoderm. These data suggest that proteases play a

role in several important developmental processes, and ongoing work incorporates these "morphoeffector" proteins into the sea urchin GRN.

Program/Abstract # 598

Sexually Dimorphic Fin Development: Implications for the Evolution of Intercourse

O'Shaughnessy, Katherine (University of Florida, USA); Dahn, Randall (Madison, WI, United States); Cohn, Martin (University of Florida, USA)

Claspers are extensions of the basal skeleton (basipterygium) of pelvic fins and are the most primitive vertebrate intromittent organs. These structures are found in the fossil record extending back to arthrodires, jawed fishes that predate the origin of sharks by 25 million years. Today only male members of the class Chondricythes develop claspers, and their morphology is remarkably similar to those found in the most basal jawed vertebrates. Using the Little Skate (Leucoraja erinacea) as a model system, this study seeks to determine the molecular mechanisms involved in formation of this important evolutionary novelty. Analysis of clasper skeletogenesis using Sox9 expression and alcian blue staining demonstrates that the clasper skeleton (myxopterigium) undergoes a prolonged period of outgrowth, suggesting that the fin development circuit may remain active in the posterior region of the pelvic fin bud in males. Further analysis of Hoxd12, Hoxd13, Shh, and Fgfs by in situ hybridization also demonstrates prolonged expression in male fins when compared to stage-matched females. As claspers are sexually dimorphic organs, we next investigated whether steroid hormone receptors were present in the posterior fins of these fishes. Interestingly levels of androgen receptor (AR) transcript and protein in the posterior pelvic fin bud were higher in males, suggesting that androgen signaling may regulate clasper development. These results suggest that the earliest male copulatory organs may have evolved by a hormonally regulated modification of the fin development program.

Program/Abstract # 599

(Epi)genetic regulation of apomixis: learning from sexual experience

Jean-Philippe Vielle-Calzada, (Group of Reproductive Development and Apomixis, Laboratorio Nacional de Genomica para la Biodiversidad, CINVESTAV Irapuato, Mexico)

Although life of nearly all organisms is organized around sex and breeding, Darwinian thinking focused more on the struggle for existence than on evolutionary significance of this frantic race to reproduce. In plants, a major consequence of this search for survival is the evolution of a multitude of reproductive alternatives that have intrigued botanists, geneticists, and evolutionary biologists for more than 100 years. Because sexually derived genetic diversity is interpreted as essential for adaptation, it is often thought that sex is necessary for the perpetuation of a species; however, many organisms, including several hundred families of flowering plants, are going efficiently about propagating their kind without bothering with meiosis and mating. Apomixis is a natural form of asexual reproduction through seeds that leads to viable offspring genetically equivalent to the mother plant. New evidence from sexual model species indicates that the regulation of female gametogenesis and seed formation is dependent on epigenetic mechanisms that are crucial to regulate gametic cell specification and differentiation, a hallmark for the understanding of the developmental events that distinguish sexuality from apomixis. In the ovule of *Arabidopsis thaliana*, gametogenesis initiates from a single haploid product following meiosis of a specific subepidermal cell. We have found that the ARGONAUTE9 (AGO9) PIWI/PAZ protein defines a small RNA-dependent regulatory pathway that controls female gametogenesis by restricting the specification of gamete precursors in a non-cell autonomous manner. Mutations in several loci of this pathway lead to differentiation of multiple cells that initiate gametogenesis by a mechanism reminiscent of apomixis. I will present our latest findings related to the genetic, epigenetic and molecular characterization of the AGO9 pathway, opening the possibility that the developmental distinction between sexual development and apomixis might have evolved as an adaptive response to evolutionary forces that modulate structural variation and reproductive versatility in flowering plants.

Program/Abstract # 600

The somites act as a signalling centre during the emergence of haematopoietic stem cells

Aldo Ciau-Uitz; Philip Pinheiro; Catherine Porcher; Roger Patient (University of Oxford, UK)

Haematopoietic stem cells (HSCs) that sustain adult blood production are created from specialised endothelial cells, haemogenic endothelium, localised in the embryonic dorsal aorta (DA). In *Xenopus* embryos, using lineage labelling experiments in conjunction with gene expression analysis and genetic manipulation, we have determined the location of the HSC precursors through development and established a cellular hierarchy leading to the generation of HSCs in the DA. A striking anatomical feature in the development of HSCs is that at any given time, HSC precursors are either in contact with or in close proximity to the somites. At the molecular level, we have found that the somites do indeed play critical roles in the programming of HSCs. The earliest HSC precursors are detected just after neurulation and they are located adjacent to the ventral side of the somites; these cells are characterised by the expression of VEGFR2 but, critically, VEGFA is made in the somites and the generation of adult haemangioblasts in the dorsal lateral plate (DLP) mesoderm is dependent on VEGFA ligand produced in the somites. Later, HSC precursors migrate from the DLP to the midline, where they coalesce to form the DA and eventually HSCs. During migration, the somites prime these precursors to be programmed as haemogenic endothelium once they arrive at the midline. Thus, the somites serve as a key signalling centre for the programming of HSCs.