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Abstracts: Society for Development Biology 64th Annual Meeting

1. MicroRNAs and Their Regulatory Roles in Plants and Animals. David P. Bartel. Massachusetts Institute of Technology and Whitehead Institute for Biomedical Research.

MicroRNAs are endogenous ~22-nucleotide RNAs that can play important regulatory roles by pairing to the messages of protein-coding genes to specify mRNA cleavage or repression of productive translation. We are using molecular and computational approaches to identify microRNAs in plants and animals and then investigate their functions. My talk will highlight recent findings, many of which result from close collaborations with other laboratories. Potential topics include: the genomics of microRNAs in invertebrates, vertebrates, and plants; expression profiling of microRNAs in mammals, which supports the idea that most microRNAs are pol II products and that many are processed from the same transcripts as neighboring microRNAs or flanking exonic elements; the antiquity of microRNA-directed regulation in plants and the essential roles of this regulation during *Arabidopsis* development; improved methods for computational identification of microRNA regulatory targets in animals, which indicate that more than a third of the human genes are conserved microRNA targets; the typical features of metazoan microRNA target sites and ideas on the purpose and scope of microRNA-mediated gene regulation.

2. Epigenetic Control of the X Chromosomes by the *C. elegans* MES Proteins Methylation of Histone Tails. Susan Strome,[1] Laurel Bender,[1] Jinkyo Suh,[1] Coleen Carroll,[1] Wenchao Wang,[1] Taryn Phippen,[1] Ru Cao,[2] and Yi Zhang[2]. [1]Dept. of Biology, Indiana Univ., Bloomington, IN 47405; [2]Dept. of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Center, Univ. of North Carolina, Chapel Hill, NC 27599.

The *C. elegans* MES proteins are required for germline survival and fertility. One role of the MES system appears to be silencing the X chromosomes in the hermaphrodite germline. MES-2 and MES-6, orthologs of the Polycomb Group chromatin repressors E(Z) and ESC, exist in a complex with their novel partner MES-3. We have demonstrated that the MES-2/3/6 complex is responsible for di- and trimethylation of histone H3 on Lys27 in the adult germline and in embryos. MES-dependent H3-Lys27 marks are lightly distributed on autosomes and concentrated on the Xs. Our results suggest that MES-mediated methylation of H3-Lys27 represses expression of genes on the X chromosome and probably at autosomal sites as well. This regulation is essential for normal early germline development. Development of the soma appears to be less dependent on H3-Lys27 methylation, as mes mutant embryos at all stages display greatly reduced levels of Lys27 methylation but nevertheless develop into healthy (albeit sterile) adults. MES-4 functions independently of the MES-2/3/6 complex and has a unique distribution: associated with the autosomes and absent from most of the length of the X. Antibody staining results suggest that MES-4 also functions to methylate histone H3 tails. Our working model is that MES-2/3/6 and MES-4 operate, perhaps antagonistically, at the level of histone modification to regulate chromatin states and gene expression patterns in the germline.

3. Solving the Signal-Regulated Enhancer: A Structure–Function Approach. Scott Barolo, Paulette Ference, Christina Rogers, Maya Subbarao, and Krista Golden. Univ. of Michigan Medical School, Ann Arbor, MI.

Signaling pathways affect cell fate mainly by controlling the expression of pathway target genes via enhancers (*cis*-regulatory elements). Although many signal-regulated enhancers have been studied, basic questions about these regulatory elements remain unanswered. This is best illustrated by the fact that synthetic versions of well-characterized enhancers nearly always fail to drive gene expression in vivo. Therefore, it seems that we do not yet know all of the component parts of the enhancer or its basic structure. We are investigating the structure and function of several well-studied signal-regulated enhancers via *Drosophila* transgenic reporter assays. We find that the known transcription factor (TF) binding sites within these enhancers are not sufficient for transcriptional activation in vivo, even when these sites are placed in their native arrangement and spacing. Therefore, additional unknown regulatory factors appear to be required for the activity of each of these enhancers. We are currently pursuing the identities and functions of these novel factors. We have also found interesting differences between enhancers that respond to signaling in a tissue-specific manner and those that respond "universally" to a pathway. We find that signal-regulated TF binding sites isolated from tissue-specific enhancers are not able to activate transcription; however, binding sites for the same TFs, when isolated from universally responding enhancers, *are* able to respond to signaling in vivo. Thus, the difference between a universal and a tissue-specific response to signaling may depend on the sequence of individual TF binding sites.

4. Lmp4 Regulates Tbx5 Transcriptional Activity by Controlling its Nuclear Localization. Hans-Georg Simon,[1] Troy Camarata,[1] Andre Kulisz,[1] Teng-Leong Chew,[2] Ben Bimber,[1] and Jennifer Yeung[3]. [1]Department of Pediatrics, Northwestern University, Feinberg School of Medicine, and Children's Memorial Research Center; [2]Northwestern University, Feinberg School of Medicine; [3]Northwestern University.

Congenital malformations of the limbs and heart as seen in Holt–Oram syndrome are caused by mutations in the Tbx5 gene. The encoded Tbx5 transcription factor has been shown to play essential roles during the development of limbs and heart in virtually all vertebrates. In order to elucidate how Tbx transcription factors interact with other proteins within a regulatory network, we have identified a novel PDZ-LIM domain scaffolding protein, Lmp4, that specifically binds Tbx5. The co-expression in the limbs and heart and co-localization within a cell suggest a conserved Lmp4 function in both developing organs. In the presence of Lmp4, Tbx5 shuttles out of the nucleus and via Lmp4 localizes to actin filaments. To test

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whether the re-localization from nuclear to cytoplasmic sites interferes with downstream gene expression, we employed limb and/or heart-specific Fgf10- and Anf-promoters fused to luciferase reporters and demonstrate that Lmp4 acts as a repressor of Tbx5 activity. In addition, we show that an intact actin cytoskeleton is essential for this transcriptional repression. These studies reveal a previously unknown mechanism for transcription factor regulation in vertebrate limb and heart development and provide a better understanding of the molecular basis of hand/heart birth defects. This work is supported by NIH grant ES012725-01.

5. Studies on an RNAi-like Mechanism of Germline DNA Elimination in *Tetrahymena thermophila*. Kazufumi Mochizuki and Martin A. Gorovsky. Department of Biology, University of Rochester, Rochester, NY 14627.

During formation of the somatic macronucleus (MAC) from the germline micronucleus (MIC) in *T. thermophila*, ~15% of the DNA is eliminated by deletion of ~6000 internal eliminated sequences (IESs). We proposed a "scan RNA model" based on our discovery of conjugation-specific 28 nt (scn)RNAs that hybridized to IESs and an argonaute family protein, Twi1p, that migrated from the old to the new MAC and was required for accumulation of the scnRNAs and sequence elimination. In this model, MIC transcripts formed dsRNAs that were cleaved to form scnRNAs; scnRNAs entered the old macronucleus and scanned the sequences therein; scnRNAs homologous to MAC DNAs were destroyed; the remaining, MIC-specific scnRNAs, were transferred to the developing new MAC to target IESs for elimination. Subsequent studies support the model. (1) A dicer-like protein was shown to enter the MIC when it is transcribed during conjugation and is required to produce scnRNAs from bidirectional IES transcripts. (2) Twi1p was shown to be associated with scnRNAs in both the old and the developing MAC. (3) Specificity of the small RNAs for IESs was shown to increase as conjugation proceeds. (4) Foreign sequences introduced specifically into the MIC were shown to be eliminated during MAC development by a mechanism whose efficiency improved as the foreign sequence became more repeated. Additional studies showed that the RNAi-like mechanism by which IESs are identified for elimination involves methylation of K9 on histone H3 and has other features of a highly conserved pathway for gene silencing/heterochromatin formation in fungi, plants and animals.

6. Geminin Regulates Neuronal Differentiation by Antagonizing Brg1 Activity. Kristen L. Kroll,[1] Anabel Herr,[2] Jong-Won Lim,[1] Genova A. Richardson,[1] Helena Richardson,[2] and Seongjin Seo[1]. [1]Washington University School of Medicine, St. Louis, MO 63110; [2]Peter MacCallum Cancer Centre, East Melbourne, Victoria, 300, Australia.

Precise control of cell proliferation and differentiation is critical for organogenesis. Geminin (Gem or Gmnn) has been proposed to link cell cycle exit and differentiation as a pro-differentiation factor and plays a role in neural cell fate acquisition. Here, we identified the SWI/SNF chromatin remodeling protein Brg1 as an interacting partner of Gem. Brg1 has been implicated in cell cycle withdrawal and cellular differentiation. Surprisingly, we discovered that Gem antagonizes Brg1 activity during neurogenesis to maintain the undifferentiated cell state. Down-regulation of Gem expression normally precedes neuronal differentiation and gain- and loss-of-function experiments in *Xenopus* embryos, and mouse P19 cells demonstrated that Gem was essential to prevent premature neurogenesis. Gem's differentiation-blocking activity depended upon its ability to bind Brg1 and could be mediated by Gem's inhibition of proneural bHLH–Brg1 interactions required for target gene activation. Our data demonstrate a novel mechanism of Gem activity, through regulation of SWI/SNF chromatin remodeling proteins, and indicate that Gem is an essential regulator of neurogenesis that can control the timing of neuronal progenitor differentiation to maintain the undifferentiated cell state.

7. Baf60c Integrates Notch Signaling at the Mouse Node to Initiate Left–Right Asymmetry and Regulate Node Morphology. Benoit G. Bruneau,[1] Jun K. Takeuchi,[1] Heiko Lickert,[2] Masamichi Yamamoto,[3] Hiroshi Hamada,[3] and Janet Rossant[2]. [1]Hospital for Sick Children, and University of Toronto, Canada; [2]Samuel Lunenfeld Research Institute, and University of Toronto, Canada; [3]Developmental Genetics Group, Osaka University, and CREST, Japan Science and Technology Corporation (JST).

Smarcd3 encodes the BAF chromatin remodeling complex subunit Baf60c. Mouse embryos in which Smarcd3 function is reduced by RNA interference had randomized heart situs and lost expression of key left–right (LR) asymmetry genes in the lateral plate mesoderm (LPM). Expression of the secreted protein Nodal around the node is a key early step in the initiation of LR asymmetry in the mouse embryo. The LPM of Smarcd3 knockdown embryos was competent to respond to Nodal, suggesting that LR defects arose at the node. Indeed, Nodal expression at the node was defective, as was node formation, including alignment of the node at the midline and nodal cilia function. Smarcd3 is expressed in Nodal-expressing perinodal cells, and its expression overlaps that of Notch signaling molecules, which are required for Nodal expression in perinodal cells. In cell culture, Baf60c potentiated activation of the Nodal node enhancer by activated Notch signaling and strengthened a three-way interaction between the intracellular component of Notch, its DNA-binding partner RBPj, and Brg1, the catalytic subunit of the BAF complex. Thus, the chromatin remodeling factor Baf60c integrates Notch signaling at the Nodal node enhancer to initiate the LR cascade and direct nodal morphogenesis, providing a mechanism for enhancing cell type specificity of morphogenetic signaling mechanisms. Funding: CIHR.

8. EGF Receptor Signaling and Dorso-Ventral Axis Establishment in *Drosophila*. Trudi Schupbach,[1] Jennifer S. Goodrich,[1] K. Nicole Clouse,[1] Uri Abdu,[2] Martha Klovstad,[1] Attilio Pane,[1] and Kristina Wehr[1]. [1]HHMI, Dept. of Molecular Biology, Princeton University, Princeton, NJ 08544; [2]Dept. of Life Sciences, Ben Gurion University, Beer Sheva 84105, Israel.

The spatial patterning of the egg and embryo of *Drosophila* depends on signaling events that occur during oogenesis. In particular, the gene *gurken* (*grk*), which encodes a secreted molecule with homologies to TGF-alpha like growth factors, plays an important role in transmitting pattern information from the oocyte to the overlying follicle cells. The *Drosophila* EGF receptor (Egfr) is expressed in the follicle cells, and its spatially restricted activation is crucial for patterning of egg shell and embryo. We have found that this signaling process is highly regulated. The localization of *grk* RNA to the dorsal side requires the activity of the hnRNP protein Squid, as well as Hrb27c and Otu. We have identified other proteins which interact with Squid, among those the protein Cup. In addition, we have found that the novel protein Spindle-F is also required for the localization of the grk mRNA. The production of Gurken protein is dependent on several factors, in particular, we have found that it is regulated by the meiotic cell cycle via two checkpoint kinases (*Drosophila* ATR and CHK-2). (Supported by NIH funding and by the Howard Hughes Medical Institute).

9. Regulation of Cortical Microfilament Contractility in Early *Caenorhabditis elegans* Embryos. Bruce Bowerman, Thimo Kurz, John H. Willis, and Danielle R. Hamill. Univ. of Oregon, Eugene, OR 97403.

Upon fertilization, the *Caenorhabditis elegans* zygote completes two meiotic divisions before the first mitotic division. The meiotic and mitotic spindles differ dramatically in the size and organization of microtubules that capture and segregate chromosomes. The acentrosomal meiotic spindle is small, while the first mitotic spindle, with centrosomes at the poles, is large.

Long astral microtubules project from the mitotic spindle poles to make extensive contact with the cell cortex. The microtubule severing complex katanin is required for meiosis: in mutants lacking katanin, the meiotic spindle is disorganized, and chromosome segregation fails. Mitosis, in contrast, requires the ubiquitin-mediated degradation of katanin following meiosis. If katanin persists, it localizes to the mitotic spindle, producing fragmented and short astral microtubules, and unstable spindle positioning. In addition, if katanin persists, ectopic cleavage furrows form and persist throughout the cortex. These findings indicate that astral microtubules normally prevent cleavage furrows from forming outside the one furrow positioned midway between the two spindle poles. Presumably the single furrow that normally forms receives signals that over-ride negative regulation by astral microtubules. Our findings further implicate a second microtubule-independent mechanism that contributes to negative regulation of cortical contractile activity during mitosis.

10. Rotation of 1-cell *C. elegans* Embryos Inside the Egg Shell Reveals an Early Step in Establishment of Left–Right Polarity and Embryonic Handedness. William B. Wood[1] and Stephanie Schonegg[2]. [1]MPI-CBG, Dresden, Germany; MCDB, U. Colorado, Boulder, CO, USA; [2]MPI-CBG, Dresden, Germany.

Most animals establish L–R asymmetry with invariant handedness early in embryogenesis, but the initial symmetry-breaking mechanism is not understood in any embryo. *C. elegans* embryos first exhibit obvious L–R asymmetry at the 6-cell stage; however, asymmetry is present already during second cleavage, when the mid-body between the two AB daughter cells invariably moves to the right, apparently due to asymmetric closure of the furrow. This suggests a pre-existing L–R asymmetry in the AB-cell cortex at the 2-cell stage. In searching for possible earlier asymmetry cues, we observed that, just prior to initiation of first cleavage, the entire embryo rotates for ~50 s, through an angle of ~120°, inside the vitelline membrane of the egg shell. The screw sense of the rotation is invariant relative to the already established anterior–posterior (A–P) polarity and is independent of future dorsal–ventral polarity, indicating that the 1-cell embryo has an intrinsic chirality. Strong maternal knock-down (KD) of gene products required for actomyosin function or microtubule formation blocks the rotation. KD of PAR-2, PAR-3, or PAR-6, required for maintenance of A–P polarity, results in apparent randomization of the direction and extent of rotation as well as the handedness of the later embryo. Based on additional KD experiments, we propose a model for determination of this early chirality, which requires integrity of A–P polarity and appears to be an initial cue for establishment of L–R polarity with correct choice of handedness.

11. Cilia Driven Fluid Flow in Kupffer's Vesicle Directs Left–Right Patterning in Zebrafish. Jeffrey D. Amack, Jeffrey J. Essner, and H.J. Yost. Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA.

The heart, digestive tract and brain develop essential left–right (LR) asymmetries. Specialized cilia have been proposed to establish the LR axis in vertebrates by generating a directional fluid flow that initiates asymmetric gene expression. In zebrafish, dorsal forerunner cells (DFCs) express a ciliary dynein gene left–right dynein-related 1 (lrdr1) and give rise to a ciliated epithelium in an organ called Kupffer's vesicle (KV). Using videomicroscopy, we show that KV cilia are motile and create a directional fluid flow inside KV that precedes and then overlaps with the onset of asymmetric gene expression in cells near KV. Antisense morpholino (MO) knockdown of lrdr1 disrupted flow in KV and altered LR development. More specifically, lrdr1 MO targeted to DFC/KV cells perturbed LR patterning, indicating lrdr1 function in KV is necessary for LR development. Analyses of two T-box transcription factors expressed in DFCs-No tail (Ntl) and Spadetail (Spt) revealed that both of these factors play a cell-autonomous role in DFCs that is required for normal KV organogenesis and LR patterning. Targeting MO against either gene to DFCs prevented these cells from properly forming KV. Importantly, Ntl is not required for Spt expression in DFCs (and vice versa), indicating both factors are involved in regulating 'KV organogenesis genes.' In addition, lrdr1 expression in DFC/KV cells is dependent on Ntl but not Spt. These results suggest that Ntl and Spt control distinct steps of the formation of KV, which functions as an 'organ of asymmetry' that directs zebrafish LR development by establishing a directional fluid flow.

12. Regulative Development and Acquiring Spatial Patterning in the Early Mouse Embryo. Magdalena Zernicka-Goetz. Wellcome Trust/Cancer Res UK Gurdon Inst, Cambridge, UK.

Both orientation and symmetry of cell division are important in development of pattern in the early mammalian embryo, and yet the embryo is able to regulate its development if perturbed. Time-lapse observations of normal mouse embryo development on multiple focal planes and experimental manipulations indicate that orientation of the first cleavage is not random but relates to the animal pole and sperm entry point, itself related to egg shape. The orientation of the first cleavage can be altered by changing the egg's shape. Non-invasive lineage tracing shows that, even when the orientation of the first cleavage is changed, the progeny of the resulting 2-cell blastomeres tend to populate the respective embryonic and abembryonic parts of the blastocyst. The relative orientation and order of the second cleavage divisions are also not pre-determined but do have consequences on blastocyst pattern and developmental properties of 4-cell blastomeres. Chimeric embryos comprised entirely of certain types of 4-cell blastomeres develop to term, but chimeras of one specific cell type cannot. Thus, unexpectedly, cells start to differ in their developmental potential according to their origin in the embryo from as early as the 4-cell stage. One cell property that appears in the progeny of 4-cell blastomeres is a capacity to undertake either symmetric or asymmetric divisions. These are essential to generate inside and outside cell lineages that develop into ICM and trophectoderm respectively. It now appears that the conserved Par proteins control cell polarity and division orientation in the early mouse embryo. Par3 and aPKC adopt a polarized localization from the 8-cell stage, and when they are down-regulated in individual, random 4-cell blastomeres, the fate of their progeny is directed towards the ICM. It therefore appears that division orientation influences several stages of the early development of the mammalian embryo to direct cells to particular fates within these regulative embryos.

13. Polarity of the Mouse Embryo is Established at Blastocyst and is not Prepatterned. Takashi Hiiragi, Nami Motosugi, Tobias Bauer, Zbigniew Polanski, and Davor Solter. Max-Planck Institute, Freiburg, Germany.

Polarity formation in mammalian preimplantation embryos has long been a subject of controversy. Mammalian embryos are highly regulative, which has led to the conclusion that polarity specification does not exist until the blastocyst stage; however, some recent reports have suggested polarity predetermination in the egg. Our recent time-lapse recordings have demonstrated that the first cleavage plane is not predetermined in the mouse egg (Hiiragi and Solter, 2004). Morphologically, the mouse blastocyst possesses an obvious asymmetry, with the inner cell mass (ICM) at one end of the long axis of the ellipsoidal embryo and the blastocoel surrounded by the trophectoderm (TE) at the other. Thus, the intriguing question remains as to whether this asymmetry in the embryonic axis, the embryonic–abembryonic (Em–Ab) axis, is anticipated at an earlier point in development, as previously suggested. Here, we show (Motosugi et al. in press) that, in contrast to previous notions, 2-cell blastomeres do not differ and that their precise future contribution to the ICM and/or the TE cannot be anticipated. Thus, all evidence so far strongly suggest the absence of predetermined axes in the mouse egg. We observe that the ellipsoidal zona

pellucida exerts mechanical pressure and space constraints as the coalescing multiple blastocoel cavities are restricted to one end of the long axis of the blastocyst. We propose a novel model wherein these mechanical cues, in conjunction with the epithelial seal in the outer cell layer, lead to specification of the Em–Ab axis, thus establishing polarity for the first time in mouse embryonic development.

14. Regulation of Egg and Early Embryo Polarity of Sea Urchins by Disheveled. A. Wikramanayake, J. Bince, R. Xu, C. Peng, M. Hong, and Y. Marikawa. University of Hawaii, Honolulu, HI 96822.

Selective nuclear entry of β-catenin regulates early axial polarities in embryos of animals as diverse as cnidarians and chordates. Nuclear entry of β-catenin in early embryos is likely regulated by maternal factors, but these are poorly understood. We have previously shown that localized "activation" of Disheveled (Dsh) in vegetal cells regulates nuclear entry of β-catenin in early sea urchin embryos. The Dsh-DIX domain blocks endomesoderm specification by preventing nuclear entry of β-catenin, presumably via a dominant-negative (D/N) mechanism. Here, we have investigated the mechanisms that specifically regulate Dsh activation in vegetal cells. Using an anti-Dsh antibody, we show that endogenous Dsh is localized to vesicular structures at the vegetal cortex of unfertilized eggs. During early development, Dsh remains localized to the cortex of cells with nuclear β-catenin. After fertilization, Dsh becomes post-translationally modified (PTM), suggesting that this modification is important for early Dsh function. Preventing Dsh-DIX from interacting with membrane vesicles abolishes its D/N activity and also blocks PTM of this domain. Point mutations of two potential PTM sites on Dsh-DIX abolish its D/N activity. Assays done in mouse P19 cells indicate that these mechanisms are conserved in vertebrates. Together, these results suggest that PTM of Dsh-DIX that occurs within a membrane vesicle domain "activates" Dsh to transduce canonical Wnt signals in vegetal blastomeres. Thus, the localized determinant in the sea urchin egg may be a molecule involved in PTM of Dsh in the vegetal vesicle domain.

15. Hermes is a Component of the Xcat2 RNP Complex Localized within *Xenopus* Germ Plasm. Mary Lou King,[1] Yi Zhou,[1] Malgorzata Kloc,[2] Patrick Chang,[3] Evelyn Houliston,[3] and Laurence Etkin[2]. [1]Univ. of Miami School of Medicine, Miami, FL 33136; [2]Univ. Texas, M.D. Anderson Cancer Center, Houston, TX 77030; [3]CNRS/UPMC, Observatoire Océanologique, Villefranche sur mer, France.

The germ cell lineage in *Xenopus* is specified by the inheritance of germ plasm. Germ plasm assembles within the mitochondrial cloud in stage I oocytes and contains localized RNAs such as Xcat2, a nanos family member and germinal granule component. Previous work has shown that Xcat2 accumulates within the germ plasm through a diffusion/entrapment mechanism that requires UGCAC motifs found in the 3′UTR. Vg1 is involved in specifying somatic cell fates, does not enter the germ plasm. To understand how RNAs are selected for localization, we are characterizing the Xcat2 ribonucleoprotein complex. Trans-acting factors defining the germline pathway are unknown. We have identified Hermes, an RNA binding protein that co-localizes with Xcat2 within germinal granules and is also found in the nucleus and cytoplasm of stage I oocytes. Hermes binds Xcat2 in vivo and in a UGCAC-dependent manner but does not bind Vg1. In vitro binding data suggest Hermes requires an additional factor(s) to bind Xcat2. In vitro and in vivo data confirm that hnRNPI is part of the Xcat2 complex in stage I oocytes and may be one of these factors. Finally, we show that Hermes binds Xcat2, but not Vg1 RNA, in the nucleus of stage I oocytes. Hermes may function in the nucleus as part of a selection process that sorts germline RNAs to the germ plasm. (Supported by grants from the NSF and NIH to MLK and LE and by ACI 167 to EH.)

16. Application of Fluorescence Lifetime Imaging to Cell and Developmental Biology. John G. White, Damian Bird, and Eliceiri Kevin. Univ. Wisconsin, Madison, WI 53706.

The combination of fluorescent protein reporters and optical sectioning microscopy has provided developmental biologists with a powerful tool for studying both cellular and intracellular dynamics in vivo. Recent technical developments in this area have focused on extracting as much information as possible from the weak fluorescent signals typically encountered. Spectral imaging is becoming a rapidly established method of separating out overlapping signals from different fluorophores that have significant overlap; however, the wavelength of fluorescence photons is not the only information that they potentially carry. The excited state lifetime of a fluorescent molecule is an intrinsic property of a fluorophore that can be measured and used for identification purposes. In addition, excited state lifetimes are also dependant on the microenvironment of the fluorophore. Factors such as the reactive oxygen species, Ph and the close proximity of a resonant absorber can all act to reduce the characteristic lifetime of a fluorophore. This feature can be used to make fluorescent reporters that can measure aspects of their local environment. The great advantage of these types of reporter is that they yield absolute values, unlike reporters that give relative changes in fluorescence intensity that are difficult to calibrate. For this reason, fluorescence lifetime measurements are likely to provide a less ambiguous readout in experiments that use fluorescence resonant energy transfer (FRET) to detect intracellular molecular associations. The combination of spectral and lifetime imaging may well open up exciting new applications for in vivo fluorescence microscopy.

17. Multiphoton Fluorescence Lifetime Imaging Microscopy (FLIM) Using a Streak Camera. Brian Herman, Krishnan Ramanujan, Victoria Centonze, and Marisa Lopez-Cruzan. University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78229, USA.

We report the development and calibration of a Multiphoton Fluorescence Lifetime Imaging Microscopy (FLIM) system using a streak camera. The scanning and detection unit consists of a set of galvanometer mirrors (–for XY scan) to scan the femtosecond pulsed Ti:Sapphire beam into an Olympus IX-70 inverted microscope. The fluorescence emission from the specimen after the multiphoton excitation is directed through specified barrier filters into a Hamamatsu streakscope. The output of the streakscope, amplified by a microchannel plate, is coupled to a fast CCD camera (Hamamatsu, Argus HiSCA). The data acquisition and analysis is facilitated by Aquacosmos (Hamamatsu) software. The FLIM system has been calibrated with standard fluorophore solutions and is found to have a high temporal resolution (~50 ps) and a good accuracy in lifetime calculations. We demonstrate the applicability of our system in quantitative imaging including Fluorescence Resonance Energy Transfer (FRET) imaging to examine mitochondrial caspase-2 activity and compare the performance of this streak camera system with the conventional approaches such as the multigate detection and the time correlated single photon counting (TCSPC) approach. Although streak cameras have been earlier employed in the studies of semiconductor phenomena and picosecond spectroscopy, we report, for the first time, its application in biomedical research.

18. Nonlinear Optics and Multidimensional Imaging of Developing Tissues. William A. Mohler, Sergey V. Plotnikov, Ariel B. Isaacson, Andrew C. Millard, Paul J. Campagnola, and Vaibhav Juneja. UConn Health Center, Farmington, CT 06030-3301.

Development occurs in 3 dimensions over time. Vital imaging using optical sectioning microscopes has become a crucial tool in observing the cell biological processes that underlie development of embryos and tissues.

Some endogenous proteins – tubulin, myosin, collagen – produce strong second-harmonic generation (SHG), a form of contrast that can be imaged by optical-sectioning microscopically. By combining SHG with fluorescent dyes and transgene reporters, specific molecules can be co-localized with intrinsic protein structures in live specimens. The resulting multidimensional image sets are being analyzed by a combination of animation, morphometry, and pattern analysis to follow histogenesis and regeneration.

19. The Road to the Professorate: Getting a Job and Writing a Dynamite Grant. Karen L. Bennett. University of Missouri, Columbia MO 65212.

This career development workshop is especially targeted to postdoctoral fellows and new faculty. The session is modeled after those in the Lab Scientific Management course offered to Howard Hughes/Burroughs Wellcome fellows (a great manual "Making the Right Moves: a Practical Guide to Scientific Management for Postdocs and New Faculty" is available free online at http://www.hhmi.org/labmanagement). Two senior investigators, Chris Wylie, U Cincinnati and Keith Yamamoto, UCSF will offer suggestions regarding the job hunting and grant writing process. In addition, two junior faculty, Daniela Drummond Barbosa, Vanderbilt, and Phil Newmark, U Ill, will give their advice based on recent experience. Karen Bennett will moderate the panel. Half of the time will be allotted to questions and participation from those attending the session.

20. Job Interviews: Handling the Stress and Finding a Good Fit. Daniela Drummond-Barbosa. Vanderbilt University Medical Center, TN 37232-8240.

Going through the job interview process is one of many challenges that we face as we advance through our scientific careers. Sorting through our concerns and focusing our energy on aspects of the process that are under our control can help us be our best during each interview and, at the same time, identify a good fit. As part of the "Road to the Professorate: getting a job and writing a dynamite grant" workshop, we will discuss strategies that can be applied before, during, and after the interview to increase the odds that a job talk will be effective, that interactions with potential future colleagues and students will be productive, and that we will be prepared to weigh the pros and cons of each institution we visit.

21. Genome-Wide RNAi Screening for the Study of Cell Polarity in *C. elegans*. Julie Ahringer. The Gurdon Institute, University of Cambridge, Cambridge, England.

One of the major challenges currently facing biologists is how to connect the complete DNA sequence of an organism to its development and behaviour. To address this problem, we constructed an RNAi feeding library to generate knockdowns for 16,757 (~86%) of the predicted genes of *C. elegans*. We identified mutant phenotypes for 1722 (10.3%) of genes examined; ~1200 of these were not previously known. I will illustrate how we have used the RNAi library for systematic identification of genes involved in cell division, cell polarity and asymmetric cell division.

22. Generation of Temporal Identity in the *Drosophila* CNS. Chris Doe, Bret Pearson, Ruth Grosskortenhaus, Amanda Marusich, and Mike Cleary. Univ Oregon/HHMI, Eugene, OR 97403.

Temporal patterning is an important aspect of embryonic development, but the underlying molecular mechanisms are not well understood. *Drosophila* neuroblasts are an excellent model for studying temporal identity: they sequentially express four transcription factors (Hunchback → Kruppel → Pdm1 → Castor) whose temporal regulation is essential for generating neuronal diversity. I will discuss the "timers" that regulate neuroblast transcription factor switching, which is essential to generate neuronal diversity in the CNS. Interestingly, cytokinesis is required for Hunchback → Krüppel switching, whereas Krüppel → Pdm1 → Castor switching can occur in G2-arrested neuroblasts or in neuroblasts isolated in vitro. I will also present data on the role of the Hunchback zinc finger transcription factor in maintaining neuroblasts in a "young" multipotent state, as well as evidence that Hunchback acts by recruiting a specific Polycomb protein complex to heritably silence genes within early-born neurons.

23. Cilia and Hedgehog Signaling in the Mouse. Danwei Huangfu, Tamara Caspary, and Kathryn Anderson. Developmental Biology Program, Sloan-Kettering Institute.

Nearly all cells in vertebrates have a single, nonmotile primary cilium, and it has long been speculated that primary cilia have a role in receiving extracellular signals. In the course of a forward genetic screen in the mouse, we found that intraflagellar transport (IFT) proteins, which are required for the production and maintenance of cilia, are also required for the activation of the mouse Hedgehog pathway. We present evidence that IFT proteins are required for both the ability of Hedgehog proteins to promote activator Gli transcription factors and to regulate production of Gli3 repressor. Mouse mutants that lack either Dnchc2, a subunit of the retrograde IFT motor, or Kif3a, a subunit of the anterograde IFT motor, block Gli activation and processing. This finding suggests that the role for IFT proteins is to promote formation of cilia and that cilia are organelles that are required for Hedgehog signaling. Studies of another gene, hennin, which is required for normal structure of the ciliary axoneme, suggest that cilia have complex functions in integrating and processing signal inputs to the cell.

24. Interactome Networks. Marc Vidal. Harvard Medical School and Dana-Farber Cancer Institute, Boston, MA 02115.

Despite the considerable success of molecular biology to understand diseases such as cancer, many fundamental questions remain unanswered. Most importantly, since the majority of gene products in the cell mediate their function together with other gene products, biological processes should be considered as complex networks of interconnected components. In other words, for any normal biological process, or any disease mechanism, such as cancer, one might consider a "systems approach" in which the behavior and function of such networks are studied as a whole, in addition to studying some of its components individually. The draft of the human genome sequence is likely to help such a transition from molecular biology to systems biology. Our laboratory uses a model organism, the nematode *C. elegans*, to study the role of protein networks in development and, doing so, develop the concepts and technologies needed for a transition to systems biology. Our goals are to: (i) generate protein–protein interaction, or 'interactome', maps for *C. elegans* networks involved in development, (ii) develop new concepts to integrate such interactome maps with other functional maps such as expression profiles (transcriptome), global phenotypic analysis (phenome), localization of expression projects (localizome), etc. . ., and (iii) use such integrated information to discover novel network properties.

25. Signaling and Transcriptional Network Controlling Patterns of Cell Type in *C. elegans* Organogenesis. Paul W. Sternberg, Takao Inoue, Byung J. Hwang, Jolene S. Fernandes, Ted O. Ririe, Jennifer Sanders, David Sherwood, and Adeline Seah. HHMI and Caltech, Pasadena, CA 91125.

We seek to understand how the genome specifies a signaling and transcriptional network underlying organogenesis in *C. elegans*. The adult vulva comprises 7 cell types arranged in a pattern mirror symmetric about

the gonadal anchor cell. We have identified 17 genes expressed in subsets of these 7 cell types. This pattern depends upon multiple signaling events involving inductive EGF signaling, Notch-mediated lateral signaling and WNT polarizing signaling via both Frizzled- and Ryk-class receptors. Over 20 transcription factors are involved in specifying this pattern. Some factors were identified by classical developmental genetics followed by positional cloning. We have identified others using RNAi against transcription factors, searching for alterations in expression of several different GFP reporters. The phenotypes, expression patterns, and genetic interactions of these transcription factors suggest models of this regulatory network. The anchor cell organizes the formation of the vulva by serving as a source of signaling proteins: EGF to induce vulva patterning; WNT to establish the mirror symmetry of vulval cells; and the DSL ligand for LIN-12 to pattern the ventral uterus. The anchor cell also expresses genes that degrade the basement membrane and allow it to invade the vulval epidermis. We are thus examining the transcriptional network controlling anchor cell gene expression. For example, separate transcriptional controls over the genes necessary for basement membrane breakdown versus vulval induction.

26. The Impact of Cell Geometry on Planar Cell Polarity. Dali Ma,[1] Keith Amonlirdviman,[2] Claire J. Tomlin,[2] and Jeffrey D. Axelrod[1]. [1]Dept. of Pathology, Stanford; [2]Dept. of Aeronautics and Astronautics, Stanford.

Many epithelial cells display planar cell polarity (PCP) along the proximal–distal axis within the plane of the epithelia. Around 34 h APF, PCP is evident in the *Drosophila* wing as each cell projects a distal-pointing trichome, or hair, out of its distal vertex. The cadherin Fat provides a distal-polarizing cue to individual wing cells; this cue is interpreted by a cell–cell alignment mechanism mediated by a Frizzled feedback loop that amplifies and propagates PCP across the wing. As the epithelial cells align their polarity, their cortical domains concomitantly undergo remarkable changes in cell geometry as the cells form regular hexagonal arrays. We had shown that polarity information can propagate across the tissue in the absence of the global Fat signal. We now show that irregularities in cellular packing prevent correct polarity propagation without a broadly distributed Fat signal. We find that the extent of failure to achieve regular packing correlates to elevated frequency and severity of polarity defects in fat clones and that this frequency can be modified by altering cell geometry. We further show that our mathematical model, based on the Frizzled feedback loop and a Fat-dependent global bias, captures the geometry dependence of the propagation mechanism when Fat is removed, indicating that the defects seen in fat clones with altered geometry are a simple consequence of the inherent behavior of the Frizzled feedback mechanism when confronted with irregular cell packing. Interestingly, loss of Fat results in increased errors in cell packing, suggesting interplay between cellular packing and PCP signaling.

27. Endocytic Control of Cell Signaling and Epithelial Polarity by the *Drosophila* Syntaxin Avalanche. David Bilder and Han Lu. Dept. of Molecular & Cell Biology, UC-Berkeley, Berkeley, CA.

Signaling pathways are regulated not only by canonical receptor-associated ligands and transduction components but also by the spatial and subcellular organization of the cell. *Drosophila* 'neoplastic' or malignant tumors, in which loss of epithelial architecture is coupled to a failure in the control of cell proliferation, provide a dramatic example of the latter form of regulation. To understand how growth control signaling and cellular organization are linked, we have carried out a mosaic screen to identify new mutations that cause *Drosophila* neoplastic tumors. In this screen, we have isolated mutations in the novel gene avalanche (avl). avl loss of function transforms columnar monolayered epithelia into rounded and multilayered cells in which apical proteins as well as adherens junctions are ectopically localized throughout the basolateral cell surface. Even more dramatically, avl mutant tissues exhibit massive overproliferation to form tumor-like growths. These phenotypes are remarkably similar to mutations in scrib, one of the three known *Drosophila* neoplastic tumor suppressor genes (nTSGs), indicating that Avl is the 4th member of this class. avl encodes a previously uncharacterized *Drosophila* syntaxin that is localized to and required for entry into early endosomes, thereby demonstrating a role for endocytic trafficking in regulating epithelial architecture and growth control. We find that a subset of membrane proteins and receptors are specifically upregulated at the cell surface in avl mutant cells. We will report on the signaling pathways whose misregulation in avl mutants leads to overproliferation and polarity disruption.

28. Probing Wnt Signaling Using Small Molecule Regulation of Protein Stability and Localization. Karen J. Liu, Joseph R. Arron, Jason E. Gestwicki, Kryn Stankunas, J. Henri Bayle, Vidusha Devasthali, Julie C. Baker, and Gerald R. Crabtree. Stanford University Medical Center/HHMI.

The study of signal transduction processes in development is complicated by the precise spatial and temporal coordination required to properly pattern an embryo. In vivo analyses are difficult since often the response of many tissues is involved in a developmental process and these tissues are necessarily signaling to each other, thus making it difficult to distinguish immediate molecular and cell biological changes from later secondary effects. To date, what has been lacking is a general method for precisely controlling the activity of a protein. We have developed a general method of making conditional protein alleles that allow the rapid and reversible regulation of specific proteins. When fused to an 89 amino acid tag, FrbPLF, proteins are rapidly destabilized. In the presence of rapamycin or rapamycin analogs, FrbPLF-tagged proteins dimerize with endogenous FKBPs. This interaction stabilizes the fusion proteins and restores both protein levels and activity. By tagging and expressing FrbPLF-fused components of the Wnt signaling pathway in *Xenopus*, as well as using knockin strategies in mice, we are dissecting the subcellular requirements for GSK3-mediated Wnt activity during development.

29. A Pbx1-Dependent Genetic and Transcriptional Network Regulates Spleen Ontogeny. Licia Selleri and Andrea Brendolan. Cornell University Medical School, New York, NY 10021.

Little is known about spleen ontogeny and the genetic networks that control its development. Studies of mutant mice implicate various transcription factors in spleen development, but the hierarchical relationships among these factors have not been explored. In this study, we establish a genetic network that regulates spleen ontogeny by analyzing asplenic mice mutant for the transcription factors Pbx1, Hox11 (Tlx1), Nkx3.2 (Bapx1) and Pod1 (Capsulin). We show that two of the earliest known markers for splenic progenitor cells, Hox11 and Nkx2.5, are absent in the splenic anlage of Pbx1 homozygous mutant embryos, implicating Pbx1 in splenic cell specification. We demonstrate that Pbx1 and Hox11 genetically interact in spleen formation, as Pbx1/Hox11 double heterozygous mice have hypoplastic and malformed spleens, while single heterozygotes have normal spleens. Furthermore, loss of either Pbx1 or Hox11 is found with a similar reduction of progenitor cell proliferation and failed expansion of the splenic anlage. Our ChIP assays show that Pbx1 binds to the Hox11 promoter in embryonic primary spleen cells in culture, which co-express Pbx1 and Hox11. We also show that Hox11 binds its own promoter in vivo and acts synergistically with TALE proteins to activate transcription, supporting its role in an auto-regulatory circuit. These studies establish a Pbx1–Hox11-dependent genetic and transcriptional pathway in spleen ontogeny. Additionally, we demonstrate that, while Nkx 3.2 and Pod1 control spleen development via separate pathways, Pbx1 genetically regulates key players in both pathways and thus emerges as a central hierarchical co-regulator in spleen genesis.

30. Exploring Smad Signaling Pathways in Normal and Cancer Cells. Xuedong Liu, Soncheng Zhu, Tom Cheung, Richard Erickson, Wei Wang, and David Clarke. University of Colorado-Boulder, Boulder, CO 80309.

Smads are the principal intracellular signal transducers for TGF-beta signaling. Upon ligand stimulation, Smad2 and Smad3 are phosphorylated by the type I receptor kinase and subsequently form a complex with Smad4 to bind the promoter region of TGF-beta responsive genes and target gene expression. The Smad signaling pathway is one of the major signaling networks controlling cell proliferation and tumor suppression. Three independent approaches were employed to explore novel Smad functions in normal and cancer cells. First, using a unique computational method, we analyzed and compared sequence compositions of the regulatory regions of TGF-beta-responsive genes in human, mouse and rat genomes. Our analysis revealed that a distinct set of sequence elements conserved across species is either unique or occurs at a much higher frequency in TGF-beta-regulated genes. These regulatory elements include some of the previously well-characterized TGF-beta-responsive elements as well as a number of novel transcription factor binding sites that had not yet been implicated in TGF-beta signaling. Second, to identify potential genetic events in tumor cells that alter cellular response to TGF-beta, we conducted a cDNA library screen to isolate genes that, when ectopically expressed, disrupt the TGF-beta signal transduction pathway. This screen led to the isolation of several coding sequences whose products were found in association with Smads and antagonize their function. The mechanisms by which these gene products inhibit Smad function will be discussed. Finally, we designed a novel tandem affinity purification method in mammalian systems and purified a novel Smad signaling complex. The composition of this signaling complex was analyzed by LC/MS/MS and MALDI-TOF mass spectrometry. Characterization of this signaling complex revealed an unexpected function of the Smad signaling pathway in mitotic spindle checkpoint regulation.

31. Polarity and Proliferation in the *Arabidopsis* Meristem. Kathryn Barton. Carnegie Institution, Stanford, CA 94305.

The angiosperm shoot apical meristem includes both a small population of stem cells and their recent derivatives. These derivatives give rise to leaves, stem and additional meristems. Leaves form at predictable positions in the meristem. In addition, subdomains of the leaves acquire polarly distinct fates, while the leaf is still closely associated with the meristem. *Arabidopsis* class III HD-ZIP transcription factor genes affect both stem cell specification as well as leaf patterning. *Arabidopsis* class III HD-ZIP factors in turn are regulated by small RNAs through a mechanism that involves chromatin alterations and mRNA destabilization. Observations on the phenotypes resulting from over and underexpression of *Arabidopsis* ARGONAUTE-like proteins further underscore the importance of small RNAs in specifying the locations and activities of meristems.

32. Stem Cell and Regeneration Regulators in the Planarian *Schmidtea mediterranea*. Alejandro Sánchez Alvarado,[1] Peter W. Reddien,[1] Adam Bermange,[2] Kenneth Murfitt,[1] and Joya R. Jennings[1]. [1]University of Utah School of Medicine; [2]London Research Institute.

Planarians have been a classic model system for the study of regeneration, tissue homeostasis, and stem cell biology. Historically, however, planarians have not been accessible to extensive genetic manipulation. Here, we report the results of the first planarian RNA-mediated genetic interference (RNAi) screen, which introduces large-scale gene inhibition studies to this classic system. We screened a total of 1065 genes chosen from head and stem cell-enriched (neoblast) cDNA libraries. Phenotypes associated with the RNAi of 240 genes identify a gamut of specific defects in the process of regeneration and define the major categories of defects planarians display following gene perturbations. We assessed the effects of inhibiting genes with RNAi on tissue homeostasis in intact animals and stem cell proliferation in amputated animals identifying candidate stem cell, regeneration, and homeostasis regulators. Our study demonstrates the great potential of RNAi for the systematic exploration of gene function in understudied organisms and establishes planarians as a new and powerful model for the molecular genetic study of stem cells, regeneration, and tissue homeostasis.

33. Fgf-20 is Required for Blastema Formation in Zebrafish Caudal Fin Regeneration. Geoffrey G. Whitehead, Shinji Makino, Ching-Ling Lien, Soo Kim, Payal Jwahar, and Mark T. Keating. HHMI, Harvard Medical School, Children's Hospital, Boston, MA.

A fundamental root for many degenerative human diseases is our inability to regenerate. In contrast to humans, zebrafish regenerate many tissues and are amenable to genetic analyses. We have shown that caudal fin regeneration in zebrafish involves many processes. Two processes are thought to be unique to regenerative species: dedifferentiation and blastema formation. To identify the genetic requirements for dedifferentiation and blastema formation, we performed a forward genetic screen in zebrafish to identify caudal fin regeneration mutants. Frd5 mutants fail during blastema formation, primarily due to a drastic reduction in mesenchymal cell proliferation. Positional cloning has identified that the frd5 mutation is in the highly conserved FGF core-domain of Fgf-20. In accordance with the early mutant defect, this Fgf is upregulated during the initial stages of regeneration in proliferative blastemal cells. Microarray analysis of frd5 mutants has identified a number of interesting targets downstream of Fgf signaling during regeneration. Since FGFs play many roles throughout development, it is surprising that this mutation has no developmental phenotype, aside from asymmetric lobe patterning in the caudal fin. These results show that frd5 encodes the newly identified fgf-20 that is required specifically for regeneration during blastema formation. The mechanism of the defect in regeneration is a lack of blastema proliferation and formation, likely through decreased signaling via traditional and nontraditional downstream targets.

34. Stem Cells for the Anterior Pituitary Identified by Nestin-GFP Transgene Expression. Anatoli S. Gleiberman,[1] Juan M. Encinas,[2] Jose L. Roig,[2] Tatyana Michurina,[2] Peter Krasnov,[2] Michael G. Rosenfeld,[1] and Grigory Enikolopov[2]. [1]Univ. of California, San Diego, CA 92093; [2]Cold Spring Harbor Laboratory, NY 11724.

The presence of stem cells in the adult anterior pituitary has been postulated but had never been demonstrated. We now present evidence of their existence. Transgenic mice which express GFP under the control of regulatory elements of the nestin gene contain a very small population of epithelial GFP-expressing cells in the adult anterior pituitary. These cells first appear in the Rathke's pouch at 11.5 days of embryonic development. They reside in the periluminal space, the remnant of Rathke's pouch, throughout the later stages of embryonic development and in the adult organ and are capable of generating terminally differentiated pituitary cell types. Upon isolation from the adult pituitary, these cells can generate colonies of epithelial cells in vitro. They express several general epithelial markers, together with markers characteristic for neural stem cells and Lhx3, a transcription factor highly specific for the anterior pituitary. These cells maintain a very high proliferative potential and can undergo at least 20 divisions. At high cell density, they form aggregates that contain ACTH-, αGSU- and Pit-1-positive cells. Transcriptional profiling confirms that isolated nestin-GFP cells can differentiate into cells of the anterior pituitary lineages. Thus, the periluminal space of the adult anterior pituitary is a specific niche for the pituitary stem cells. Our results describe a new type of stem cells and highlight the use of nestin-driven transgenes for the

identification and isolation of stem cells from the developing and adult tissues.

35. Neural Crest Stem Cell Depletion Mediated by Loss of Tcof1 Causes Treacher Collins Syndrome Craniofacial Abnormalities. Paul A. Trainor,[1] Natalie C. Jones,[1] Jill Dixon,[2] and Michael Dixon[2]. [1]Stowers Institute for Medical Research, Kansas City, MO 64110; [2]University of Manchester, M13 9PT, UK.

Treacher Collins Syndrome is a rare autosomal dominant human congenital disorder of craniofacial development. Characterised by numerous developmental anomalies including hypoplasia of the facial bones, middle and external ear defects and cleft palate, Treacher Collins Syndrome has been hypothesised to arise due to abnormal cell migration and/or improper cellular differentiation during embryonic development. To date, however, there has been no genetic or biochemical evidence to support any of these hypotheses. We have generated a mouse model of Treacher Collins Syndrome via null mutation of the mouse homologue of Tcof1. The majority of craniofacial structures affected in Treacher Collins Syndrome are derived from multipotent migratory neural crest stem cells. Characterisation of early neural crest cell patterning events in Tcof1 mutant embryos reveals that cranial neural crest cells migrate normally ruling out migration anomalies as a cause of Treacher Collins Syndrome. Instead, through whole embryo culture, cell lineage tracing and transplantation assays, we have determined that Tcof1 acts cell autonomously and that haploinsufficiency of Tcof1 causes massive neuroepithelial apoptosis. Consequently, this severely depletes the neural crest stem cell population causing hypoplasia of the cranioskeletal elements characteristic of Treacher Collins Syndrome. Further analyses reveal that minimising apoptosis and neural crest stem cell regeneration can rescue the malformations associated with Treacher Collins Syndrome.

36. Roles for Myc and the Cell Cycle Machinery in Self-Renewal of Murine ES Cells. Stephen Dalton, Matthew Bechard, and Cameron McLean. University of Georgia, Athens, GA 30602.

Murine ES cells have an unusual mode of cell cycle regulation and can be maintained as a pluripotent, self-renewing population by LIF/STAT3-dependent signaling. The downstream effectors of this pathway have not been previously defined. We describe a key target of the LIF self-renewal pathway by showing that STAT3 directly regulates expression of the c-*myc* transcription factor. Murine ES cells express elevated levels of c-*myc*, and following LIF withdrawal, c-*myc* mRNA levels collapse and c-*myc* protein becomes phosphorylated on threonine 58 (T58), triggering its GSK3b-dependent degradation. Maintained expression of stable c-*myc* (T58A) renders self-renewal and maintenance of pluripotency independent of LIF. In contrast, expression of a dominant negative form of c-*myc* antagonizes self-renewal and promotes differentiation. Transcriptional control by STAT3 and suppression of T58 phosphorylation are crucial for regulation of c-*myc* activity in ES cells and therefore in promoting self-renewal. Together, our results establish a mechanism for how LIF/STAT3 regulates self-renewal and suggests a link between this and the mechanisms underpinning ES cell cycle control.

37. Stem Cell Self-Renewal and Aging Reflect a Balance Between Repression and Age-Related Activation of the INK4A and ARF Senescence Pathways. Ricardo Pardal, Anna V. Molofsky, Shenghui He, Genevieve M. Kruger, and Sean J. Morrison. HHMI, Departments of Internal Medicine and Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan, 48109-0934.

A general question is how self-renewal mechanisms regulate stem cells throughout life. BMI-1 is required for the postnatal maintenance of stem cells in the central (CNS) and peripheral (PNS) nervous systems. BMI-1-deficient mice die by early adulthood with stem cell depletion, growth retardation, ataxia, and seizures. These phenotypes correlate with increased expression of the cyclin-dependent kinase inhibitor p16Ink4a and the p53 agonist p19Arf, which promote cellular senescence. Deletion of INK4a or ARF from BMI-1−/− mice partially rescued stem cell self-renewal and stem cell frequency in the CNS and PNS as well as forebrain proliferation and gut neurogenesis. ARF deficiency, but not INK4a deficiency, partially rescued cerebellum development. INK4a-ARF deficiency did not affect the growth or survival of BMI-1−/− mice, demonstrating that stem cell function can be uncoupled from growth in the nervous system. Bmi-1 thus prevents the premature senescence of neural stem cells by repressing INK4a and ARF. Bmi-1 does not indefinitely prevent senescence as neural stem cell frequency and self-renewal potential decline with age and p16Ink4a expression increases. Neural stem cell self-renewal reflects a balance between the repression and age-related activation of the p16Ink4a and p19Arf tumor suppressors. The regulation of stem cell self-renewal and aging by tumor suppressors emphasizes the link between stem cell self-renewal and cancer cell proliferation.

38. The Neural Crest and the Development of the Vertebrate Head. Nicole M. Le Douarin. Inst d'Embryol Cell et Molec du CNRS et Coll de France, France.

Tracing the migration of the neural crest cells (NCCs) in the avian embryo through the quail-chick chimera system has revealed their prominent role in the construction of the vertebrate head. NCCs participating in the facial skeleton and hyoid cartilages arise from the diencephalon (posterior half only), the mesencephalon and the rhombencephalon. They can be divided into an anterior domain (extending down to r2 included) in which genes of the *Hox* clusters are not expressed (*Hox*-negative NCC) and a posterior domain including r4 to r8 in which Hox genes of the four first paralogue groups are expressed. The NCCs which form the facial skeleton belong exclusively to the anterior *Hox*-negative domain.

39. Turning Over a New Leaf. Ian Sussex. Yale University, New Haven, CT 06511.

The mature vein system of plant leaves performs several functions: support of the expanded leaf blade to maximize light interception, import of water and inorganic nutrients via xylem cells, and export of photosynthate via phloem cells. To perform these functions effectively, the vascular system conforms to the shape of the leaf blade. But, how the vascular system is established and develops in conformity with expansion and shape changes of the blade has not been investigated in detail. To analyze this relationship, we have begun an interdisciplinary study involving a paleobotanist (Leo Hickey), two computational biologists (Pavel Dimitrov and Steven Zucker), a molecular biologist (Timothy Nelson), and two developmental biologists (Nancy Kerk and Ian Sussex). In this talk, I will present the historical foundations of our research and the progress that we have made to date.

40. The Great Challenges of Science Education. Bruce Alberts[1,2]. [1]Former President (1993–2005), National Academy of Sciences, Washington, DC; [2]Dept. of Biochemistry and Biophysics, UCSF, CA.

The National Science Education Standards (1996) propose that science should become a core subject that is taught along with reading, writing, and mathematics in every school year, starting in kindergarten. I am not referring to science as most students currently experience it, as the memorization of science facts and terms. Science education should instead emphasize empowering experiences in problem-solving that take advantage of the curiosity in children and increase each student's understanding

of the world. Through activities carefully designed through research and development cycles by experts, schooling can become exciting even for those students who currently find little to attract them or motivate them in schools. This type of hands-on inquiry learning can also serve as a core from which meaningful mathematics, writing, and reading lessons are spun off. But, there are many challenges ahead. We need outstanding curricula, continuously improved and disseminated widely through the Worldwide Web. Parents need to be convinced that this type of learning makes sense for their children and to understand its nature and its value. Teachers need to be reeducated to teach this type of science, which means that inservice activities of high quality must be designed and become an integral part of school districts. But most importantly, none of this will happen without the energetic and informed participation of our colleges and universities. If our first year science courses for undergraduates do not change to emphasize science as inquiry, there is little hope that the nature of the science we teach at lower levels can be reinvigorated. The National Academies have been focused on "making a science out of education", which means creating continuously improving education systems based on evidence-based analyses of how people learn and what works in our schools. For free access to the full text of our many studies and books on this subject, see http://www.nas.edu/subjectindex/edu.html.

41. Morphogen Gradients and Scaling in Insect Embryos. Eric Wieschaus,[1] Thomas Gregor,[1] Alistair McGregor,[2] William Bialek,[3] and David Tank[3]. [1]HHMI, Molecular Biology Department, Princeton University, Princeton, NJ 08544; [2]Department of Ecology and Evolutionary Biology, Princeton Unversity; [3]Lewis Sigler Institute for Integrated Genomics, Princeton University, Princeton, NJ.

Cells in the *Drosophila* embryo are assigned anterior fates based on a gradient of the maternal morphogen Bicoid that activates transcription of downstream gap genes like Hunchback. We have used quantitative antibody staining and dynamic analyses of EGFP Bicoid distributions in living embryos to characterize the movement of Bcd protein and how the gradient arises during early embryonic development. The Bcd distributions are compared to those produced by injecting fluorescently labeled dextrans in the anterior end of the egg. We have investigated how the Bcd system has been adjusted during evolution to pattern eggs from other dipteran species (*Musca*, *Calliphora*, *Lucillia*) that are 2.5 to 3 times longer than that of *Drosophila*. Bcd distributions are broader in longer eggs, compensating for the increases in egg length. These results contrast with the insensitivity of Bcd distribution to egg length within the *Drosophila melanogaster* species itself and suggest that, during the course of evolution, the organism has altered the properties of the Bcd protein itself, or the movement of molecules within the egg cytoplasm, to allow morphogen gradients to scale. To investigate these mechanisms responsible for these changes, we have cloned the Bcd and Hb genes from various dipteran species and will introduce EGFP tagged versions into *D. melanogaster* to follow distributions of foreign Bcds in *Drosophila* and test their ability to generate normal patterns of gene expression.

42. Spatial Bistability of Dpp-receptor Interactions During *Drosophila* Dorsal-Ventral Patterning. Chip Ferguson and Yu-Chiun Wang. University of Chicago, Chicago, IL 60637.

In many developmental contexts, a locally produced morphogen specifies positional information by forming a concentration gradient over a field of cells. Conversely, during embryonic dorsal–ventral patterning in *Drosophila*, two members of the Bone Morphogenetic Protein (BMP) family, Decapentaplegic (Dpp) and Screw (Scw), are broadly transcribed but promote receptor-mediated signaling in a restricted subset of expressing cells. Here, we use a novel immunostaining protocol to visualize receptor-bound BMPs and show that both proteins become localized to a sharp stripe of dorsal cells. We demonstrate proper BMP localization involves two distinct processes. First, Dpp undergoes directed, long-range extracellular transport. Scw also exhibits long-range movement but can do so independently of Dpp transport. Second, an intracellular positive feedback circuit promotes future ligand binding as a function of previous signaling strength. These data elicit a model whereby extracellular Dpp transport initially creates a shallow gradient of BMP binding, which is acted on by positive intracellular feedback to produce two stable states of BMP-receptor interactions, a spatial bistability in which BMP binding and signaling capabilities are high in dorsal-most cells and low in lateral cells.

43. The Role of BMPs in Commissural Axon Guidance. Samantha J. Butler,[1] Keith D. Phan,[1] and Jane Dodd[2]. [1]Univ. of So. California, LA; [2]Columbia Univ., NY.

Molecules used as morphogens to induce the formation of distinct neuronal subtypes can also function as axon guidance cues. This paradigm has been best described for the commissural (C) interneurons in the developing spinal cord. C neurons are induced by Bone Morphogenetic Proteins (BMPs) present in the roof plate. Our work has shown that the initial trajectory of C axons away from the roof plate is also directed by the activity of BMPs. Thus, BMPs have a role determining the identity of C neurons and a subsequent role guiding their projections. These observations raise questions about how morphogens guide axons and whether this activity is distinct from their patterning and inductive capacities. Our findings suggest that, for the BMPs, their guidance activities may be different from their morphogenetic activities. BMP homodimers important for inductive activity are not the principal mediators of guidance activity; rather, this function is carried out by BMP heterodimers. Moreover, the conventional view of BMP signaling is that a complex of BMP receptors (BMPRs) mediates the inductive activities of BMPs by changes in transcriptional activity. However, C growth cones rapidly collapse on exposure to BMPs, a reaction that requires a fast, local cytoskeletal response. Thus, the signaling pathway transducing BMP guidance cues may either consist of novel components or may use the classical components in an unorthodox way. To resolve how the axonal responses to BMPs differ mechanistically from the morphogenetic activities, we will present results from experiments examining the effect of constitutively active and dominantly negative forms of BMPRs on the guidance of C axons.

44. Whole Genome Analysis of Foregut Development in *C. elegans*. Susan E. Mango. Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112.

A critical question in developmental biology is how complex programs of gene expression are orchestrated by a unique class of regulators known as selector genes. Selector genes code for transcription factors that autonomously govern the fates of groups of cells related to each other by virtue of their cell type, position, or affiliation to an organ. In *C. elegans*, the selector gene PHA-4/FoxA dictates the identity of cells within the foregut or pharynx. Embryos that lack pha-4 fail to generate pharyngeal cells, and conversely ectopic pha-4 is sufficient to drive non-pharyngeal cells towards a pharyngeal fate. These dramatic cell fate transformations reflect the global regulatory role of PHA-4 within pharyngeal cells. Many genes selectively expressed within the pharynx are activated directly by PHA-4, including genes expressed in different pharyngeal cell types and at different developmental stages. This strategy raises the question of how a single transcription factor can mediate diverse transcriptional outcomes within different cellular contexts. Our data implicate three strategies. First, the affinity of PHA-4 for its DNA binding site influences the timing of the onset of expression. Second, we have devised bioinformatic approaches to identify additional *cis*-regulatory motifs that function in combination with PHA-4 to establish temporal or cell type specific expression. Third, we have begun to analyze factors that interact with PHA-4 and modulate its expression or activity. These data will be presented.

45. A FOG-1/CPEB Gradient Controls the *C. elegans* Germ Line. Beth E. Thompson,[1] David S. Bernstein,[1] Jennifer L. Bachorik,[1] Andrei G. Petcherski,[1] Marvin Wickens,[1] and Judith Kimble[2]. [1]UW-Madison; [2]UW-Madison & HHMI.

The *C. elegans* germ line divides mitotically in young larvae; later, it balances stem cells with entry into meiosis and gametogenesis. We focus on two key regulators: FBF, a PUF RNA-binding protein, and FOG-1, a CPEB homolog. Previous work showed that FBF promotes germ line mitoses and that FOG-1 specifies the sperm fate. Here, we report that FOG-1 also promotes mitoses and that it does so redundantly with FBF. Whereas *fog-1* and *fbf-1 fbf-2* mutants make ~2000 and ~120 germ cells respectively, *fog-1; fbf-1 fbf-2* triple mutants make only ~10 germ cells. By contrast, *fog-1/+; fbf-1 fbf-2* animals make ~500—far more than *fbf-1 fbf-2*. This counterintuitive result demonstrates that wild-type *fog-1* promotes mitosis more effectively in one dose than two—at least in the absence of FBF; conversely, two *fog-1* doses promote spermatogenesis more effectively than one. In wild-type germ lines, FOG-1 occurs in a temporal and spatial gradient: FOG-1 is low in mitotic germ cells, both in early larvae and adult males, and abundant in germ cells destined for spermatogenesis, both in mid-stage hermaphrodite larvae and adult males. The FOG-1 dosage effect together with its graded distribution suggests that FOG-1 may act in a gradient, with low FOG-1 promoting mitosis, and high FOG-1 specifying sperm fate. FBF directly represses fog-1 expression. In *fbf-1 fbf-2* germ lines, the FOG-1 gradient is compressed in time and abolished in space; all germ cells arrest mitosis and differentiate as sperm. We suggest that FBF represses *fog-1* to establish a FOG-1 gradient and thereby balance growth and differentiation in the *C. elegans* germ line.

46. Dissecting the Roles of RA as a Morphogen in the Zebrafish Hindbrain. Richard J. White and Thomas F. Schilling. Univ. of California, Irvine, CA 92697.

Retinoic acid (RA) is an important signaling molecule during vertebrate embryogenesis and has been proposed to act as a morphogen. To test this hypothesis, we are investigating the ability of RA to act at long range in a concentration-dependent manner in the developing zebrafish hindbrain. We have taken advantage of an RA-responsive transgene (RARE-YFP) to monitor cells responding directly to RA in living embryos. Using transplants of cells into RA-deficient embryos, we have shown that RA from the somitic mesoderm signals directly to cells over at least 12 cell diameters without intervening RA synthesis. In response to a local source of RA (RA-coated bead), RARE-YFP is initially activated in cells close to the bead, and expression expands progressively further away over the course of several hours, consistent with simple diffusion.

We are now investigating the role of RA in establishing boundaries of gene expression by studying the responses of hoxb4 and hoxb5 to local sources of RA. These two genes have differing expression boundaries along the anterior–posterior axis and require RA for their expression. Our results suggest that they are activated at different thresholds, consistent with the morphogen model of RA action.

However, asymmetries in the response of genes to exogenous RA suggest that there are also intrinsic differences in competence in the responding neurectoderm. To address this, we have focused on members of the RA signaling pathway such as the RA receptors and the cyp26 enzymes, which negatively regulate the pathway by catabolizing RA. By disrupting these individually and in combination, we can address how multiple boundaries of genes expression are established.

47. Chance Caught on the Wing: *Cis*-Regulatory Evolution and the Origins of Novelty. Sean B. Carroll,[1] Nicolas Gompel,[2] Benjamin Prud'homme,[1] Trisha Wittkopp,[3] and Victoria Kassner[1]. [1]Howard Hughes Medical Institute and University of Wisconsin-Madison; [2]University of Cambridge; [3]Cornell University.

The gain, loss or modification of morphological traits is generally associated with changes in gene regulation during development. However, the molecular mechanisms underlying these evolutionary changes have remained elusive. We have identified one of the molecular bases for the evolutionary gain of a male-specific wing pigmentation spot in *Drosophila* biarmipes, a species closely related to *Drosophila melanogaster*. We have found that the evolution of this spot involved modifications of an ancestral *cis*-regulatory element of the yellow pigmentation gene. This element has gained multiple binding sites for transcription factors that are deeply conserved components of the regulatory landscape controlling wing development, including the selector protein Engrailed. The evolutionary stability of components of regulatory landscapes, which can be co-opted by chance mutations in *cis*-regulatory elements, may explain the repeated evolution of similar morphological patterns, such as wing pigmentation patterns in flies. Similarly, the loss of functional sites may account for the loss of characters within lineages. The modularity of *cis*-regulatory elements thus enables the evolution of form to evolve in individual body parts, independently of other parts.

48. Partial Redundancy of Regulatory Elements of duplicated *Hoxb5* Genes in Teleosts. Olga Jarinova,[1] Christelle Prudhomme,[2] Lucille Joly,[3] Lucie Jeannotte,[2] and Marc Ekker[1]. [1]CAREG, Dept. of Biology & Dept. of Cellular and Molecular Medicine, U. of Ottawa, Canada; [2]Centre de Cancérologie de l'Hôtel-Dieu de Québec, Université Laval, Canada; [3]Dept. of Biology, U. of Ottawa, Canada.

The Duplication–Degeneration–Complementation (DDC) model predicts that division of the original function between duplicate genes (subfunctionalization) is a common mechanism for their preservation. The *Hox* genes constitute a good system to test this hypothesis as they underwent significant expansion during evolution. The mouse has one *Hoxb* complex, whereas zebrafish and *Takifugu* have two *hoxb* complexes, each containing a *hoxb5* gene. The zebrafish *hoxb5a* and *hoxb5b* genes are expressed in overlapping, yet distinct, domains during development with their combined expression patterns similar to that of the single mouse *Hoxb5* gene. To determine if changes in *cis*-acting regulatory elements account for *hoxb5a* and *hoxb5b* subfunctionalization, we compared the sequences of the *Hoxb5* loci of human, mouse, zebrafish and *Takifugu*. We identified four conserved non-coding sequences (CNSs); all but one were present in both teleost loci. We are testing the enhancer activity of the CNS, together or individually, in transgenic animals. When tested in isolation, the enhancer activity of paralogous sequences from the *hoxb5a* and *hoxb5b* loci extensively overlapped. The dynamics of changes in the *cis*-acting regulatory elements of *Hoxb5* duplicates are examined in light of the DDC model, which predicts rapid loss of redundant subfunctions. Supported by NSERC.

49. Diverse Segmental Functions of Pair-Rule Gene Orthologs in *Tribolium*. Chong Pyo Choe and Susan J. Brown. Kansas State University, Manhattan, KS 66506.

Elegant genetic studies on segmentation mechanisms in the fruit fly have contributed to our understanding on segment formation. However, in contrast to the fly which has long-germ embryos, most arthropods have short-germ embryos. For the last decade, studies have focused on revealing the extent to which fly segmentation mechanisms are conserved in other arthropods. Despite our increasing understanding of segmentation in other arthropods, it is still a matter of debate whether the pair-rule patterning seen in flies is an ancient or a derived mode of segmentation.

To address this question on the origin of pair-rule patterning, we have been investigating the expression pattern and function of newly cloned secondary pair-rule gene orthologs as well as the function of previously cloned primary pair-rule gene orthologs in the short-germ insect *Tribolium castaneum*. RNAi knockdown experiments indicate that only two of these

genes (*paired* and *sloppy paired*) have typical pair-rule functions. The remaining genes display an array of segmentation phenotypes, ranging from gap-like to almost complete loss of segments. These phenotypes suggest that pair-rule patterning does occur in this short-germ beetle, but the interactions between segmentation genes appear to be more complex than in *Drosophila*.

50. Building Divergent Body Plans with Similar Genetic Pathways. Billie J. Swalla. Friday Harbor Laboratories and Univ. of Washington, Seattle, WA 98195-1800.

We study the development and evolution of the chordate body plan within the Deuterostomes. The four major clades of deuterostomes are echinoderms, hemichordates, urochordates (tunicates) and chordates (vertebrates and lancelets). Examination of larval and adult body plans in the deuterostomes show two distinct ways of evolving divergent body plans. One scenario, seen in echinoderms and hemichordates, is to build a new adult body plan on the same lava. Echinoid echinoderms have evolved this mechanism where the adult axes are not the same as the larval axes. In contrast, in hemichordates, the adult is built onto the larva with the larval axes becoming the adult axes and the larval mouth becomes the adult mouth. A second separate scenario seen in the hemichordates and tunicates is to become colonial. We are studying closely related solitary and colonial species to examine the underlying cellular and molecular mechanisms underlying this life history switch. Finally, I will review results examining early gastrulation in all deuterostomes and show that, in the chordates, the anterior–posterior axis is established at right angles to the animal–vegetal axis, whereas in hemichordates, the animal–vegetal axis becomes the anterior–posterior axis. This shift in symmetry has consequences for elaboration of the mouth, which is formed secondarily in all deuterostomes. Some the genes that are considered vertebrate "mesodermal" genes, such as *nodal* and *brachyury T*, are likely to ancestrally been involved in the formation of the mouth and anus and later were co-opted into mesoderm during the evolution of the chordates.

51. Bat Wing Interdigit Webbing is Maintained by BMP Attenuation and Unique Domains of FGF Signaling. Scott D. Weatherbee,[1] Chris J. Cretekos,[2] Richard R. Behringer,[2] John J. Rasweiler IV,[3] and Lee A. Niswander[4]. [1]MSKCC, New York, NY 10021; [2]M. D. Anderson Cancer Ctr., Houston, TX 77030; [3]SUNY Downstate Medical Ctr., Brooklyn, NY 11203; [4]UCHSC, Aurora, CO 80045.

A prevalent trend in animal evolution is the morphological diversification of homologous structures between lineages. All tetrapod limbs derive from a common ancestral appendage, yet great divergence is evident in form and function. One of the most productive adaptations is for flight. While bats are the only mammals that evolved winged flight, they comprise more than 20% of all mammalian species. We are pursuing molecular embryological studies in bats in order to identify molecular genetic differences that contribute to the morphological diversity of vertebrate limbs. Bat wings are largely composed of membranes (dactylopatagia) that extend between the forelimb digits. Differences in the extent of interdigital cell death during vertebrate embryogenesis lead to freed or webbed digits. In chicks and mice, BMP signaling triggers this apoptosis, whereas in ducks, BMP signaling is attenuated by BMP antagonists. We have studied the phyllostomid bat *Carrollia perspicillata* to determine the mechanism for maintaining interdigit tissue in the wings. Similar to chick and mice, the bat interdigit mesoderm exhibits expression of Bmp signaling components and bat hindlimbs undergo interdigital apoptosis. However, the bat forelimb retains interdigit webbing and, intriguingly, this tissue also displays a unique pattern of *Fgf8* expression. By manipulating FGF and BMP signaling, we show that a combination of FGF signaling and attenuated BMP signaling appears to maintain interdigit tissue in the bat wing.

52. The Development of Flight in Chiroptera: The Morphologic and Genetic Evolution of Bat Wing Digits. Karen E. Sears,[1] Richard R. Behringer,[2] and Lee A. Niswander[1]. [1]Univ. of CO Health Sciences Center; [2]U.T. M.D. Anderson Cancer Center.

Anatomically modern bats suddenly appear in the fossil record 50 mya. The absence of transitional fossil forms forces us to look elsewhere to understand the mechanisms driving the evolution of the unique morphology of the bat wing. With this as our goal, we compared the development of the digits of the bat (*Carollia perspicillata*) wing with the more generalized digits of the mouse (*Mus musculus*). Histological results indicate that the initial cartilage condensations and segmentation patterns of bat digits are similar in size and position to those observed in mouse, suggesting that bat digit elongation is due to post-segmental processes. Longitudinal growth of post-segmental digits is dependent upon the relative proliferation and differentiation of chondrocytes in the growth plate. The area in which chondrocytes undergo differentiation (hypertrophic zone) is expanded in the bat growth plate, relative to mouse. Limb culture experiments reveal that the application of *BMP2* protein can stimulate expansion of the hypertrophic zone in both bat and mouse digits. In addition, initial in situ hybridization and RT-PCR results suggest that the spatial distribution and level of *BMP2* expression are increased in bat digits relative to mouse. Taken together, these results suggest that a simple change in the expression of a single key genetic regulator of limb development was sufficient to drive the rapid evolutionary elongation of bat wing digits. By linking small changes in molecular patterning to dramatically different phenotypes, we provide a potential explanation for the rapid evolution of flight in bats.

53. Deciphering the Instructions for Building a Hydra. Rob Steele. Univ. of California, Irvine, CA 92697.

In order to understand the evolution of metazoans, we need to have detailed explanations of how animals in diverse phyla are assembled during development and how they function. Toward this end, we are engaged in an effort to understand the molecular circuitry underlying developmental processes in Hydra, a member of the early-diverging animal phylum Cnidaria. Hydra has been the subject of experimental studies for over 200 years. Because of the tissue dynamics of the adult Hydra polyp, pattern formation, morphogenesis, cell division, and cell differentiation are continuously active. In addition, the adult polyp has remarkable regenerative capacities. To identify the genes used to build and maintain the adult Hydra polyp, a large-scale EST project (150,000 sequences to date) and a genome project (projected to yield 8× coverage) are being carried out. From these projects, we have identified a number of genes which are of interest. Studies of these genes in experimentally manipulated Hydra are underway.

54. Viva La Difference: Profiling Changes in the Proteome and Transcriptome of *Arabidopsis* Caused by Genetics and Pharmacology. Michael R. Sussman. 425 Henry Mall, Biotechnology Center, Univ. of Wisconsin-Madison, WI 53706.

In the model higher plant, *Arabidopsis thaliana*, reverse genetics is easy. There are knockout mutants publicly available, for ca. 75% of the known genes, from the Ohio State University Seed Stock Center. Identifying morphological changes caused by null mutations can be as simple as ordering seeds and planting them in your (indoor) garden. However, since only a small percentage of any single gene knockout displays an obvious growth defect, more sensitive methods to detect changes at the molecular level are needed. In this talk, I will describe genomic technologies being developed in my laboratory to explore deep into the transcriptome and proteome. High density oligonucleotide arrays are created using a "bench-top" Affymetrix machine called a maskless

array synthesizer (cf. Stolc et al., 2005, Identification of transcribed sequences in *Arabidopsis thaliana* by using high-resolution genome tiling arrays PNAS 102: 4453–4458). As the name implies, this instrument uses a digital micromirror device, such as that used in powerpoint projectors, to control photolithography and create microscope slides containing hundreds of thousands of different sequences of long oligonucleotides (e.g., 60 mers). For exploring deep into the proteome, we are growing wildtype plants in 14N-nitrate and mutant plants in 99% 15N-nitrate and grinding them together. The ratio of natural to heavy isotope in individual proteins is determined to high precision in a tandem mass spectrometer (e.g., ESI-QTOF). By this procedure, we quantify changes in the proteome and also in the phosphorylome. Similar heavy isotope methods are also being used to identify contaminants in subcellular fractions and rigorously characterize the proteome of the plant plasma membrane. In general, despite sophisticated and expensive instruments, analysis of the proteome remains a difficult and slow process, particularly when compared to transcriptome profiling and reverse genetics.

55. Systems Level Analysis of the Aging Process. Stuart K. Kim. Stanford University.

Aging affects nearly all organisms and is a major risk factor in most human diseases. Recent work has begun to uncover molecular mechanisms that specify lifespan and to identify alterations in cellular physiology that occur at the end of life. We have begun our studies of human aging by focusing on the kidney, an organ that shows a quantifiable decline in function with age. We present a molecular portrait of the aging process in the human kidney by analyzing gene expression as a function of age on a genome-wide scale. We show that age regulation is similar in the cortex and the medulla and that age-regulated genes in the kidney are broadly expressed. We show that the expression profiles of age-regulated genes correlate well with the morphological and physiological state of the kidney in old age. Finally, we analyze the set of age-regulated genes to identify specific metabolic processes and cellular functions that change as a function of age and discuss their possible roles in specifying the functional lifespan of the kidney.

56. Mapping the Genome's Second Code. Bing Ren. Ludwig Institute for Cancer Research, and University of California, San Diego School of Medicine, Department of Cellular and Molecular Medicine, 9500 Gilman Drive, La Jolla, CA 92093-0653.

With the complete sequence of the human genome in hand, researchers now need to decode the instruction sets embedded in the nucleotide sequences. While much has been learned about the genetic code for making proteins, the code that instructs gene expression programs is only beginning to be elucidated. We will introduce a general experimental approach for mapping transcriptional regulatory code in the human genome. This approach, known as ChIP-on-chip, is designed to map in vivo protein binding sites in the genome by combining Chromatin Immunoprecipitation (ChIP) with the DNA microarray technologies (chip). The lecture will be focused on the methodology of ChIP-on-chip and its application in mapping transcriptional regulatory elements in eukaryotic genomes. Examples will be given illustrating the use of this method to map transcription factor binding sites in yeast and in human cells. The ChIP-on-chip method is one of several technology platforms being explored by the ENCODE (Encyclopedia of DNA Element) Consortium, which was organized by the United State's National Human Genome Research Institute (NHGRI) to identify functional elements in the human genome. Results will be presented demonstrating the use of ChIP-on-chip analysis to obtain a high-resolution map of active promoters in the human genome.

57. Why Should Undergraduates be Engaged in Research? Robert L. DeHaan. Emory University, Atlanta, GA 30322.

Recent progress in science and technology has been dramatic, with the emergence of whole new fields such as bioinformatics, proteomics, and nanotechnology driven by the digital revolution and innovations in information processing. Equally impressive has been the research in the "learning sciences", expanding our understanding of how people learn and how the mind converts information into useful knowledge. Despite these achievements, the impact on many college science courses has been modest. Life science instructors in too many university classrooms continue to teach as they always have, spending most of their time telling students what to learn, while their students spend most of their time memorizing the required information. Growing evidence, too often ignored, shows that students need more active personal engagement in their learning to meaningfully incorporate new knowledge. They also need greater involvement in the research process and much more exposure to quantitative aspects of biology to prepare them for more advanced courses, graduate study, and real world careers. In this workshop, speakers will explore strategies that have proven successful in engaging undergraduates in meaningful research experiences.

58. An Education Group Experiment: Inspiration and Engagement of Students. Graham C. Walker. Massachusetts Institute of Technology, Cambridge, MA 02139.

MIT has had a long tradition of student involvement in research. Their highly successful UROP (Undergraduate Research Opportunities Program) was founded in 1970; currently, about two thirds of MIT undergraduates engage in research. The teaching of "Project Labs" (Advanced Undergraduate Research Labs) in the Department of Biology was also begun in the early 1970s and continues today, providing an exciting, highly supportive environment for students to learn to do research. A recent innovation for students using UROP instead of Project Labs to satisfy their degree requirements has been the introduction of a special class to develop the communication skills the students gain in Project Labs. An HHMI Education Group founded at MIT is building on MIT experience in engaging students in research and in developing ways of exciting and engaging students in large lecture classes (http://www.cfkeep.org/html/snapshot.php?id=22816209). A variety of different approaches are being tried. In an attempt to organize the many concepts introduced in an Introductory Biology class, a Hierarchical Concept Framework (http://www.cellbioed.org/articles/vol3no2/article.cfm?articleid=105) has been created. This Concept Framework was used to develop a volunteer laboratory, taught in conjunction with the lecture course, that enables students to explore the connections between different topics in the course in a hands-on way (http://www.cfkeep.org/html/snapshot.php?id=23219984).

59. Undergraduate Research Experiences and the Epigenesis of a Science Career. David E. Lopatto. Grinnell College, Grinnell, IA 50112.

The undergraduate research experience is widely supported as a vehicle for attracting talented students to careers in science. The career orientation goal of the undergraduate research experience is so entrenched that some science educators consider the undergraduate researcher who does not continue in science to be a waste of resources. Recent studies have clarified the benefits of undergraduate research. These studies show that, while career clarification and preparation are benefits of undergraduate research, the experience alone does not compel a career in science. The intended science career is a conjoint outcome of the experience and the cognitive, motivational, and emotional expectations brought to the experience by the student. In addition, the benefits of the undergraduate research experience are ubiquitous, including gains in oral communication, writing, and

working with colleagues, and thus afford preparation for many careers. These benefits include significant gains in personal development characteristics such as self-confidence and independence. The importance of personal development as a feature of undergraduate research suggests that mentoring, including the management of individual and group work, assumes a key role in student success.

60. Developmental Biology: How to Engage Engineering Students. Kristi S. Anseth. Howard Hughes Medical Institute and the University of Colorado, Boulder, CO 80309-0424.

Tissue engineering is an interdisciplinary field that marries the principles of engineering and developmental biology toward the creation of biological substitutes that restore, maintain, or improve tissue function. This talk will illustrate approaches to prepare and integrate undergraduate researchers in this highly interdisciplinary field and highlight areas related to quantitation, vocabulary, and disciplinary boundaries that are key components to successfully traversing this dynamic landscape. For example, engineering students are often intimidated by the basic terminology of the developmental biology field, but once understood, can use their strong mathematical skills to apply simple diffusion–elimination models and understand how gradients of morphogens can provide spatial information during development. Typically, tissue engineers use advanced biomaterials that serve as scaffolds for the delivery of cells, and the scaffolds further provide cues and signals to the cells to guide the re-growth or healing of a tissue. From an engineer's perspective, this is the quintessential design problem involving the control of processes on length scales ranging from nanometers to centimeters and timescales ranging from milliseconds to months. More simply put, however, the aim is to recapitulate critical events that occur naturally during development. Engaging engineering undergraduates in research projects in this area offers them opportunities to gain wholly new insights into developmental processes.

61. Mentoring Undergraduate Research Projects. Graham F. Hatfull. University of Pittsburgh, Pittsburgh, PA 15260.

The research laboratory is the perfect environment for introducing students to science: what science is, how it is done, and who scientists are. While undergraduate students frequently have opportunities to participate in scientific research as juniors or seniors, many students have already lost interest in science by this time. Providing research opportunities to beginning undergraduates, to high school students, and even middle schoolers, offers a means of introducing students to scientific research while their curiosity is strong and they are willing to experiment. While not all projects are well-suited to these situations, bacteriophage discovery and genomics has unique features that makes it particularly suitable. The high degree of phage diversity and the abundance of phage particles in the environment ensure that each student has a good chance of isolating a completely novel bacteriophage that they can discover, purify, and name. Isolation of phage DNA and genomic sequencing provides insights into the genetic diversity of viruses and the evolutionary mechanisms that give rise to them. Many stages of this project are technically feasible while offering important scientific insights into bacteriophage biology. While discovering new phages, students have opportunities to learn about many aspects of biology including microbiology, ecology, genetics, and evolution.

62. Computer Modeling of Cells and Early Development: Getting Students Involved. Garrett M. Odell. Friday Harbor Laboratory, U Washington, WA.

Much of modern human technology would not exist or function absent understanding gained from math/computer modeling which few biologists currently regard as a useful research tool. But evolved biological phenomena – from molecular biology to cell biological mechanism to ecological community dynamics and especially development – are far more complex than any human-wrought technology. To figure out quickly how natural selection's technology works, tomorrow's biologists will need math/computer simulation tools more urgently than today's engineers. Because of modern fast affordable computers and powerful software such as Mathematica, Maple, MatLab, and Java, these tools are accessible to any interested biologist with the determination to learn to use them. Courses I have taught at the University of Washington's Biology department on mathematical modeling techniques useful in biology required independent and extensive projects in which students used Mathematica to solve equations they formulated to account for ecological or cell biological or biochemical phenomena. These courses, open to undergraduate and graduate students, have been populated chiefly by biology majors who found standard issue algebra and calculus courses repellent. My courses had no prerequisites. They concentrated on what theorems meant rather than on how to prove them. They offloaded all calculations and formula manipulation to Mathematica. Spanning two quarters, they began with elementary arithmetic, progressed through linear algebra, and ended with non-linear ordinary and partial differential equations. Many students pursued their projects after the courses ended, to publication in biology journals. In this talk, I will show a few examples of student work to illustrate how mathematical modeling can shed light on biological mysteries that would otherwise remain obscure.

63. Controlling Cell Proliferation During *Drosophila* Wing Development. Bruce A. Edgar, Laura Buttitta, Ling Li, Huaqi Jiang, David O'Keefe, Derek Nickerson, Ortrud Wartlick, and Aida Flor A. de la Cruz. Fred Hutchinson Cancer Research Center, Seattle, WA 98109.

A central, unsolved mystery of development concerns the control of growth and form. The control of cell proliferation is central to this problem, and the mechanisms that execute cell cycle arrest upon differentiation are particularly relevant. While cell cycle exit has been studied in cell culture and in several model organisms, no general paradigm exists explaining the ubiquitous coupling of cell differentiation to G1 arrest. We are studying cell cycle exit in the *Drosophila* wing. By manipulating cell growth rates using MYC and TOR pathway components, which regulate protein synthesis and nutrient storage, we find that cell cycle exit in the wing is controlled independently of cell growth. We have also tested each of the components of the E2F/RB and Cyclin/CDK/CKI cell cycle control systems for regulatory roles in cell cycle exit. Although E2F/RBF activity does ensure the repression of cell cycle gene expression at differentiation, the E2F/RBF system is dispensable for cell cycle exit. *Drosophila*'s sole G1-specific cyclin-kinase inhibitor, dacapo, is also not required. These results prompt us to hypothesize that differentiation signals dominantly suppress the transcription of multiple cell cycle control genes using an E2F/RB-independent mechanism. Hence, we are analyzing the *cis*-regulatory control region of the cyclin E gene, which must be silenced for timely cell cycle exit. We are also performing genetic screens for novel factors involved in the exit process. Progress in each of these areas will be reported.

64. Dynamic Genetics: A New View of Plant Growth. Elliot M. Meyerowitz,[1] Venugopala Reddy,[1] Marcus Heisler,[1] Henrik Jonsson,[2] Bruce Shapiro,[3] and Eric Mjolsness[4]. [1]California Inst. Technol., Pasadena, CA; [2]Lund University, Lund, Sweden; [3]Jet Propulsion Lab, California Inst. Technol., Pasadena, CA; [4]Univ. California Irvine, Irvine, CA.

The shoot apical meristem (SAM) is a permanent population of stem cells that provides for all of the above-ground tissue in a growing *Arabidopsis* plant. Despite consisting of only a few hundred cells, the SAM is highly structured, and planes, patterns, and numbers of cell divisions are tightly regulated. The cells in the SAM control their division and patterns of

gene expression based upon cell–cell communication—the SAM is a network of interacting cells that maintain their states and activities dynamically. We have developed a new set of methods for studying gene expression within, and cell division in, the SAM, and are developing computational methods for modeling it. The new methods combine fluorescent reporter genes, time-lapse confocal imaging, and regulatable versions of key regulatory genes such as those coding for ligands and transcription factors involved in the communication network. Computational models of the interaction network suggest new experiments, and real-time analysis of meristem activities after changes in gene activity tests the computational models, leading iteratively to refined computational models of meristem activities.

65. MicroRNAs Controlling Neuronal Development. Oliver Hobert. Columbia University Medical Center, New York, NY 10033.

MicroRNAs (miRNAs) have recently surfaced as abundant regulators of gene expression, yet the in vivo function of the vast majority of miRNAs is still poorly defined. I describe here an intricate network of gene regulatory factors including microRNAs and transcription factors, which regulate a bistable cell fate decision between two alternative neuronal fates, the ASE left (ASEL) and ASE right (ASER) fate. ASEL and ASER are the primary taste receptor neurons of *C. elegans* and share many bilaterally symmetric features. However, in the adult stage, each cell expresses a distinct set of chemoreceptors and neurotransmitters that define an "ASEL fate" or an "ASER fate". These distinct fates endow the gustatory system with the capacity to sense and discriminate specific environmental inputs. These left/right asymmetric fates develop from an equipotent precursor state in which both ASEL and ASER express mixed features. Mutant analysis reveals that, after the initial expression of mixed features, the ASE cells must subsequently adopt either the ASEL or ASER fate, thus defining the ASE cell fate decision as a bistable system. This bistable system is controlled by a feedback regulatory loop that involves two miRNAs, lsy-6 and mir-273, and their respective transcription factor target genes, cog-1 and die-1. Similar regulatory loops are found as common motifs in many gene regulatory networks, but a role for miRNAs in such networks had not yet been identified. The regulatory architecture defined here may present a paradigm for how neurons select among alternative fates and lock into stable states.

66. The Plant Exosome: Role in Embryo/Endosperm Identity Choice and Imprinting, Functional Specialization of its Subunits, and Novel RNA Substrates. Dmitry A. Belostotsky,[1] Sergei V. Reverdatto,[1] Julia A. Chekanova,[1] Nikolai P. Skiba,[2] Jose M. Alonso,[3] Vladimir B. Brukhin,[4] Joseph R. Ecker,[5] and Ueli Grossniklaus[4]. [1]State University of New York, Albany, NY 12222; [2]Harvard Medical School, Boston, MA 02114; [3]North Carolina State University, Raleigh, NC 27695; [4]University of Zurich, Switzerland; [5]Salk Institute, La Jolla, CA 92037.

Exosome is an evolutionarily ancient macromolecular complex composed of numerous hydrolytic and phosphorolytic RNases and auxiliary factors. It plays a key role in numerous, mechanistically distinct reactions of RNA processing, proofreading, and degradation in nucleus and cytoplasm. To date, the effect of exosome depletion has not been investigated at the whole-organism level in any multicellular species of life. Here, we present the proteomic analysis of the *Arabidopsis* exosome and characterization of several exosome subunit mutants. The loss of the RRP4 subunit leads to an ectopic activation of endosperm-specific genes in the embryo, loss of parental imprinting, and embryo lethal phenotype. In contrast, the loss of RRP41 results in an arrest of the female gametophyte development. This is the first evidence that the individual subunits of the exosome core have specialized developmental functions. A novel genetic depletion system for RRP4 and RRP41 exosome subunits via inducible RNA interference (iRNAi) has been established and helped reveal the essential requirement for exosome at the stages of plant life cycle beyond reproductive development, the role in rRNA processing, and a novel function in processing and/or degradation of the RNAP III transcripts. Supported by grants from USDA and NSF to D.A.B.

67. An RNA–Protein Complex that is Required for Efficient TGF-α Secretion in *Drosophila*. James E. Wilhelm and Suzanne Sayles. Carnegie Institution of Washington.

Proper trafficking of proteins through the secretory pathway is critical for a number of signaling events that establish the primary body axes. It has become increasingly apparent that a number of signaling molecules require specialized chaperones or processing for proper transit through the secretory pathway. During *Drosophila* oogenesis, the dorsal–ventral axis is established by localized signaling between the oocyte and the follicle cells by *gurken* (*grk*), a TGF-α homolog. We have identified a novel gene, *trailer hitch*, that is required for proper secretion of Grk. Mutations in *trailer hitch* cause Grk protein to accumulate in large membrane vesicles that appear to be derived from the ER. Consistent with a role in ER-Golgi trafficking of Grk, we have found that Trailer Hitch localizes to domains that overlap ER exit sites. Surprisingly, *trailer hitch* contains an atypical Sm domain and an FDF domain, suggesting that it plays a role in regulating RNA metabolism. Biochemical purification of Trailer Hitch complexes has revealed that Trailer Hitch is part of a large RNA–protein complex that includes the RNA helicase, Me31B, and the eIF4E binding protein, Cup. Me31B and Cup also colocalize with Trailer Hitch at ER exit sites, confirming the in vivo relevance of their biochemical association with Trailer Hitch. We propose that the normal secretion of Grk requires the local translation of proteins at ER exit sites and that *trailer hitch* is required for this process. Because *trailer hitch* orthologs are present in virtually all eukaryotes, these results raise exciting new possibilities for how the "RNA world" could interface with the classical secretory pathway.

68. To Find, Modify, and Control the Messenger RNA: New PAPs, PUFs, and 3′UTRs. Marvin Wickens,[1] Labib Rouhana,[1] Jae Eun Kwak,[1] Laura Opperman,[1] Natascha Buter,[1] and Charles Landry[2]. [1]Dept. Biochemistry, University of Wisconsin, Madison, WI 53706; [2]Dept. Psychiatry, University of Wisconsin, Madison, WI 53706.

Regulatory elements in the 3′UTR of eukaryotic mRNAs often control mRNA stability, translation, and localization. Changes in poly(A) length, dictated by these elements, often are critical. Two protein families – PUF proteins and the GLD-2 poly(A) polymerases – provide paradigms for mRNA controls mediated by the 3′UTR and poly(A). We will describe our studies of PUF proteins in *C. elegans*, focusing on the basis of the mRNA binding specificity. The binding by these proteins is strikingly modular, permitting modifications that alter specificity predictably. From the biological standpoint, the PUF proteins accelerate poly(A) loss and cause deadenylation. Thus, they can either repress translation or cause mRNA decay. A new family of Poly(A) adding enzymes, termed the GLD-2 (or CID1/13) family, are also critical in RNA controls. We will discuss the roles of these GLD-2 proteins in *C. elegans*, *Xenopus*, and mouse cells, We will discuss their activities, mRNA targets, and biological functions.

69. RNA Recognition by the STAR Proteins GLD-1 and Quaking. James R. Williamson,[1] Sean P. Ryder,[1] Leah A. Frater,[2] and Elizabeth B. Goodwin[2]. [1]The Scripps Research Institute, La Jolla, CA 92014; [2]The University of Wisconsin, Madison, WI 53706.

STAR domain RNA binding proteins have been implicated in a variety of post-transcriptional gene regulation processes in many organisms. We have taken a quantitative biochemical approach to study the RNA binding

by the two prototypical STAR domain proteins GLD-1 and Quaking. GLD-1 is a key translational regulator involved in *C. elegans* germline development, and GLD-1 binding to tra-2 mRNA has been localized to the 3′-UTR. Quaking is a murine protein that binds to the mRNA for myelin basic protein and is involved in regulation of myelination in the central nervous system. Quantitative analysis of the binding to RNA oligonucleotides has identified the minimal binding site for these proteins and defined the consensus recognition motifs as a hexanucleotide element. Using this consensus binding site, we have identified putative novel targets for these proteins. Several challenges remain, including defining the minimal functional element in vivo, and verification of putative targets and experiments are ongoing to address these important issues.

70. miR-196 Acts Upstream of Hoxb-8 and Shh in Limb Development. Eran Hornstein,[1] Jennifer H. Mansfield,[1] Soraya Yekta,[2] Brian D. Harfe,[3] Michael McManus,[4] Scott Baskerville,[2] David P. Bartel,[2] and Clifford J. Tabin[1]. [1]Department of Genetics Harvard Medical School Boston, MA 02115; [2]Whitehead Institute for Biomedical Research Cambridge, MA 02142; [3]University of Florida, FL; [4]UCSF San Francisco, CA 94143.

MicroRNAs are an important, recently identified class of molecules that regulate gene activity. However, to date, miRNAs in vertebrates have not been associated with any specific embryonic processes. We find that miR-196 acts upstream of Hoxb-8 and Sonic hedgehog in vivo in the context of limb development, representing a previously identified but uncharacterized activity operating specifically in the hind limb. Our data suggest that the role of miRNAs in vertebrate development is to act as a fail-safe mechanism to assure fidelity of expression domains regulated primarily at the transcriptional level.

71. A New Mechanism for Translational Repression: Tethering of *Caudal* mRNA 5′ and 3′ Ends by Bicoid and 4EHP. Francis Poulin,[1] Park F. Cho,[1] Yoon A. Cho-Park,[1] Ian B. Cho-Park,[1] Jarred D. Chicoine,[2] Paul Lasko,[2] and Nahum Sonenberg[1]. [1]Department of Biochemistry, McGill University, Montréal, QC H3G 1Y6, Canada; [2]Department of Biology, McGill University, Montréal, QC H3A 1B1, Canada.

Translational control is a key genetic regulatory mechanism implicated in the regulation of cell and organismal growth and early embryonic development. Initiation at the mRNA 5′ cap structure recognition step is frequently targeted by translational control mechanisms. In the *Drosophila* embryo, cap-dependent translation of the uniformly distributed *caudal* (*cad*) mRNA is inhibited in the anterior by Bicoid (Bcd) to create an asymmetric distribution of Cad protein. Here, we show that d4EHP, an eIF4E-related cap-binding protein, specifically interacts with Bcd to suppress cad translation. Translational inhibition depends on the Bcd binding region (BBR) present in the cad 3′ untranslated region. Thus, a simultaneous interaction of d4EHP with the cap structure and Bcd interaction with BBR renders *cad* mRNA translationally inactive. This example of cap-dependent translational control that is not mediated by canonical eIF4E defines a new mechanism for translational inhibition involving tethering of the mRNA 5′ and 3′ ends.

72. The Genetic Mechanisms Underlying Brain Ventricle Morphogenesis in Zebrafish. Laura A. Lowery and Hazel Sive. Whitehead Inst./MIT.

The mechanisms by which the vertebrate brain develops its characteristic three-dimensional structure are poorly understood. The brain ventricles are a conserved system of cavities that form very early during brain morphogenesis and are required for normal brain function. However, the molecular basis for brain ventricle development has not been defined. We have initiated a study of zebrafish brain ventricle formation and show that the neural tube expands into primary forebrain, midbrain, and hindbrain ventricles rapidly, over a 4-h period during mid-somitogenesis. Circulation is not required for initial ventricle formation, only for later expansion. In order to define the genetic mechanisms underlying initial brain ventricle formation, we are analyzing thirty-three brain ventricle mutants identified in several mutagenesis screens (Jiang et al., 1996; Schier et al., 1996; Amsterdam et al., 2004), but not generally studied further. Two mutants that do not develop brain ventricles are nagie oko and snakehead. Mutants in nagie oko, which encodes a MAGUK family protein, fail to undergo ventricle morphogenesis. This correlates with an abnormal brain neuroepithelium, with no clear midline and disrupted junctional protein expression. In contrast, the snakehead neural tube undergoes normal ventricle morphogenesis, however, the ventricles do not inflate, likely due to impaired ion transport and disrupted osmotic gradient formation. We show that snakehead is allelic to small heart, which has a mutation in the Na+K+ ATPase gene atp1a1a.1. We are using transplantation techniques to generate mosaic snakehead embryos in order to examine the tissue requirement for atp1a1a.1 function during brain ventricle formation. This study defines three steps required for brain ventricle development and that occur independently of circulation: (1) morphogenesis of the neural tube, requiring nok function; (2) lumen inflation requiring atp1a1a.1 function, and (3) localized cell proliferation. We suggest that mechanisms of brain ventricle development are conserved throughout the vertebrates.

73. Zebrafish *from beyond* Establishes Left–Right Asymmetry of the Zebrafish Brain. Joshua T. Gamse,[1,2] Kiran Santhakumar,[2] Christine Thisse,[3] Bernard Thisse,[3] and Marnie E. Halpern[2]. [1]Vanderbilt University; [2]Carnegie Institution of Washington; [3]IGBMC.

Differences between the left and right sides of the brain are found throughout the vertebrate phylum; however, the developmental basis of these left–right (L–R) differences is largely unexplored. We have identified a family of genes in zebrafish, including *leftover* (*lov*), *right on* (*ron*) and *dexter* (*dex*), that show different expression patterns in the left and right habenulae, bilaterally paired nuclei in the forebrain. Lov and Ron proteins are differentially distributed in cell bodies and also in efferent axonal connections from the L–R habenular neurons to the midbrain target, the interpeduncular nucleus (IPN). Lov^{+} and Ron^{+} projections innervate different regions along the DV axis of the IPN. Since the function of Lov and Ron is not obvious from their sequence, we are using two-hybrid screening of an expression library to identify interacting proteins and antisense depletion in zebrafish embryos. To gain insight into the mechanisms that determine brain laterality, we performed a screen for new mutations, using expression of *lov* in the habenulae as a read-out and isolated *from beyond* (*fby*). In 4-day-old *fby* mutants, the forebrain develops symmetrically, and D–V differences in habenular connectivity are perturbed. However, laterality of the visceral organs is unchanged. The *fby* phenotype is significant for two reasons. First, unlike other mutants, it appears to affect brain laterality exclusively, revealing a brain-specific L–R developmental pathway. Second, *fby* affects the formation of laterality, while other known mutations only randomize the direction of laterality.

74. Pattering the Endoderm into Organ Progenitor Domains. Kenneth S. Zaret,[1] Amelie Calmont,[1] Kimberly Tremblay,[1] Gail R. Martin,[2] and Roque Bort[1]. [1]Cell and Devel. Biology, Fox Chase Cancer Center, Philadelphia, Pa; [2]Univ. Calif. San Francisco.

The endoderm germ layer is the progenitor cell population that gives rise to the liver, pancreas, and other digestive organs. The mechanisms by which these tissues are specified and initiate morphogenesis should provide insight into tissue regenerative responses, tumorigenesis, and

stem cell differentiation. Using dye labeling and culture of whole mouse embryos, we created a fate map of the foregut endoderm. The fate map allows us to investigate tissue progenitor cells prospectively, prior to and during their specification. Surprisingly, we found that two distinct types of endoderm-progenitor cells, from three spatially separate embryonic domains, converge to generate the epithelial cells of the liver bud. Previous studies from our laboratory showed that FGF signaling from the cardiogenic mesoderm induces liver genes and suppresses pancreatic genes in the endoderm. We now find that, prior to specification, the lateral prospective liver domains of endoderm cells, adjacent to cardiac mesoderm, contain phosphorylated ERK and that P-ERK remains activated for the hours it takes for the liver to be specified. We have employed cre-activated, dominant negative inhibitors of FGF signaling in endoderm and signal inhibitory drugs on mouse embryos and endoderm–mesoderm explants, along with scaled-down biochemical analysis of proteins in endoderm cells. Together, these studies have revealed FGF-dependent signal pathways within endoderm cells that lead to cell type specification and include the unexpected finding that post-transcriptional induction of a tissue-specific transcription factor is involved.

75. Genetic Analysis of Sprouty Gene Function in the Developing Mouse Embryo. Gail R. Martin, George Minowada, Benjamin Yu, Ophir Klein, and Katherine Shim. University of California, San Francisco.

Members of the Sprouty gene family encode negative feedback antagonists of receptor tyrosine kinase (RTK) signaling. In mice, three of the four Sprouty genes, *Spry1*, *Spry2*, and *Spry4*, are expressed during embryogenesis in a variety of developmental settings. Using mice carrying null or conditional null alleles of these Sprouty genes, we are exploring the developmental consequences of eliminating their function. We have found that *Spry2* null mutants display abnormalities in both tooth and inner ear development, the latter resulting in deafness, and that *Spry4* null mutants have abnormal limbs. All of these defects can be rescued by reducing FGF or FGF receptor gene dosage, thereby demonstrating the importance for normal development of regulating responsiveness to FGF signaling via Sprouty gene function.

76. Conditional Inactivation of Fgfr1 in Mouse Defines its Role in Limb Bud Establishment, Outgrowth and Digit Patterning. Xin Sun,[1] Mark Lewandoski,[2] Chuxia Deng,[3] Brian Harfe,[4] and Jamie Verheyden[1]. [1]Laboratory of Genetics, University of Wisconsin, Madison, WI 53706; [2]National Cancer Institute, Frederick, MD 21702; [3]NIDDK, NIH, Bethesda, MD 20892; [4]Univ. of Florida, Gainesville, FL 32610.

Previous studies have implicated Fibroblast growth factor receptor 1 (Fgfr1) in limb development. However, the precise nature and complexity of its role have not been defined. Here, we dissect Fgfr1 function in mouse limb by inactivating a conditional allele of Fgfr1 using two different Cre recombinase-expressing lines. Use of the Tcre line led to Fgfr1 inactivation in lateral plate mesoderm (LPM) cells including limb bud mesenchymal precursors prior to limb initiation. This mutant reveals two essential roles of Fgfr1 in limb development. In a nascent limb bud, Fgfr1 restricts mesenchymal cell number and organizes these cells into three axes with defined parameters. Later on during limb outgrowth, Fgfr1 is essential for the expansion of precursor population along the P/D axis by maintaining cell survival. Use of mice carrying the Sonic Hedgehogcre (Shhcre) allele led to Fgfr1 inactivation in posterior mesenchymal cells prior to autopod development. This mutant allows us to test the role of Fgfr1 in patterning without disturbing growth. Our results show that Fgfr1 influences digit number and identity, likely through cell-autonomous regulation of Shh expression. We will present data from the characterization of these two Fgfr1 conditional mutants that allowed us to define the multiple roles of Fgfr1 in limb bud establishment, growth and patterning.

77. Regulation of Branching Morphogenesis in the Mammary Gland by MMP and FGF Signaling. Andrew J. Ewald, Pengfei Lu, Justin Trumbull, Gail Martin, and Zena Werb. Dept. of Anatomy, UC, San Francisco.

The mammary gland forms through the invasion and branching of the epithelium through a fatty stroma. We seek to understand how the invasion is initiated and how the morphogenetic program is accomplished at the cellular level. We have developed an in vitro culture model of mammary branching morphogenesis as a complement to in vivo genetic analyses. We have reconstructed the basic program of morphogenesis in these cultures from extensive long-term time lapse movies. We observe epithelial remodeling, lumen formation through cell death, ductal initiation, extension, and bifurcation. Ligands of the FGF, EGF, and HGF families are all sufficient to induce this program but result in different final branched states and in different cell behaviors during branching. To characterize our model system, we examined MMP2 null mice. Previous work from our laboratory demonstrated that MMP2 was required for primary invasion in vivo. We observe a deficit in branch initiation in the MMP2 knockout in vitro and a corresponding increase in cell death in the luminal epithelium, accurately replicating the in vivo phenotype in a 1 week culture model. We then tested the requirement for FGFR2. Mosaic deletion of FGFR2 in the luminal epithelium resulted in severely delayed invasion in vivo and a near absence of branching in vitro. Confocal analysis revealed that the FGFR2 null cells are progressively excluded from the epithelium and die. These results together validate our in vitro culture model and identify a required role for both MMP2 and FGFR2 in branching morphogenesis.

78. Shoots and Leaves, an Evo–Devo Perspective. Neelima R. Sinha,[1] Helena M. Garces,[2] Connie E. Champagne,[1] Kook-Hyun Chung,[1] Sumer J. Seiki,[1] and Brad T. Townsley[1]. [1]Section of Plant Biology, University of California, Davis, CA 95616; [2]Centro de Biotecnologia Vegetal, Faculdade de Ciencias, Universiade de Lisboa, Campo Grande, P-1749-016, Lisboa, Portugal.

The Class I Knotted-like homeobox (KNOX 1) genes are highly expressed in the shoot apical meristem but not expressed in the emerging leaf primordium in tobacco, maize, or *Arabidopsis*. These genes play a role in generating atypical leaf morphology in the genus *Kalanchoe*. We have analyzed compound leaf producing shoot apices in clades with independently derived compound leaves and shown that with one exception (a derived clade in the Fabaceae) compound leaves always show expression of KNOX genes (Bharathan et al., 2002). In the derived pea clade, the LFY/FLO gene regulates this function of generating leaf complexity. We have explored the function of LFY in the basal and derived clades in the Fabaceae. In addition, we find that other genes like PHANTASTICA might play a role in determining the form of the compound leaf generated. Global gene expression differences between simple and compound leaves are also being analyzed using *Neobeckia aquatica* RNA on the *Arabidopsis* gene chip. In addition, we are exploring the evolution of KNOX genes and their role in shoot morphology across Angiosperms.

79. Gene-Regulatory Interactions in Neural Crest Evolution and Development. Marianne Bronner-Fraser. Div. of Biology, California Institute of Technology, Pasadena, CA 91125, USA.

The neural crest is an embryonic cell population defined by its origins at the neural plate border, migratory capacity, multipotency, and characteristic gene expression profile. The appearance of this tissue was likely a turning point in vertebrate evolution since many of the structures which define the vertebrate clade are derived from neural crest. Consistent with a key role in vertebrate origins, a bona fide neural crest is present in every extant vertebrate species including the most basal jawless fish, lamprey, but not in the most vertebrate-like invertebrates. I will present data on the conserved

role of SoxE genes in craniofacial patterning of the lamprey and both conserved and divergent aspects of its protein function in amphioxus—a vertebrate-like chordate that lacks neural crest. Finally, I will compare gene-regulatory circuits involved in neural crest formation in vertebrates to putatively homologous networks operating in amphioxus. These comparisons suggest novel gene-regulatory relationships that may have potentiated the evolution of definitive neural crest.

80. Hemichordates and the Origin of Chordates. John Gerhart,[1] Christopher Lowe,[2] and Marc Kirschner[3]. [1]University of California, Berkeley, CA 94720-3200; [2]University of Chicago, Chicago, IL 60637; [3]Harvard Medical School, Boston, MA 02115.

Hemichordates are the phylum of adult bilateral animals closest to chordates. Comparisons of the two groups reveal aspects of development and organization that are ancestral to chordates. We find similar anterior–posterior maps in the two groups for the domains of expression of 32 genes implicated in chordate neural patterning. In this dimension, four signaling centers are remarkably alike, for example, one of Wnt1 and Fgf8 at the first gill slit level of hemichordates and at the midbrain–hindbrain level of chordates. In the dorsal–ventral dimension, both groups have a Bmp–Chordin axis, but inversely oriented. Hemichordate patterning in this dimension can be systematically altered by siRNA elimination of endogenous Bmp and by exposure of the embryo to exogenous Bmp protein, whereas neurogenesis does not appear inhibited by Bmp. We conclude that the common ancestor possessed a complex anterior–posterior domain organization, gill slits, and a post-anal tail that were conserved with little change in both lines, whereas dorsal–ventral organization was altered greatly in the chordate line in connection with the centralization of the nervous system (which is diffuse in hemichordates) and the evolution of the notochord.

81. Pipe Cleaners and Beads: Modeling Molecular Interactions Involved in Anterior–Posterior Pattern Formation in *Drosophila*. Kristin R. Douglas. Augustana College, Rock Island, IL 61201.

The only prerequisite for my developmental biology course is an introductory cell biology course. Although students appreciate the fact that proteins have three dimensional structure, they often fail to recognize how protein interactions with other cellular components can lead to very specific responses. One of the first topics covered in my developmental biology course is anterior–posterior axis determination in *Drosophila*. In the past, I have taught this subject with a series of graphs demonstrating mRNA and protein concentrations along the anterior–posterior axis. These graphs made perfect sense to me, but the ideas were too conceptual for the vast majority of my students. To aid their understanding, I have created a kinesthetic model of the molecular interactions involving *bicoid*, *nanos*, *hunchback*, and *caudal* transcripts and proteins using colored pipe cleaners and beads. Students model molecular interactions between proteins (beads) and transcripts (pipe cleaners) by placing the appropriate bead on the appropriate pipe cleaner. After working with the model, the concept of molecular interactions became much more concrete to students, and they were able to conceptualize anterior–posterior axis determination in *Drosophila* more clearly. Throughout the rest of the course, students were able to understand molecular interactions without the aid of additional models.

82. Molecular Laboratory Activities for Undergraduate Developmental Biology Courses: Examining the Expression of Spicule Matrix Protein 30 (SM30) in *Lytechinus variegatus* Embryos Using RT-PCR. Deborah D. Ricker and Jeffrey P. Thompson. York College of Pennsylvania, York, PA 17405.

Studies in developmental biology have reached an amazing level of complexity in recent years, especially those examining the expression, regulation, inhibition, and/or manipulation of specific genes throughout embryogenesis. Unfortunately, this type of intensive, hands-on, molecular study is often far removed from the more basic laboratory activities commonly available to students at the undergraduate level. To address this inconsistency, we sought to bridge the gap between current trends in developmental biology research and the undergraduate laboratory experience. This study utilized current research in the field of sea urchin (*Lytechinus variegatus*) spicule matrix proteins conducted in the laboratory of Dr. Fred Wilt at the University of California-Berkley. The specific protein, SM30, exhibits tissue-specific expression beginning at the mid-gastrula stage. Using the Cells-To-cDNA® RT-PCR kit available from Ambion, Inc. and primer sequences published by the Wilt laboratory, students were able to witness the stage-specific expression of the SM-30 gene throughout sea urchin embryo development. The protocol available in the RT-PCR kit eliminated the time and difficulty often associated with RNA isolation and provided more consistent results between laboratory groups. Students not only gained valuable experience with a powerful molecular technique, but they also were able to extend the classical morphological assessment of sea urchin embryo development to a more advanced molecular level.

83. Molecular Laboratory Activities for Undergraduate Developmental Biology Courses: Examining the Expression of TIE-2 Protein and mRNA During Zebrafish (*Danio rerio*) Embryo Development Using ELISA and RT-PCR. Deborah D. Ricker and Jeffrey P. Thompson. York College of Pennsylvania, York, PA 17405.

This molecular activity was designed for use in an undergraduate developmental biology course, where oftentimes laboratories are restricted to basic morphological assessments. This activity allowed students to use ELISA and RT-PCR to examine TIE-2 protein and gene expression in developing zebrafish (*Danio rerio*) embryos. TIE-2 is the tyrosine kinase receptor for angiopoietin, an angiogenic protein. Using an antibody against zebrafish TIE-2 and maleic anhydride plates coated with embryonic extracts, students performed an ELISA to identify the TIE-2 protein throughout zebrafish development. These data were then correlated with TIE-2 gene expression using Cells-to-cDNA® RT-PCR. This laboratory allowed students to witness the onset of zebrafish TIE-2 protein synthesis and correlate this event with gene expression. Moreover, students were able to integrate two techniques to better understand the molecular events surrounding zebrafish embryo development. By combining commercially available resources, set-up time for this activity was significantly reduced. While the initial cost was notable, using these materials in additional laboratories justified the expense. Obtaining adequate numbers of zebrafish embryos was also a consideration. Overall, this laboratory activity provided students with valuable hands-on experience using current molecular techniques. Students could also use this model system to investigate the developmental effects of various angiogenic or anti-angiogenic drugs on TIE-2 protein and gene expression.

84. Engaging Undergraduates in a Laboratory Course Using Human Adult Stem Cells and Osteoblasts: Approaches and Assessments. John S. Doctor and Katie M. Gallagher. Duquesne University, Pittsburgh, PA 15282.

The excitement of stem cell research and cell culture was brought directly into an undergraduate laboratory course at Duquesne U. Commercially available human adult mesenchymal stem cells (hAMSC) and osteoblast cells (line MG-63) were the focus of a 5-week-long module in an advanced (junior/senior level) laboratory course in Cellular and Molecular Biology, one of four "SuperLab" courses offered at Duquesne. The laboratory meets twice a week for 3–4 h per session and once a week for a 1 h recitation. A total of seventy-eight students in the course, during the springs of 2002 through 2005, learned the basics of cell culture and aseptic technique and set up experiments to examine the developmental potential of hAMSC and

proliferative/differentiative capacity of MG-63 cells. Cell culture was a component of almost all laboratory sessions during the module. Several experiments by small teams of students evaluated hAMSC morphology, proliferation and osteogenic differentiation via fluorescent microscopy, cell counting, MTT vital dye staining/colorimetric quantitation, alkaline phosphatase histochemistry, and measurement of calcium deposition. Portions of several laboratory sessions were devoted to journal club discussions and oral presentations. The students analyzed and presented their research results in written papers (prepared, reviewed, and re-submitted, as if for publication) and at a poster session for the laboratory course. Understanding and technical facility were measured to assess student learning.

85. The Whole Developmental Class Does One 10-week Research Project. Judith E. Heady. University of Michigan-Dearborn, Dearborn, MI 48128.

My embryology (developmental biology) class is certainly not the first or only class that has used a single long original research project for the primary laboratory activity for the whole class, but this is my first time. There are many advantages: all students are engaged in a common problem that is at least moderately exciting; there are many more repetitions of the variations of conditions than any single group could do routinely; there is only one preparation to be done for the project part of the laboratory; and there is a reasonable chance that the data will yield an interesting presentation at a local, regional, or even national meeting. The only disadvantages are the need for large numbers of animals at the same stages rather constantly and the need for a large number of glass finger bowls perhaps never to be used in such quantity again. Another drawback might be that the possibility for individuality and small group ingenuity is minimal. The third time the students set up experiments, the individual groups were allowed to have a small, but unique variation that would add information to the main project. This did allow for some creativity. The poster will explore the pluses, minuses, and the outcomes of the project. Several students have pledged to write abstracts for local Michigan meetings to be held in April and May, 2005.

86. Integration of Research Projects in Standard Undergraduate Courses as a Strategy to Teach Analytical Skills and Increase Faculty Research Productivity. Brian J. Avery and Bonnie K. Baxter. Dept. of Biology, Westminster College, Salt Lake City, UT 84105, USA.

Integrating faculty research projects into courses is a potentially successful strategy to accomplish two of the goals of most undergraduate teaching institutions. These goals are to help our students acquire the research and critical thinking skills that they need to succeed and to find time for faculty to pursue their own research endeavors while teaching 3–4 classes each semester. We have developed courses that involve students in related faculty research projects instead of short, individual laboratory exercises. This approach benefits the students since they are gaining valuable experience including experimental design and technique, reading and interpreting primary research literature, and a taste of how immersive and rewarding research can be. We start this process in our second year genetics course and continue it in most of our upper division biology courses. Faculty members gain the hard work and insight of a larger group of students to work on their research projects, and they also may recruit research students for their laboratories. We will present our strategy and experience from the past few years.

87. Ernest Everett Just (1883–1941)—An Early Ecological Developmental Biologist. W. Malcolm Byrnes[1] and William R. Eckberg[2]. [1]Biochemistry and Molecular Biology and; [2]Biology Departments, Howard University, Washington, DC.

Ecological developmental (Eco–Devo) biology involves the study of development in its natural environmental context as opposed to the laboratory setting. E.E. Just was an early twentieth-century African-American embryologist. Born in the South and educated in New England and the Midwest, he devoted his career to studying the early development of marine invertebrates in the United States and abroad. Through detailed study of the fertilization process, he came to see the cell cortex as playing a central role in development, inheritance, and evolution. This poster, after describing the world in which Just worked and presenting some of his scientific and philosophical contributions, will argue that Just was an Eco–Devo biologist. Three lines of evidence will be given. First, Just believed that intimate knowledge of the natural history of the marine animal under study – hence, the natural setting in which fertilization occurs – was essential. Second, he stressed the importance of the egg's "normality" — how well its condition in the laboratory corresponds to the natural, fertilizable state. Finally, Just was an organicist, believing that living things are holistic systems with emergent properties that arise from their organization and complexity, a view common among Eco–Devo biologists today. The conclusion is that, although other scientists may stand out more clearly as founding architects of Eco–Devo, E.E. Just, with his unwavering insistence on the normality and holistic integrity of the egg cell, was one of its purest early adherents. Supported in part by the Howard University Fund for Academic Excellence.

88. Spatio-Temporal Manipulation of Signaling Pathways at Rosa26 Locus. Junhao Mao,[1] Jeffery Barrow,[2] Jill McMahon,[1] Joe Vaughan,[1] and Andrew P. McMahon[1]. [1]Harvard University, Cambridge, MA 02138; [2]Brigham Young University, Provo, UT 84602.

We have developed a versatile genetic system for spatio-temporal regulation of signaling pathways at Rosa26 locus. In this, loss of either *E. coli lacZ* or YFP activity in a new embryonic cell line, 3–1, enables the rapid identification of targeted alleles at the ubiquitously expressed Rosa26 locus. We introduced a dominant allele of Smoothened that results in constitutive activation of Hedgehog signaling into 3–1 cells. By coupling Cre-loxP-dependent activation with tetracycline-dependent transcription in a single allele at Rosa26 locus, we established a novel conditional method to control Smoothened activity and neural progenitor specification in differentiating ES cell in vitro and in the neural tube of G0 chimeras in vivo and in spatio-temporal control of cell lineage activity when crossed to an appropriate Cre driver strain in the developing mouse embryo. Given the increasing evidence linking Hedgehog, Wnt, and BMP signaling pathways to the regulation of stem cell biology and tumor formation, the cell autonomous manipulation of dominant active or dominant negative forms of key members of the signaling pathways is likely to be wildly applicable. We have established Cre-LoxP and Doxycycline regulated ES cell lines with R26 knock-in of a dominant active beta-catenin, dominant negative mutant of FGFR2 and Noggin-CD4-GFP fusion, which is predicted to inhibit BMP2/4 signaling. The cellular and genetic tools we generated will facilitate the analysis of the regulatory function of major signaling pathways in their normal and pathological roles.

89. Spatiotemporal Gradients of Retinoic Acid and Identification of Target Tissues. Gregg Duester, Natalia Molotkova, Andrei Molotkov, and Ovidiu Sirbu. Burnham Institute, La Jolla, CA.

A spatiotemporal requirement for retinoic acid (RA) signaling during vertebrate development has been revealed by genetic analysis of retinaldehyde dehydrogenase-2 which catalyzes RA synthesis. In the mouse, RA is first synthesized at E7.5 prior to somitogenesis by Raldh2 expressed in paraxial mesoderm and later in somites themselves. Although it is clear that Raldh2 expression in somites serves as a source of RA required for development of hindbrain rhombomeres 4–8, for spinal cord motor neurons and for bilateral symmetry during somitogenesis, it is unclear when and where RA acts. This is being addressed by examination of the location of RA activity in wild-type, Raldh2 knockout, and RA-rescued Raldh2 knockout

mouse embryos carrying an RA-reporter transgene. In E8.0–E8.5 Raldh2−/− embryos rescued for posterior neural development and somitogenesis by maternal dietary RA treatment, we find that the administered RA results in RA activity that becomes localized to posterior neuroectoderm but that RA activity is surprisingly absent in somites which are normally the source of RA in wild-type embryos. We provide evidence that the unique spatiotemporal expression patterns of retinoic-acid-binding proteins in the posterior neuroectoderm and Cyp26 RA-degrading enzymes in the anterior neuroectoderm may account for preferential RA sequestration to the posterior neuroectoderm of rescued mutants and absence of RA activity in somites. Our findings suggest that, while RA is normally synthesized in the paraxial mesoderm and later in somites, it does not need to act in mesoderm in order to fulfill its role in posterior neural development and somitogenesis.

90. Deciphering Roles of PDGF Targets in Vivo. Jennifer P. Schmahl and Philippe Soriano. Fred Hutchinson Cancer Research Center, Seattle, WA 98109.

PDGF receptors and their ligands are essential for the development and normal function of many cell types and tissues including vascular smooth muscle cells, neural crest cells, the kidneys, testes, lung and skeleton. Despite the broad requirements for PDGF, most critical downstream targets of PDGF signaling remain unknown. Recently, several PDGF target genes were identified using a novel screen combining microarrays and gene trap mutagenesis in ES cells. To elucidate the in vivo function of these genes and their relevance to PDGF mutant phenotypes, mice carrying null mutations of twelve target genes were generated and characterized. These twelve genes include five putative transcription factors (Arid5b, BC055757, Mzf6D, Tiparp and Zfp216), three genes potentially involved in cytoskeletal remodeling or adhesion (Cdh11, MyoIe and Schip1) and three genes that may regulate cellular metabolism (Axud1, AA960558 and Sgpl1). We have determined that mice with null mutations for all but one of these genes are homozygous viable, however, further characterization revealed that many of these genes are critical for the patterning and normal function of PDGF-dependent systems, including the kidneys, testes, lungs and skeleton.

91. Context-Specific Requirements for FRS2-Mediated Signaling Downstream of FGFR1 During Mouse Development. Renee V. Hoch and Philippe Soriano. FHCRC, UW, Seattle, WA 98109.

Loss-of-function studies have highlighted essential developmental functions of fibroblast growth factors and their receptors (FGFRs). However, the mechanisms by which FGFRs transduce signals in vivo are still poorly understood. Biochemical experiments have implicated FRS2 (α,β) adaptor proteins as major effectors of FGFR signaling. These proteins interact constitutively with FGFRs and mediate transduction of FGF signals to various pathways. To determine which developmental functions of FGFR1 require signaling through FRS2 adaptors, we have generated mice harboring a deletion in the FRS2 binding site on FGFR1. This mutation results in embryonic lethality, but the phenotypes of $FGFR1^{\Delta FRS2/\Delta FRS2}$ embryos indicate that this signaling pathway is required to mediate only a subset of FGFR1 functions during development. In addition to signaling through FRS2, FGFR1 may signal in vivo through previously unidentified pathways or may transduce additive signals by concurrently activating multiple pathways.

92. IFT Proteins Regulate Both the Activator and Repressor Activities of Gli Proteins. Aimin Liu,[1] Baolin Wang,[2] and Lee A. Niswander[3]. [1]University of Colorado Health Sciences Center, Aurora, CO 80045; [2]Cornell University Medical College, New York, NY 10021; [3]HHMI and University of Colorado Health Sciences Center, Aurora, CO 80045.

Hh signaling has been shown to be critical for regulating numerous patterning events during mammalian embryonic development, and abnormal Hh signaling is accounted for many human birth defects and cancer. However, despite intensive study for the last decade, very little has been known about the transduction and regulation of the Hh signaling in mammal. Through a forward genetic approach in the mouse, we identified IFT88, a protein implicated in ciliogenesis, as a novel regulator of Hh signaling in the mouse. Genetic epistasis analyses indicate that IFT88 acts inside the Hh receiving cells to regulate the activities of Gli transcription factors. More interesting, we found that, different from all the previously identified Hh signaling regulators in the mouse (Disp1, Ptc1, Smo, Rab23, etc), IFT88 is required for both the activator and repressor functions of Gli transcription factors. Preliminary biochemical study indicates that IFT88 is required for the proteolytic cleavage of Gli3 protein. We also generated mouse mutants for another mouse IFT gene, IFT52, and showed that, similar to IFT88, IFT52 is also required for Hh signaling and Gli3 processing. Therefore, there is a general requirement for IFT proteins in Hh signaling. In summary, we have found a previously unappreciated link between IFT and Hh signaling, and understanding the unique way by which IFT regulate Hh signaling would greatly advance our knowledge on the Hh signaling regulation.

93. A Novel Secreted Regulator of the Hedgehog Pathway Required for Both Activation and Inhibition. Jeremy F. Reiter. Univ. of California, San Francisco, CA 94143.

Through a large-scale insertional mutagenesis of the mouse genome, we identified a number of new regulators of mouse development. One of these encodes a secreted factor that participates in Hedgehog-mediated patterning of the neural tube and limb buds. This protein, named Tectonic, is the founding member of a previously undescribed family of evolutionarily conserved proteins. During neural tube development, mouse Tectonic is required for formation of the most ventral cell types and for full Hedgehog (Hh) pathway activation. Epistasis analyses reveal that Tectonic acts in the Hh signal transduction pathway downstream of Sonic hedgehog (Shh) and its receptor Patched1. Interestingly, characterization of *Tectonic Shh* double mutants indicates that, apart from its role mediating Hh signal transduction, Tectonic has an additional role in repressing Hh pathway activity, also reflected by the essential role of Tectonic in restricting digit number. Identification of a factor required for both full Hh pathway activation and repression provides genetic evidence that the mammalian Hh signal transduction pathway exists in three discrete states corresponding to absent, intermediate and high Hh concentrations.

94. Analysis of the Interactions Between Wnt-3a and Wnt-5b Signaling Pathways During Chick Development. K.J. Sanders,* Y. Martinez,* M. Meyerzon,* and L.W. Burrus, *Equal contribution; SFSU, 1600 Holloway Avenue, SF, CA 94132.

Wnt proteins are a family of secreted signaling molecules that are important in many vertebrate developmental processes, including neural tube and somite patterning. Wnts signal through two main pathways, the best understood being the β-catenin-mediated pathway. β-catenin-independent pathways, though less studied, provide additional insight into the various functions of Wnts. While Wnt-3a generally signals through the β-catenin-mediated pathway and plays a role in somite patterning in early chick development, Wnt-5a/5b generally signal through β-catenin-independent pathways and are linked to myogenesis. As Wnt-3a and Wnt-5b both have documented roles in somites, we sought to characterize the interaction between these two signaling pathways in chick embryos, cell culture, and somite explants. Analysis of proliferation in overexpression studies in the neural tube of chick embryos suggests an antagonistic relationship between Wnt-3a and Wnt-5b, as does a β-catenin dependent Tcf/Lef reporter assay in 293T cells. Conversely, somite explants treated with Wnt-3a and Wnt-5a conditioned media both exhibited increased proliferation, suggesting the possibility that they may have additive or synergistic effects. This potentially agonistic relationship was also observed in somites in vivo where Wnt-3a and

Wnt-5b synergize to expand the area of the dermomyotome. Thus, we have identified potentially agonistic and antagonistic relationships between Wnt-3a and Wnt-5a/5b signaling pathways in different embryonic structures, suggesting tissue-specific interactions between the pathways.

95. Induction of the Opthalmic Trigeminal Placode is Regulated by Wnt/Frizzled Signaling. Rhonda T. Lassiter and Michael R. Stark. Brigham Young Univ., UT.

Cranial placodes are focal regions of ectoderm that contribute extensively to the peripheral nervous system in the vertebrate head. The trigeminal and epibranchial placodes give rise only to sensory neurons in cranial sensory ganglia. The paired-domain homeobox transcription factor Pax-3 is an early marker of the avian ophthalmic trigeminal (opV) placode. This placode gives rise to sensory neurons in the ophthalmic lobe of the trigeminal ganglion (opV). It has previously been shown that a diffusible signal from the midbrain/rostral hindbrain is necessary for Pax-3 induction or maintenance in the opV placode. In this study, we have identified Wnt/Frizzled signaling as a candidate pathway that regulates opV trigeminal placode induction. We demonstrate that inhibition of canonical Wnt signaling results in cell-autonomous downregulation of Pax-3 protein in the trigeminal placode of chick embryos.

96. Wnt-7a is Involved in BMP-2-Induced Chondro-Inhibitory Mechanism in Chick Wing Bud Mesenchymal Cells. Eun-Jung Jin,[1] Sun-Young Lee,[1] Young-Bae Lee,[1] Ok-Sun Bang,[2] and Shin-Sung Kang[1]. [1]Department of Biological Sciences, Kyungpook National University, Daegu, 701-702, South Korea; [2]Department of Life Sciences, Seoul National University, Seoul 151-742, South Korea.

The bone morphogenetic protein (BMP) family of signaling molecules has been implicated in the regulation of cartilage development. Here, we demonstrate a direct role for BMP-2 signaling in chondrogenic differentiation of chick wing bud mesenchymal cells. In vitro studies reveal that BMP-2 represses precartilage condensation and chondrogenesis. The presence of BMP-2 induces down-regulation of cellular condensation-related molecules, such as fibronectin, FAK, and paxillin, induces apoptotic cell death via activation of caspase-cascade signaling, and subsequently results in down-regulation of chondrogenesis. BMP-2 induces sustained protein levels of N-cadherin and β-catenin as well as mRNA level of Wnt-7a, and phosphorylation pattern of GSK3, at the time their expressions are extinguished during normal chondrogenesis. Consistent with these results, a sustained activation of Wnt signaling by transfection of constitutively active β-catenin also induces a chondro-inhibitory effect suggesting that BMP-2-induced persistent up-regulation of Wnt-7a signaling is responsible for chondrogenic inhibition in mesenchymal cells. Taken together, BMP-2 acts as a negative regulator of precartilage condensation and survival via cross-talk with Wnt-7a signaling during the chondrogenesis of HH stage 22/23 chick wing bud mesenchymal cells. Key words: BMP-2, Wnt-7a, chondrogenesis, chick.

97. Planar Cell Polarity Patterns the Actin-Based Cell Protrusions that Form Ventral Denticles in the *Drosophila* Embryo. James Walters and Stephen Dinardo. Univ. of Pennsylvania, Philadelphia, PA.

Planar cell polarity (PCP) allows epithelial cells to coordinate their development to their surrounding tissue. PCP occurs in many tissues including orientation of hairs on the fly wing and, in vertebrates, the gastrulation movements of convergence–extension (CE). In the embryonic epidermis of *Drosophila*, segments elaborate actin-based protrusions (APs) that are oriented and coordinated along cell rows in the denticle field (DF). We found that the DF exhibits PCP and that this contributes to proper alignment of APs. Several features point to a novel mechanism for establishing DF PCP. First, APs initiate broadly on the apical face of the epithelial cell, only later migrating to or stabilizing at its posterior edge. This contrasts with wing prehair initiation. We are using live imaging to differentiate two AP deployment models: guided placement vs. selective stabilization. Second, we found denticle orientation to be distinct from PCP, unlike wing PCP. Third, neither core PCP component frizzled or disheveled is involved in DF PCP, while non-muscle myosin II (zipper) exhibited a striking phenotype. Zip is polarized to posterior edges of DF cells as is its regulatory light chain, Spaghetti squash (Sqh). We are using inhibitors of the activating kinase for Sqh to confirm its role in DF PCP. Zip and Sqh have recently been implicated in CE events earlier in development, we suggest that they are re-deployed to affect DF PCP. We are imaging their re-alignment on cells between the period of CE and DF polarity. Finally, fringe may play a role in DF PCP. We suggest that DF cells exhibit PCP and may use novel principles and genes to execute it.

98. TGF-β-Induced Up-Regulation of MMP-2 Depends on MAPK Signaling in the Chondrogenic Differentiation of Chick Leg Bud Mesenchymal Cells. Young-Ae Choi, Jae-Han Park, Ji-Hye Son, Eun-Jung Jin, and Shin-Sung Kang. Department of Biological Sciences, Kyungpook National University, Daegu, 701-702, South Korea.

Transforming growth factor-β (TGF-β) can inhibit or stimulate chondrogenic differentiation. In the present study, treatment of TGF-β3 inhibited chondrogenenesis of chick leg bud mesenchymal cells and also induced a sustained expression level of both active MMP-2 protein and MMP-2 mRNA at later stage, implicating the role of active MMP-2 in chondro-inhibitory mechanism of TGF-β3. To verify the role of active MMP-2 on chondrogenesis, MMP-2 activity was modulated by using GM6001 (an inhibitor) or bafilomycin A1 (an activator). The treatment of GM 6001 under TGF-β3 stimulus compensated the chondro-inhibitory effect of TGF-β3. However, activation of MMP-2 by bafilomycin A1 following the stimulus of TGF-β3 synergistically increased inhibition of chondrogenesis. These results demonstrate that the down-regulation of enzymatic activity of MMP-2 is necessary at later stage of chondrogenesis. Moreover, blocking Erk1/2 phosphorylation with U0126 inhibited MMP-2 activation, whereas inhibiting p38 MAPK activity using PD169316 stimulated proteolytic potential of MMP-2. These observations demonstrate the opposing effects of ERK1/2 and p38 MAPK in the regulation of MMP activity during chondrogenesis of leg mesenchymal cells. Collectively, these data suggest that TGF-β3 induces the sustained enzymatic activity of MMP-2 which involves the modulation of MAPK signaling, and this persistent activation of MMP-2 might be responsible for the chondro-inhibitory effect.

99. Methyl-Beta-Cyclodextrin Inhibits Chicken Embryo Somitic Myogenesis Possibly through Inactivation of Lipid Raft Signaling. Wendy L. Rosenthal and Wilfred F. Denetclaw. San Francisco State University, San Francisco, CA 94132.

The ectoderm is an important signaling source for dermomyotome development and myotome differentiation. Our previous studies showed stable dermomyotome filopodial contact with its overlying ectoderm and membrane microdomain movement between these tissues suggestive of lipid raft signaling for early myotome formation. We investigated this possibility by treating chicken embryos (HH14–15) in ovo with 2.5 mM methyl-β-cyclodextrin (MβC), an inhibitor of lipid raft activity, and assessing effects on myogenesis by desmin immunolabeling and by myoD in situ hybridization. When MβC was added for 6–8 h, myotome formation was completely blocked in somite stages VII to X, and myoD expression was lost in newly formed somites (stages II to V). In contrast, control embryos (no MβC) were positive for myotome and myoD at somite stages indicated. When MβC treatment was followed by washing and 36 h embryo re-incubation, somites previously inhibited for myotome and myoD

expression recovered normally. However, somites produced from the cranial half of the segmental plate mesoderm during MβC treatment (4–5 new somites) exhibited a unique loss of myotome at the dermomyotome dorsomedial cranial corner, although it was normally expressed from other dermomyotome lip locations. In more caudal somites, myotome formation was again normal. These results suggest that lipid rafts regulate myotome development in somites and that they participate in the specification of myotome precursor cells in the cranial segmental plate mesoderm. Supported by NIH-RIMI 1P20MD000544, 9P20MD000262.

100. Dermomyotome Filopodia Contacts Surface Ectoderm During Early Epaxial Myotome Development in the Chicken Embryo. Gustavo A. Gomez, Emi Okada, and Wilfred F. Denetclaw Jr. Department of Biology, San Francisco State University, San Francisco, CA 94132.

The tissues surrounding somites in chick embryos secrete molecular factors that result in somitogenesis and myotome development. Previous studies in vitro suggest that the surface ectoderm (SE) signals the myogenic precursor cell population of the dermomyotome (DM) in a contact-dependent manner. However, the evidence has not been examined in the natural environment of these two tissues. To investigate filopodia–ectoderm interactions, we electroporated both the DM and SE with a farnesyl membrane tagged EGFP reporter construct and imaged the positively transfected cells in vivo by confocal microscopy. Their interaction and the transfer of membrane materials between them was also examined at the ultrastructural level by Transmission Electron Microscopy (TEM) after DiI labeling of the SE. With both confocal and TEM imaging, the evidence confirms that filopodia are DM derived and contact the SE basal lamina without penetrating it, and we have also observed DM filopodia extended perpendicular to the apico–basal axis of DM cells. While the confocal images alone show that DM filopodia are both unbranched and branched, and their dynamic nature, as revealed by time lapse imaging, suggests that filopodia may play an active role in the development of myotome cell progeny from the DM. With TEM, DiI photoconversion with DAB showed small membrane particles both in the SE and DM. These findings show membrane transfer between SE and DM possibly by signaling to the dermomyotome via filopodia. Supported by NIH Grants 1P20MD000544, 9P20MD000262, 5R25GM59298-06.

101. Interaction of Contactin and Notochord-Derived Chondroitin Sulfate Proteoglycans Induces Repulsive Axon Guidance of Primary Sensory Neurons in *Xenopus* Embryo. Naoko Fujita and Saburo Nagata. Faculty of Science, Japan Women's Univ., Tokyo, Japan.

Contactin, a member of the immunoglobulin (Ig) superfamily neuronal recognition molecule, regulates axonal growth, guidance and myelination through interaction with various signal molecules in the environment. In *Xenopus* embryos, Contactin is expressed in spinal primary somatosensory neurons and plays an essential role in guidance of their growing peripheral axons. Here, we show data suggesting that this axon guidance involves recognition by Contactin of the notochord-derived repulsive signal mediated by chondroitin sulfate proteoglycans (CSPG). In an alkaline-phosphatase (AP)-tagged receptor-binding assay, Contactin-AP specifically bound to the notochord of whole-mounted or sectioned tailbud embryos. This binding was abolished either by chondroitinase ABC digestion of the embryo sections or preincubation of Contactin-AP with chondroitin sulfate A (CS-A). Immunohistochemical examination of the embryo showed localization of CS in the notochord and its vicinity. When the spinal cord and the notochord explants were co-cultured in collagen gel matrix, the Contactin-positive spinal axons were repelled by notochord-derived diffusible factors. After cultivation, CS was immunochemically detected in the collagen gel matrix around the notochord. Addition of the anti-Contactin monoclonal antibody or CS-A into the culture medium inhibited the repulsive response of the spinal axons to the notochord. These results suggest that the notochord secretes CSPGs with CS-A or related glycosaminoglycan side chains and repels the growing Contactin-positive axons.

102. Ectodermal GATA-2 Functions as Part of a Transcriptional Complex Downstream of BMPs During Primitive Hematopoiesis. Gokhan Dalgin and Jan L. Christian. Oregon Health and Science University, Portland, OR 97239.

In *Xenopus*, primitive blood originates primarily from the ventral mesoderm, but signals from the ectoderm are required during gastrulation for differentiation of erythrocytes. We have previously shown that Bone Morphogenetic Proteins (BMPs) function non-cell autonomously in the ectoderm to regulate primitive hematopoiesis. Specifically, BMP function is required in ectodermal cells to generate a secondary signal(s) that acts across germ layers to enable hematopoietic progenitors to commit to the erythroid pathway and to protect committed progenitors from apoptosis. Moreover, we have shown that calmodulin-dependent kinase IV (CamKIV) negatively regulates BMP-mediated transcriptional responses during hematopoiesis. In the present study, we demonstrate that the transcription factor GATA-2 is also required in ectodermal cells for normal primitive hematopoiesis. Morpholino-mediated knockdown of GATA-2 expression in whole embryos phenocopied downregulation of BMPs or upregulation of CamKIV, causing a loss of RBCs due to increased apoptosis. Furthermore, ectoderm derived from control embryos was able to support globin expression in recombined ventral mesoderm, whereas that derived from GATA-2 MO injected embryos was not. This demonstrates that GATA-2 function is required specifically in ectodermal cells. Finally, epistasis analysis was performed, and the results are consistent with the hypothesis that GATA-2 functions as a component of a transcriptional complex downstream of BMP/CamKIV in ectodermal cells to regulate primitive hematopoiesis.

103. The Role of fgf Receptors in Otic Placode Development. Robert Esterberg and Andreas Fritz. Emory University, Atlanta, GA 30322.

In vertebrates, the inner ear arises from the otic placode. Development of the otic placode requires both intrinsic and inductive factors. The signaling molecules Fgf3 and Fgf8 have been implicated in the induction and specification of the zebrafish inner ear. *fgf3* and *fgf8* are expressed in early mesendoderm as well as the hindbrain, but not in otic precursor cells, and are redundant in otic vesicle formation. We have identified the intrinsic transcription factor Foxi1 as being necessary for this process. It has been suggested that foxi1 may provide competence to the ectoderm to respond to inductive signals. Foxi1 is the earliest known gene to be expressed in the otic primordia and is essential for normal ear development. To begin to understand the nature of competence in otic placode formation, we are focusing on the role of Fgf receptors. There are four known Fgf receptors in zebrafish. We are examining the expression patterns of these receptors in *foxi1* mutant backgrounds. We are also using genetic interaction analyses and morpholino knock-down to determine which receptors are necessary to specify competence to otic precursor cells. Using strains of *fgf3* or *fgf8* null fish, we propose that it may be possible to dissect ligand–receptor interactions in vivo.

104. An Early Role for Notch Signaling During Development of the Zebrafish Inner Ear. Adam Bermange, Nicolas Daudet, Jonathan Leslie, Rachael Brooker, and Julian Lewis. Vertebrate Development Laboratory, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3PX, UK.

The Notch signaling pathway is best known for its role in lateral inhibition; cells embarking on differentiation express Notch ligands and

signal to adjacent cells to both prevent them from expressing these ligands and from adopting the same fate. In the vertebrate inner ear, the fine-grained patterning of the sensory patches, comprising of mechanosensory hair-cells surrounded by supporting cells, requires Notch-mediated lateral inhibition with the cells differentiating as hair cells expressing Notch ligands such as Delta1 and Serrate2/Jagged2. Perhaps the clearest evidence for this role comes from the zebrafish mutant *mind bomb*, which is defective in Delta-mediated signaling; all sensory precursor cells differentiate as hair cells at the expense of supporting cells. Recently, however, Notch activity has also been implicated in the specification of sensory patches, prior to their differentiation, conferring prosenory character on groups of cells within the otocyst, a process that may be mediated by another ligand, Serrate1/Jagged1 (Jagged1b in the zebrafish). Here, we present our initial observations of Notch activity in the early development of the zebrafish inner ear and assess the effects on sensory patch formation when the Notch pathway is overactivated at this stage by heat-shock-induced expression of a constitutively active Notch. Furthermore, we examine the role of Jagged1b in early Notch signaling and investigate, both in vivo and in cell culture, whether signaling mediated by this ligand is dependent on the activity of Mind bomb.

105. Fz Activity in Planar Cell Polarity Signaling is Regulated by Wnt(s) Gradient. Jun Wu and Marek Mlodzik. Mt Sinai School of Medicine, NY 10029.

Frizzled (Fz) activity plays a crucial role in establishing planar cell polarity (PCP). In the *Drosophila* wing, wing hairs point away from high Fz activity levels toward low activity levels. Fz is not expressed in a concentration gradient and in fact, ubiquitous Fz expression rescues fz-mutant phenotypes, suggesting Fz does not need to be expressed in a gradient in order to generate an activity gradient. This raises the issue of how an Fz activity gradient is generated in vivo. Since several Wnt gene(s) are expressed at the distal end of the wing, Wnt proteins are secreted and diffuse out from the distal to form a Wnt(s) protein gradient along the distal and proximal axis of the wing. This raises the possibility of Wnt(s) gradient might play a role in generating an Fz activity gradient. Based on our loss of function and gain of function analyses, we report that 2 Wnt genes of *Drosophila* can be involved in generating the Fz activity gradient by inhibiting Fz activity in the wing. fz-mutant clones cause PCP defects both in mutant clones and in neighboring wild type cells. Therefore, it was proposed that Fz shares two types of signaling: autonomous and non-autonomous signaling. Our data suggest that Wnt(s) might inhibit the non-autonomous Fz signaling function.

106. Teaching An Old Junction New Tricks: Apical Polarization of the Septate Junction Patterns the *Drosophila* Wing Independently of Frizzled Signaling. Dennis R. Venema[1] and Vanessa J. Auld[2]. [1]Trinity Western University, Langley BC Canada; [2]University of British Columbia, Vancouver BC, Canada.

In *Drosophila*, wing hairs are oriented from proximal to distal and aligned in parallel. Tissue polarity under the control of Frizzled (Fz) signaling is required for hair orientation, however, in the absence of Fz signaling, wing hairs remain parallel. Thus, the final hair pattern requires Fz-dependent and Fz-independent events. We have identified components of the septate junction (SJ) as the first members of an Fz-independent, parallel alignment mechanism. Mutations in two SJ proteins, Gliotactin (Gli) and Coracle (Cora), disrupted hair alignment without altering hair polarity. During early wing development, Gli and Cora were restricted to basolateral membranes in a pattern identical to that of embryonic epithelia. This pattern persisted until after prehairs were extended. After prehair extension, Gli and Cora were apically polarized in ribbons aligned beneath prehair bases. Gli and Cora remained apically polarized for several hours before returning to the embryonic pattern. In a Gli mutant, polarization of Gli and Cora was disrupted, and prehairs became unstable. Polarization of Gli and Cora was unaffected by a loss of Fz signaling. Mutations in the SJ components Discs-large, Neurexin IV and Scribble dominantly enhanced the Gli parallel alignment phenotype, and Discs-large protein was found to polarize apically in concert with Gli. Taken together, these results demonstrate a role for SJ components in Fz-independent tissue patterning and suggest that the SJ complex is reassembled on apical membranes to stabilize and align developing prehairs.

107. Specificity vs. Redundancy: *Rhomboid1*, Not *Roughoid*, is Required for *Epidermal Growth Factor Receptor* Mediated Photoreceptor Cell Patterning. Denise A. Birkholz,[1] Wen-Hai Chou,[2] Meridee Phistry,[1] and Steven G. Britt[1]. [1]Univ. Colorado School of Medicine, Aurora, CO 80045; [2]Univ. Texas Health Science Center, San Antonio, TX 78245.

The *Drosophila* compound eye is highly patterned, and opsin gene expression is tightly coordinated. The expression of *Rh5* in R8 cells appears to be regulated by a signal from adjacent *Rh3* expressing R7 cells, while *Rh6* expression in R8 cells adjacent to *Rh4* expressing R7 cells occurs as a default fate. We identified the *Scutoid* mutant, which has a dramatic alteration in this pattern. This appears to result from the generation of an inappropriate signal from *Rh4* expressing R7 cells that induce the expression of *Rh5* in the adjacent R8 cell. At a molecular level, we show that the *Scutoid* phenotype is due to the ectopic expression of *snail*, a transcriptional repressor that requires the co-repressor CtBP for this effect. These two genes repress the expression of *rhomboid1* in a narrow band of the developing eye. Interestingly, complete loss of *rhomboid1* in the eye results in the absence of *Rh5* induction, indicating that *rhomboid1* is essential for signaling. We show that inappropriate signaling in *Scutoid* likely results from two waves of *rhomboid1* expression passing over the eye, which can be mimicked in WT animals by ectopic expression of *rhomboid1*. Furthermore, we show that EGFR is required during pupal development for *Rh5* induction. This signal is specifically mediated by *rhomboid1* and not its paralogue *roughoid*. These studies demonstrate that, while *rhomboid1* and *roughoid* are interchangeable and redundant for some aspects of eye development, they each have specific and distinct functions. Supported by NEI.

108. dLgl Functions in Differentiating CNS Cells. Svetlana Trunova and Allen Shearn. Johns Hopkins Univ., Biology Dep., Baltimore, MD 21218.

The *Drosophila* neoplastic tumor suppressor *lethal* (2) *giant larvae gene* (*lgl*) is required for epithelial and neuroblast polarity, neuroblast asymmetric division and proliferation control. Our data about effects of *lgl* mutations on differentiation abilities of CNS cells show that Lgl dysfunction does not totally prevent neuron and glia differentiation even in highly overgrown brain lobes. We found a tissue-specific *lgl* effect on eye disc development and used it to apply the developing *Drosophila* visual system as a model to examine Lgl functions in CNS cells. Normally, the precursors of retinal basal glia (RBG) proliferate in the optic stalk and migrate into eye discs towards axons projected by photoreceptor cells. *Lgl*$^{-}$ photoreceptors had defects in axonal fasciculation and direction of axonal growth. An extreme *lgl* mutant (*lgl4/net62*), predicted to be missing the entire protein, still has RBG differentiation, but these glial cells lose the ability to migrate to the proper position even when photoreceptors differentiate and project axons. In a less extreme lgl mutant (*lgl-DV275/lgl-m32*), predicted to be missing the C-terminus of the protein, some RBG follow additional targets (Bolwig nerve and others). In both cases, RBG fail to sense some part of signaling from the morphogenetic furrow and developing ommatidia. The frequency of these defects was greater in larvae with greater delays in development at the third larval stage. These morphological defects mimic ones caused by abnormal *hh* signaling. Ongoing rescue experiments with transgenic *lgl* constructs will provide data about the functional significance of other Lgl domains for neuron–glia interaction.

109. The Role of EGF and TGF-β Signaling in Patterning of Follicle Cells During *Drosophila* Oogenesis. Bhupendra V. Shravage and Siegfried Roth. Univ. of Koeln, Koeln, Germany.

In *Drosophila*, patterning of the follicle cells covering the developing oocyte is achieved by inductive signaling. The EGF/TGF-α like ligand Gurken is released from a dorsal cortical region of the oocyte defined by asymmetric position of the oocyte nucleus. This signal is responsible for the determination of dorsal follicle cell fates. It is further amplified in follicle cells by Rhomboid and fine tuned by inhibitors Argos. Together, these modulate the signaling strength across the monolayer of follicle cells. In addition, TGF-β signaling is required for inducing anterior dorsal follicle cell fates. We are interested in understanding how these signaling cascades co-operate to pattern the follicle cells. Misexpression of Gurken in the developing oocyte and of Dpp (the TGF-β/BMP2/4-like ligand) in follicle cells leads to an increase in number of follicle cells becoming dorsal in character. Moreover, combined misexpression of Grk and Dpp leads to novel egg phenotypes. To investigate the exact role of EGF signaling, the expression of BR-C, *aos* and *rho* in misexpression experiments and in mutant follicle cell clones was analyzed.

We have also cloned homologues of *ski/snoN* from *Drosophila*. Ski proteins are nuclear oncoproteins which were reported as inhibitors of TGF-β signaling. SnoN shows a striking expression pattern in follicle cells. Loss of *snoN* in follicle cells results in enlarged opercula and abnormal dorsal appendages. Double mutants of *snoN* and known Dpp inhibitors in *Drosophila* lead to strong dorsalization of eggshell. Currently, we are trying to generate mutant follicle cell clones of dpp inhibitors to understand their role in patterning.

110. Action and Components of the FAT Signaling Pathway. Eunjoo Cho and Kenneth D. Irvine. HHMI, Waksman Institute, Rutgers University, Piscataway, NJ.

Fat is a *Drosophila* tumor suppressor and also a regulator of tissue polarity. We have found that Fat is also required for signaling from distal cells to proximal cells of the wing to regulate its growth and patterning. One major target gene of this signaling is Wingless in the proximal wing, which is negatively regulated by Fat. We show that two genes, *four-jointed* and *dachsous*, which have previously shown to function together with Fat for the establishment of tissue polarity, influence the expression of Wingless in the proximal wing and Fat protein localization. We also identify *dachs* as a gene that is genetically required downstream of Fat both for its effect on imaginal disc overgrowth and for the expression of Wingless in the proximal wing. In addition to Wingless in the wing, we show that Fat regulates *four-jointed* expression in the eye and Ser in the leg, which are non-autonomous targets of Four-jointed. The close relationship of Fat, Four-jointed, Dachsous and Dachs in the regulation of gene expression and tissue polarity suggests that they form a novel intercellular signaling pathway. To identify other key components of this novel signaling pathway, we used the expression of Wingless in the proximal wing as an output of the signaling pathway and examined genes that have similar overgrowth phenotypes to *fat* mutants. We will present an analysis of these new potential components of the Fat signaling pathway.

111. An Investigation of a Network of Protein Interactions Downstream of LRP Proteins in Mouse and *Drosophila* May Also Operate in *C. elegans*. Brian J. Avery,[1] Stacy Vroman,[1] Rylan Larsen,[1] and Gerald M. Rubin[2]. [1]Dept. of Biology, Westminster College, Salt Lake City, UT 84105, USA; [2]HHMI, Dept. of MCB, U. of CA, Berkeley, CA 94720, USA.

The Low Density Lipoprotein (LRP) family proteins are involved in Wnt signal transduction and are important during the embryonic development of many animals. Using the yeast two-hybrid assay to investigate the protein–protein interactions of the LRP family of proteins, we have found a complex network of interactions among proteins of the LRP, CAP/Vinexin, Axin, and Grb2/Drk protein families in flies, mice, and worms. Using the fly LRP as bait, we detected interactions between LRP and a CAP/Vinexin homolog and the fly Axin. These interactions were confirmed by co-IP. We then examined the interactions of the mouse LRP5 and LRP6 with the 3 members of the CAP/Vinexin protein family and found a similar pattern of interaction. Using the mouse LRP6 in yeast two-hybrid, we discovered that it interacts with Grb2. We further showed that Grb2 also binds to the mouse CAP. While the fly Drk and CAP/Vinexin proteins did interact, Drk did not interact with fly LRP.

112. Two Receptor-Like Kinases Mutually Required for *Arabidopsis* Embryo Development. Michael Nodine, Kelli Davies, Ramin Yadegari, and Frans Tax. University of Arizona, Tucson, AZ.

Intercellular signaling plays an essential role during plant embryogenesis. However, few of the components involved in intercellular signaling during embryo development have been described. We have identified two leucine-rich repeat receptor-like kinases (LRR-RLKs) that have overlapping functions during embryo development in *Arabidopsis thaliana*. Initially, we were unable to obtain plants homozygous for insertion alleles in both LRR-RLKs. Plants heterozygous for insertions in both genes give rise to atypical Mendelian ratios of defective seeds when self-pollinated. Results from genetic tests suggest that the mutant seed phenotype is due to defects during embryo development. The earliest observable phenotypes of double mutant embryos are abnormal cell divisions during the early globular stage of embryogenesis. These aberrant cell divisions result in mushroom-shaped mutant embryos, which ultimately lead to embryo lethality. Hence, we named these two genes *TOADSTOOL1* and *TOADSTOOL2* (*TOAD1/2*). Approximately, one-half of *toad1;toad2*/+ embryos are indistinguishable from *toad1 toad2* double mutant embryos, while the other half of *toad1;toad2*/+ embryos appear identical to wild-type embryos. Thus, embryo development is (ultra-) sensitive to *TOAD1/2* gene-dosage. Coupled with preliminary analyses of molecular markers specific for embryo cell types in mutant embryos and expression patterns of *TOAD1/2* translational fusions, these results suggest that TOAD1/2 have overlapping functions and are involved in intercellular signaling necessary for pattern formation and/or morphogenesis during early embryogenesis.

113. Novel Function of POSH, a JNK Scaffold, as a Specific E3 Ubiquitin Ligase for the Hrs Stability on Early Endosomes. Gun-Hwa Kim, Eunjoo Park, Young-Yun Kong, and Jin-Kwan Han. Division of Molecular and Life Sciences, Pohang University of Science and Technology.

POSH (plenty of SH3s) acts as a scaffold that links activated Rac1 and downstream c-Jun N-terminal kinase (JNK) signaling modules. However, it is unknown whether its functional domain-mediated roles include the interesting RING-finger domain or its cellular function. Here, we provide evidence that subcellular localization of POSH is regulated by a particular domain of the protein and POSH was colocalized with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) on early endosomes via interaction of Hrs with POSH's two rear SH3 domains. Moreover, the RING domain of POSH specifically regulates the stability of Hrs, but not of JNK1, via a ubiquitin–proteasomal degradation pathway. Finally, we demonstrate that JNK1 does not interact with Hrs under the conditions of POSH interacted with Hrs, but instead reduces the POSH-catalyzed ubiquitination of Hrs and their reciprocal interaction. Together, these data suggest that POSH has a distinct role as a specific E3 ubiquitin ligase for Hrs on early endosomes, and there exists a relationship between its separate activities as a scaffold and as an E3.

114. Analysis of the Five Intracellular PPPSP Motifs of LRP6. Bryan T. MacDonald, Keiko Tamai, Xin Zen, and Xi He. Division of Neuroscience, Children's Hospital, Harvard Medical School, Boston, MA 02115.

Signaling by secreted Wnt proteins plays a fundamental role in development and disease. The low-density lipoprotein receptor proteins LRP5 and LRP6 serve as Wnt co-receptors and are an essential component of the canonical pathway. The prevailing model suggests that Wnt binds to LRP5/6 and Frizzled to recruit Axin to the cytoplasmic membrane and inhibit the Axin-mediated destruction of β-catenin. As cytoplasmic levels increase, β-catenin translocates into the nucleus and forms a complex with TCF/LEF to activate transcription. Several studies indicate that Axin binds to the cytoplasmic portion of LRP5/6, although the exact mechanism of this interaction is uncertain. Our laboratory recently identified five highly conserved PPPSP motifs in LRP6 that may serve as the docking site for Axin. Mutation of all five motifs in the full-length LRP6 abolishes activity and results in a dominant negative receptor. To examine a single motif in isolation, we transferred the first PPPSP motif of LRP6 to the cytoplasmic portion of the LDLR receptor (LDLRDN). We discovered that this PPPSP motif was phosphorylated and that a single motif is capable of binding Axin in a phosphorylation-dependent manner. We have continued to analyze the prototypic PPPSP motif by alanine scanning mutagenesis to reveal which residues are critical for phosphorylation. We have also transferred the remaining PPPSP motifs into LDLRDN and generated full-length LRP6 receptors containing only one functional motif. The results from these studies will increase our understanding of the phosphorylation-dependent interaction between LRP6 and Axin.

115. Differential Control of Death and Differentiation in Myoblasts Mediated by Hic-5 Isoforms. Zhengliang Gao[1] and Lawrence M. Schwartz[1,2,3]. [1]Molecular and Cellular Biology Program, University of Massachusetts, Amherst, MA 01003, USA; [2]Biology Department, University of Massachusetts, Amherst, MA 01003, USA; [3]Pioneer Valley Life Sciences Institute, Springfield, MA 01107, USA.

Hic-5 is a LIM-only protein that was initially cloned from mouse osteoblasts as a TGF-β or H_2O_2 inducible cDNA. Several conflicting studies have reported that Hic-5 can both enhance and repress myogenesis. In order to resolve these discrepancies, we undertook a detailed analysis of Hic-5 expression and function in C2C12 myoblasts, a well established model for myogenesis. We found that: (1) myoblasts express 5 distinct Hic-5 isoforms; (2) the two predominant isoforms, Hic-5α and Hic-5β, are differentially expressed during myogenesis; (3) ectopic expression of Hic-5a is permissive to differentiation, while expression of either Hic-5β or antisense Hic-5 blocks myoblast fusion and reduces chemo-differentiation; (4) any experimentally induced change in Hic-5 expression resulted in a substantial increase in apoptosis during differentiation. These results explain the discrepancies reported by different research groups and suggest that the roles assumed by Hic-5 during development and homeostasis are complex.

116. Lipid Rafts Regulate Myoblast Differentiation and Myotube Formation in Chicken Embryo Skeletal Muscle Cultures. Andrei Odobescu, Anthonio Luna Jr., Jared Greenberg, and Wilfred Denetclaw Jr. Department of Biology, San Francisco State University, San Francisco, CA 94132.

Cellular membranes contain microdomains (lipid rafts) that are enriched in cholesterol, sphingolipids and signaling proteins to function in cell signal transduction activities. Lipid rafts label with cholera toxin B (CTxB) and are inactivated by cholesterol removal with methyl-beta-cyclodextrin (MBC). MBC inhibits early myotome formation in somites of chicken embryos. To gain further insight into the role of lipid rafts in regulating somitic myogenesis, we made breast muscle cultures from 12-day-old chicken embryos incubating in normal growth medium for 20 h before subjecting cells to medium either with or without 2.5 mM MBC. MBC treatment blocked myoblast differentiation shown by reduced desmin and myosin heavy chain (MHC) immunolabeling and by inhibition of myotube formation. In contrast, MBC-treated myoblast proliferation rates and cell viability were similar to control cultures (medium only or incubation in methyl-alpha-cyclodextrin). When normal growth medium was returned, MBC-treated myoblasts readily underwent differentiation expressing desmin and MHC and formed numerous myotubes after 24 h. When control myoblasts were incubated with fluorescent conjugated CTxB, many punctate membrane areas labeled indicating lipid rafts. However, after 60 min in MBC medium, CTxB labeling became reduced and diffuse. These findings suggest that the loss of cholesterol from the cellular membranes arrests skeletal muscle differentiation, possibly by altering lipid raft signal transduction capabilities. NIH Grants 1P20MD000544, 9P20MD000262, 5R25GM59298.

117. Interaction Between Epithelium and Myogenic Progenitor Cells is Required for Tongue Morphogenesis. Yuji Taya, Takahisa Fujitake, Takako Hirano, Daisuke Torii, Yoshihito Shimazu, Kaori Sato, Yuuichi Soeno, and Takaaki Aoba. Nippon Dental Univ., Tokyo, Japan.

The present study was aimed to analyze how myogenic cells derived from occipital somites are involved in tongue morphogenesis. ICR mouse embryos at E9.5 through E13.4 were subjected to analyze in vivo and in organ culture. Dissected mandibular arches were cultured for 1–4 days. We used antibodies to the following specific markers for muscle and cell division for immunohistochemistry: MyoD; Desmin; Ki67. In vivo Desmin-positive myogenic cells reached mandibular arches from occipital somites at E10.3. Lateral lingual swellings were formed by proliferation of non-myogenic mesenchymal cells without direct contribution of myogenic cells at E11.0. The myogenic cell aggregated beneath midline epithelium in lateral mandibular arches before fusion. Lateral lingual swellings and mandibular arches fused, and the myogenic cells aggregated beneath median sulcus of lateral lingual swellings, and part of these cells adhered to its epithelium at E11.4. After E12.3, tongue configuration was formed completely. In culture (presence of myogenic cells) at the beginning of E10.7 for 3 days, tongue configuration was formed completely. In contrast, lateral lingual swellings were formed and fused laterally, but tongue development made no further progress in culture of E9.5 mandibular arches which were absent of myogenic cells for 4 days. These findings suggest that the somite-derived myogenic cells are not responsible for the formation of lateral lingual swellings directly but is involved in triggering and modulating tongue morphogenesis and growth through epithelio-mesenchymal interaction.

118. Hedgehog Signaling Through the Smoothened Complex. Joan E. Hooper, Christine Bankers, and Hui Yang. Univ. of Colorado School of Med., Aurora, CO 80045.

In the absence of Hedgehog (Hh), the transcription factor Cubitus Interruptus (Ci) is processed to a transcriptional repressor, CiR. Low levels of Hh begin to block production of CiR, while substantially higher levels of Hh are needed to produce the transcriptionally active form of Ci, CiA. Thus, different levels of Hh generate graded states of Ci transcriptional activity-repressing, neutral or activating. Multiple thresholds for Hh activation are achieved because different target genes interpret the neutral state of Ci in the context of other transcriptional inputs. The two fates for Ci are regulated through a complex that includes the transmembrane protein, Smoothened, the divergent kinesin, Costal2, the S/T kinase, Fused, and Ci itself. In the absence Hh, this complex resides on internal

membranes. We show that low Hh, which blocks CiR production, moves Smo to the cell surface and allows accumulation of a partially phosphorylated form of Smo. High Hh, which generates CiA, induces a different phosphorylation of Smo, as well as phosphorylation of Costal2 and Fused. We tested the role of Smo phosphorylation, localization and accumulation in signaling by controlled overexpression of wild type and truncated Smo proteins in *Drosophila*. We find that Smo accumulation, within the physiological range, is not sufficient to activate signaling and that cell surface localization of Smo is not necessary for signaling. Moreover, the evolutionarily conserved region of the Smo cytoplasmic tail is sufficient for all signaling, while the cluster of phosphorylation sites identifies a divergent regulatory domain that restrains Smo activity in the absence of Hh. Supported by NIH.

119. Individual and Cooperative Activities of the Gli Transcription Factors in Hedgehog Signaling. Robert Lipinski and Wade Bushman. University of Wisconsin, Madison, WI 53792.

The Gli family of transcription factors mediate the Hedgehog (Hh) morphogenetic signal by regulating expression of downstream target genes. Aberrations in Hedgehog signaling seriously affect vertebrate development, and mutations in *Gli2* and *Gli3* are associated with distinct classes of developmental defects. Postnatally, inappropriate activation of Hh signaling has been associated with several types of cancers. To better understand both the upstream regulation of the Gli transcription factors, as well as their individual and cooperative roles in regulating expression of Hh target genes, we characterized a battery of embryonic fibroblasts (MEFs) from *Gli* mutant mice. Stimulation of wildtype MEFs by Sonic Hedgehog (Shh) peptide elicited unique profiles of induction of Hh target genes *Gli1*, *Ptc1*, and *Hip1*. Loss of *Gli2* was associated with diminished Shh-induced target gene expression, while loss of *Gli3* was associated with increased basal and Shh-induced target gene expression. The loss of *Gli1* alone had no effect on target gene induction but did diminish Shh-induced target gene expression when combined with the loss of *Gli2* or *Gli3*. Additionally, overexpression of *Gli1* induced target gene expression in $Gli2^{-/-}3^{-/-}$ MEFs, while Shh stimulation did not. Using MEFs expressing only *Gli2* or *Gli3*, we found that cyclopamine and PKA activator forskolin inhibited target gene induction mediated by Gli2 and Gli3. These results show that Gli2 and Gli3 share common regulatory mechanisms and modulate Hh target gene expression directly and independently while also regulating *Gli1* expression, which in specific contexts, coordinately contributes to target gene activation.

120. *Sister of Open Brain* Encodes an Intraflagellar Transport Protein Required to Prevent Ligand-Independent Activation of Mammalian Hedgehog Pathway. Jonathan T. Eggenschwiler and Jian Qin. Princeton University, Princeton, NJ 08540.

Sister of open brain (*sopb*) is a recessive, embryonic lethal mutation identified in a mouse chemical mutagenesis screen. *sopb* mutants exhibit ligand-independent activation of the Sonic hedgehog (Shh) signaling pathway in several tissues such as the neural tube. Epistasis experiments indicated that the wild-type *sopb* gene product antagonizes the pathway at a step downstream of both the Shh ligand and Smoothened, but upstream of the transcription factor Gli2. Positional cloning revealed that the sopb gene encodes Wdr10, whose homologs in *Chlamydamonas*, *C. elegans*, and *Drosophila* function in intraflagellar transport (IFT). IFT is a microtubule-based transport mechanism used to assemble cilia and flagella in a variety of organisms. Recent studies indicate that mammalian IFT components directing anterograde trafficking of cargo to the plus ends of microtubules found at ciliary tips are required for *activation* of the Hedgehog signaling pathway. In contrast, Wdr10 is required for *repression* of the Hedgehog pathway. Studies in *Chlamydamonas* suggest that complex A IFT proteins, such as the Wdr10 homolog IFT122, participate in retrograde trafficking of components towards the minus ends of microtubules located at the base of the cilium. Consistent with such a role, primary cilia from sopb mutant cells exhibit pronounced accumulation of IFT components at their distal tips. Based on these findings, we propose that that the localization of a key mammalian Hedgehog signaling component to the plus or minus ends of microtubules helps determine the "on" or "off" states of the pathway, respectively.

121. Wnt Pathways and Sea Urchin Development. Jenifer Croce,[1] David R. McClay,[1] Louise Duloquin,[2] Guy Lhomond,[2] and Christian Gache[2]. [1]Duke Univ., Durham, NC 27707; [2]UMR7009 CNRS-UPMC, Villefranche-sur-Mer 06230, France.

One of the key regulators of cell fate determination in sea urchin embryogenesis is the canonical Wnt pathway. Although this pathway is now well characterized, the two non-canonical Wnt pathways are less well understood. Here, we report the characterization of two proteins implicated in one or the other non-canonical pathways: a Frizzled receptor protein that signals through the PCP pathway and a Wnt5a ligand that may activate the PCP and/or the Ca2+ pathway. During embryogenesis, Frizzled is expressed uniformly during the first stages, and then its expression becomes restricted to the animal pole domain before being also detected later in the SMC territory. Inhibition of Frizzled signaling by over-expression of a truncated form of the receptor blocks formation of the archenteron by affecting the differentiation of the endoderm. A rescue of this phenotype is obtained by the co-expression of an activated form of the RhoA kinase, which is a cytoplasmic component of the PCP pathway, indicating that Fz utilizes this pathway. Wnt5a transcripts are first detected in the SMC territory, while during gastrulation, they are at the boundary between the ectoderm and the endoderm territories. Our preliminary functional results show that the overexpression of Wnt5a inhibits the second step of the archenteron elongation, which depends on convergent–extension (CE) movements. This result is consistent with studies in other organisms, in which Wnt5a regulates CE movements through activation of both non-canonical Wnt pathways. These results indicate that Wnt5a may regulate convergent–extension movements during sea urchin gastrulation.

122. SYS-1, a Novel β-Catenin, is a Limiting Transcriptional Co-Activator of POP-1/TCF in *C. elegans*. Ambrose R. Kidd III,[1] Jennifer A. Miskowski,[1] Kellee R. Siegfried,[1] and Judith Kimble[2]. [1]University of Wisconsin-Madison; [2]University of Wisconsin-Madison and HHMI, Madison, WI 53706.

The *C. elegans sys-1* gene is critical for Wnt/MAPK signaling. The SYS-1 amino acid sequence is novel but contains three divergent armadillo repeats. Nonetheless, SYS-1 is a functional β-catenin. Three lines of evidence support this idea. First, SYS-1 rescues a *bar-1*/β-catenin null mutant. Second, SYS-1 binds POP-1/TCF via its β-catenin binding domain. Third, SYS-1 co-activates POP-1-dependent transcription of a TOPFLASH reporter in tissue culture cells. Discovery of this novel SYS-1/β-catenin suggests that other genomes may also encode β-catenins not identified by sequence criteria. We also provide evidence that SYS-1/β-catenin functions in vivo as a limiting transcriptional co-activator of POP-1/TCF. First, the sys-1 locus is haplo-insufficient. Second, SYS-1 overexpression generates ectopic POP-1 activity. Third, the relative abundance of SYS-1 and POP-1 is critical for transcriptional activity in tissue culture cells. Previous studies showed that Wnt/MAPK signaling leads to a counterintuitive decrease in the level of nuclear POP-1 in those daughter cells requiring POP-1 activity. We suggest that Wnt/MAPK signaling reduces the level of nuclear POP-1 to accommodate the limiting availability of its transcriptional co-activator, SYS-1/β-catenin. We further suggest that this mechanism may be of broad significance in animal development since Wnt/MAPK signaling also controls TCF in vertebrates.

123. SUMO Conjugase, Lesswright, Regulates Larval Hematopoiesis Through Rel-Related Transcription Factors in *Drosophila melanogaster*. Soichi Tanda and Liang Huang. Ohio Univ., Athens, OH 45701.

The lesswright (*lwr*) gene encodes *Drosophila* SUMO conjugase. SUMO conjugation is known to regulate many different cellular functions such as nuclear transport and transcription. Accordingly, lwr mutants exhibit pleiotropic phenotypes. Here, we report that *lwr* function is critical in controlling blood cell (hemocyte) production in *Drosophila* larvae. The loss of *lwr* function leads to overproduction of plasmatocytes (phagocytic hemocytes) and lamellocytes (hemocytes which play a critical role against larger foreign objects). This abnormality is due to the loss of *lwr* function both in hemocytes and in the lymph gland (the hemocyte-producing organ). Our genetic and immunohistochemical analyses indicate that this phenotype is manifested through activation of the Rel-related transcription factors Dorsal (Dl) and Dorsal-related immunity factor (Dif) in the hematopoietic tissues. We observed Dl proteins accumulated in the nuclei of circulating *lwr* mutant hemocytes. In addition, the *dl* and *Dif* mutations suppressed *lwr* hematopoietic phenotypes. Interestingly, these Rel-related genes play different roles in hemocyte production. The function of *dl* is limited to plasmatocyte production, while *Dif* regulates the differentiation of both plasmatocytes and lamellocytes. Furthermore, overproduction of Dif in the hematopoietic tissues can substitute *dl* function in larval hematopoiesis. Possible molecular mechanisms of Lwr in the activation of Dl and Dif will be discussed.

124. The Adaptor Protein X11Lα/Dmint1 Interacts with the PDZ-Binding Domain of the Cell Recognition Protein Rst in *Drosophila*. Smitha Vishnu,[1] Alexander Hertenstein,[1] Gert H. de Couet,[2] and Karl-Friedrich Fischbach[1]. [1]Institut für Biologie III, Schänzlestr.1, 79104 Freiburg, Germany; [2]Department of Zoology, University of Hawaii, 2538 McCarthy Mall, Honolulu, HI 96822, USA.

The *Drosophila* cell adhesion molecule Rst plays key roles during the development of the embryonic musculature, spacing of ommatidia in the compound eye and of sensory organs on the antenna, as well as in the neuronal wiring of the optic lobe. In rst^{CT}mutants lacking the cytoplasmic domain of the Rst protein, cell sorting and apoptosis in the eye are affected, suggesting a requirement of this domain for Rst function. Yeast two hybrid screens were performed using the cytoplasmic domains of Rst and its paralogue Kirre as baits. Among several putative interactors, two paralogous *Drosophila* PDZ motif proteins related to X11/Mint were identified. X11/Mint family members in *C. elegans* (Lin-10) and vertebrates are believed to function as adaptor proteins and to regulate the assembly of multi-subunit complexes at the synapse, thereby linking the vesicle cycle to cell adhesion. Using genetic, cell biological, and bioinformatic approaches, we show that the interaction of Rst with X11La is of biological significance. The role of the X11L α-Rst interaction is discussed in the context of the proposed properties of this protein family as scaffolding proteins for larger protein complexes.

125. EphA4 Signaling Suppresses Cdc42 and RhoA Activities by Recruiting Pak1 in Order to Regulate Blastomere Association in the *Xenopus* Blastula Embryo. Nicolas Bisson, Luc Poitras, Alexander Mikryukov, Michel Tremblay, and Tom Moss. Cancer Research Centre, Laval University, Québec City, G1R 2J6, Canada.

The control of cell association is an important mechanism by which the large family of Eph tyrosine kinase receptors regulate cell migration and cell sorting during embryonic development. Activation of the EphA4 receptor in early *Xenopus* embryos induces a reversible, cell autonomous loss-of-association phenotype resulting in the collapse of the blastocoel roof. Here, we show that this mutant phenotype can be blocked by the SH3–SH2 adapter Nckβ (Grb4) dependent on its SH2 domain. *Xenopus* p21-activated kinase xPAK1 interacts with Nck, is activated in embryo by EphA4 in a Nck-dependent manner, and its ectopic expression phenocopies EphA4-induced loss-of-association. Loss-of-cell association does not require the catalytic activity of xPAK1 but does require its GTPase binding domain and is enhanced by its Nck binding site and by constitutive membrane targeting. Ectopic expression of the GTPase binding domain of xPAK1 and its membrane targeting are sufficient to induce loss-of-association. The EphA4- and xPAK1-induced mutant phenotypes can be rescued by activated Cdc42 and RhoA. The data demonstrate the existence of a signaling pathway passing via Nckβ and xPAK1 by which EphA4 activation suppresses both Cdc42 and RhoA activities and regulates cell–cell association. This novel mode of Eph signaling provides an explanation for some of the apparently contradictory effects of the Eph receptors and their ephrin ligands.

126. EphA9 Inhibits Precocious EMT During Avian Gastrulation. K.M. Hardy, R.K. Baker, T.A. Yatskievych, Y.J. Shin, S.L. Greenhut, and P.B. Antin. Univ. of Arizona, Tucson, AZ.

Epithelial–mesenchymal transformations (EMT) are common in vertebrate embryogenesis and are required for normal development. Although a number of receptor tyrosine kinases, cell–cell, and cell–ECM adhesion molecules have been implicated in regulating EMT in vitro, tumorigenesis, and some developmental EMTs, their potential functions during gastrulation are poorly understood. Using avian embryos, we determined mRNA and/or protein expression patterns of Ephs, cadherins, and integrins in gastrulating tissues. Based upon localization patterns and reported functions in other cell types, we hypothesize that they function in an interrelated fashion during gastrulation to control the timing and progression of cells from epithelial epiblast to migrating mesoderm. We used ex ovo electroporation to assess the effects of overexpressing wild type, dominant negative, and constitutively active versions of these signaling molecules. EphA9 is expressed in epiblast immediately adjacent to the streak and in involuting cells but is downregulated in emerging mesoderm. Electroporation of EphA9 siRNAs into epiblast resulted in premature delamination of cells from wild type (EphA9 positive) neighbors prior to entering the primitive streak. In contrast, overexpression of constitutively active EphA9 inhibited cells from leaving the streak and migrating into the mesoderm. Our preliminary results suggest that EphA9 is important in maintaining cells in the epiblast and that downregulation of expression or signaling activity is required for normal mesoderm emergence. Ongoing studies are investigating the relationship between Ephs, cadherins, and integrins during gastrulation.

127. Genetic Modifiers of the Anti-Apoptotic Functions of Bcl-2. Cicely A. Jette, David M. Langenau, Rodney A. Stewart, John P. Kanki, and A. Thomas Look. Dana-Farber Cancer Institute, Boston, MA 02115.

Mechanisms that regulate apoptosis during development are critical for normal ontogeny and tissue homeostasis, especially in the hematopoietic system where defects in apoptotic regulators can lead to myeloproliferative or autoimmune disorders, as well as cancer. We are studying apoptosis in the zebrafish hematopoietic system, an attractive vertebrate model for studying apoptosis during development and disease processes. We cloned the zebrafish orthologue of the anti-apoptotic BCL-2 gene and created a transgenic line in which the zebrafish *rag2* promoter drives expression of an EGFP-zbcl-2 fusion protein in T- and B-lymphoid cells. Expression of EGFP-zbcl-2 in developing lymphocytes led to a 2.5-fold increase in thymocyte numbers and a 1.8-fold increase in GFP-labeled B cells in the kidney marrow. Fluorescent microscopic analysis of living *rag2-EGFP-zbcl-2* transgenic embryos showed that their thymocytes are resistant to γ-radiation- and dexamethasone-induced apoptosis. The ability to monitor GFP-positive thymocytes in real time, combined with the forward genetic

capacity of the zebrafish system, makes this model ideal for identifying interacting mutations that suppress bcl-2 function. By monitoring bcl-2-mediated resistance to γ-radiation-induced apoptosis in thymocytes, we have so far screened over 20,000 ENU-induced gene mutations and have identified two dominant bcl-2 suppressor mutants. We will describe the role of zebrafish bcl-2 in regulating developmental apoptosis, our genetic modifier screen to identify bcl-2 suppressor mutations, and our functional characterization of these mutants.

128. Nrarp Functions to Modulate Neural Crest Cell Differentiation by Regulating LEF1 Protein Stability. Motoyuki Itoh,[1] Tohru Ishitani,[1] Kunihiro Matsumoto,[1] and Ajay B. Chitnis[2]. [1]Univ. of Nagoya, Aichi 464-8602, Japan; [2]NIH, Bethesda, MD 20892.

Nrarp (Notch-regulated ankyrin repeat protein) is a small protein with two ankyrin repeats. Although Nrarp is known to be an inhibitory component of Notch signaling pathway in different developmental processes, roles of Nrarp in vivo have not been fully characterized. Here, we show that Nrarp is a positive regulator in the Wnt signaling pathway. In zebrafish, knockdown of Nrarp function by antisense morpholino results in disturbed Wnt signaling-dependent neural crest cell development. Nrarp stabilizes LEF1 protein, a pivotal transcription factor in Wnt signaling cascade, by blocking LEF1 ubiquitination. In accordance with this, lef knockdown also leads to deficient neural crest development. Furthermore, LEF1 activation does not affect Notch activity and vice versa. Our findings reveal that Nrarp independently regulates Notch and canonical Wnt signaling by affecting the protein turnover of NICD and LEF1.

129. Developmental Function of Xp120-Catenin in Complex with *Xenopus* Ewing Sarcoma Protein. Kyucheol Cho, Travis G. Vaught, Hong Ji, and Pierre D. McCrea. Univ. of Texas M.D. Anderson Cancer Center, GSBS Program in Genes and Development, Houston, TX 77030.

The catenin proteins play essential roles in most animal cells and tissues, having embryonic and adult functions. To find binding partners of *Xenopus* ARVCF catenin, we undertook yeast two-hybrid screening of a *X. laevis* cDNA library using xARVCF as bait. *xEWS* was isolated as a candidate binding partner. This protein has been conjectured to be an RNA-binding protein containing an N-terminal trans-activation domain, as well as a component of the transcriptional/elongation complex. We began by authenticating the binding of xEWS with xARVCF, using a co-IP approach from *Xenopus* embryos. The xEWS protein bound to xARVCF-catenin as well as the Xp120-catenin. Northern blot results showed that *xEWS* message exists from oocyte through tadpole stages, with expression increasing after the mid-blastula transition, while in situ expression showed that *xEWS* transcripts are present in neural plate, head, and eye regions. These *xEWS*-expressing fields overlap with those reported for *Xp120*. Therefore, our binding and spatial expression results imply that Xp120 and xEWS form a complex during *Xenopus* early embryogenesis possibly at greater levels in neural tissues. To further characterize the functional relationship between xEWS and Xp120-catenin, we have used *Xenopus* animal-cap explants over-expressing xEWS and identified several putative xEWS target genes. If an xEWS:Xp120–catenin functional relationship is established in embryogenesis, it may represent a new mechanism by which catenin proteins modulate nuclear processes.

130. When Pathways Collide: Inhibitory Crosstalk and the Spatial Regulation of FGF Signaling by BMP4. A.K. Sater, H.P. Gohil, and C. Liu. Univ. of Houston, Houston, TX 77204.

BMPs and FGFs have opposing effects on vertebrate ectodermal specification. Our previous studies showed that FGF and BMP4 signals interact in *Xenopus* ectoderm via reciprocal inhibitory crosstalk involving MAPK. To test the hypothesis that FGF inhibits BMP4/Smad1-inducible gene expression, we treated gastrula ectoderm with FGF and assessed expression of several Smad1-inducible genes via RT-PCR. Treatment with FGF reduced Smad1-inducible gene expression; this inhibition was blocked by overexpression of MAP Kinase Phosphatase1 (MKP). Treatment of animal caps with FGF reduced activity of a Smad1-inducible luciferase reporter, while embryos overexpressing MKP showed increased luciferase activity. Overexpression of MKP also increased the proportion of Smad1 in the nucleus. Since our earlier work showed that the BMP4/TAK1 pathway inhibits MAPK, we asked whether inhibitory crosstalk affects the range of signaling in ectoderm by assessing the extent of MAPK activation in response to an FGF bead. Using anti-diphospho-MAPK immunohistochemistry, we found that the area of MAPK activation is expanded in ectoderm in which BMP4/TAK1 signals are reduced either by overexpression of noggin or by morpholino oligonucleotide-mediated knockdown of TAK1. These findings suggest that BMP4/TAK1 signals limit the range of FGF signaling in vivo. Experiments evaluating the effects of FGF on the spatial regulation of BMP signals are in progress. Given that the FGF/MAPK pathway inhibits Smad1 activity, this inhibitory crosstalk may help define distinct regions of FGF and BMP signaling during developmental processes governed by the opposing actions of FGFs and BMPs.

131. Role of Rap2 GTPase in Nodal/Activin Signaling Pathway. Sun-Cheol Choi and Jin-Kwan Han. Pohang Univ. of Science and Technology, Pohang, 790-784, South Korea.

Nodal/activin signaling is essential for mesoderm induction and dorsoventral patterning in early *Xenopus* embryogenesis. Currently, we are investigating the possible function of Rap2, a member of Ras-like GTPase family, in this signaling pathway. In a gain-of-function assay, Rap2 shows a dorsalizing activity during the dorsoventral patterning of early embryos. However, it cannot induce mesoderm on its own. Morpholino-mediated knockdown of Rap2 demonstrates that it is required for the transcription of target genes induced by nodal or activin ligand. Its depletion also impedes the phosphorylation and nuclear localization of Smad2 caused by nodal signaling, whereas it has no effects on BMP-4 signaling. In contrast, Rap2 knockdown does not inhibit the signaling initiated by a constitutively active form of type I receptor ALK4. The molecular mechanism by which Rap2 regulates this pathway is further being studied.

132. LPA Signaling in *Xenopus laevis* Requires Xrho and Xrac, but not Xcdc42. Robert B. Lloyd, Qinghua Tao, Stephanie A. Lang, and Christopher C. Wylie. Children's Hospital Research Foundation, Cincinnati, OH 45229.

The cortical actin cytoskeleton is critical for maintaining the shape and rigidity of early *Xenopus* embryos, but the control of its assembly and maintenance are only poorly understood. The actin network exists primarily in two states: a dense cortical network in most cells and a more coarse array in cells that have rounded up to divide. We have shown previously that LPA, an intercellular signaling phospholipid, is both sufficient and necessary to maintain the density of the actin network in cells with a dense network. The small Rho GTPases have been suggested to mediate responses downstream of LPA receptors. When LPA is added to fibroblasts in culture, stress fibers are rapidly assembled, and this process is dependent on the small Rho-GTPase RhoA. Using established dominant negative forms of the GTPases, we show that in *Xenopus* embryos dominant negative Rho and Rac are able to block the overexpression effects of XLPA2, while dominant negative forms of cdc42 are unable to block LPA signaling. Additionally, we show the Rho effector, Rock, is required for this process using a newer Rock inhibitor, H1152.

133. Sprouty1 is a Critical Regulator of Kidney Development by Antagonizing GDNF/Ret-Mediated Signals During Ureteric Bud Morphogenesis. M. Albert Basson,[1,4,5] Simge Akbulut,[1] Judy Watson-Johnson,[1] Reena Shakya,[2] Ruth Simon,[1] Thomas J. Carroll,[3] Isabelle Gross,[1] Thomas Lufkin,[1] Gail R. Martin,[4] Andrew P. McMahon,[3] Patricia D. Wilson,[1] Frank D. Costantini,[2] Ivor J. Mason,[5] and Jonathan D. Licht[1]. [1]Mount Sinai School of Medicine, New York; [2]Columbia University, New York; [3]Harvard University, Boston; [4]University of California, San Francisco; [5]King's College London, UK.

Development of the metanephric kidney is initiated by outgrowth of a ureteric bud from the caudal Wolffian duct. Ureteric bud formation is mediated by GDNF that is expressed in the nephrogenic mesenchyme. We analyzed kidney development in mice lacking the receptor tyrosine kinase (RTK) antagonist, Sprouty1 (*Spry1*). Supernumerary ureteric buds are formed in *Spry1*$^{-/-}$ embryos resulting in multiple ureters and multiplex, dysplastic kidneys. *Spry1* functions as a feedback antagonist of GDNF/Ret signaling in the Wolffian duct, thus ensuring that kidney induction is restricted to a single site. Using time-lapse analyses, we found that subsequent branching morphogenesis of the ureteric bud is also abnormal in the absence of *Spry1*. Reducing *Gdnf* gene dosage rescues the *Spry1*$^{-/-}$ phenotype. Conversely, reducing the *Spry1* gene dosage in *Gdnf*$^{+/-}$ embryos results in a significant rescue of renal hypoplasia observed in *Gdnf*$^{+/-}$ kidneys. These results demonstrate the importance of negative feedback regulation of RTK signaling during kidney development and suggest that failures in feedback control may underlie some congenital kidney malformations.

134. The Nuclear Envelope Protein Man1 is Required for Angiogenesis in the Embryonic Yolk Sac. Tatiana V. Cohen and Colin L. Stewart. National Cancer Institute at Frederick, Frederick, MD 21702.

Man1, an integral protein of the nuclear envelope that is recognized by autoantibodies from a patient with a collagen vascular disease, has recently been shown to interact with Smad transcription factors which in turn are regulated by TGFβ signaling. To understand the role of Man1 during mammalian development, we disrupted the murine Man1 gene by gene-trapping in embryonic stem cells. Southern analysis of genomic DNA from the mice showed that no homozygote mutant animals were born. Northern analysis identified a hybrid transcript in heterozygotes, consisting of the amino terminus of Man1 conjugated to the gene-trap marker. The resulting truncation of the Man1 protein eliminates the domain shown to interact with Smads. Analysis of mutant mouse embryos indicates lethality before 10 d.p.c. Examination of mutant embryos revealed that abnormal yolk sac development is the cause of lethality. Although blood islands formed in mutant yolk sacs, indicating that vascularization was initiated, there was an absence of blood vessels, demonstrated by benzidine staining and PECAM1 immunohistochemistry. In addition, there was poor attachment of the yolk sac endothelial and mesodermal cell layers. TGFβ signaling is required for yolk sac angiogenesis, and functional knockouts of TGFβ signaling molecules have resulted in similar phenotypes, suggesting that the loss of angiogenesis in the Man1 mutants may be mediated by TGFβ signaling. Thus, our studies describe a previously uncharacterized role for an integral nuclear envelope protein in mammalian development.

135. Characterization of Chibby Knockout Mice. Vera A. Voronina,[1] Ken-Ichi Takemaru,[3] Piper M. Treuting,[1] and Randall T. Moon[2]. [1]University of Washington, Seattle, WA 98195; [2]HHMI; [3]SUNY at Stony Brook, Stony Brook, NY 11794.

Chibby (Cby) is a recently discovered antagonist of Wnt/β-catenin-mediated transcription. Wnt-b-catenin signaling is involved in multiple developmental processes, and mutations that activate the pathway are linked to a variety of cancers. To study roles of Cby in the development and disease, we created a mouse knockout strain through substitution of the entire coding region of the Cby gene with neomycin-resistant cassette. On a mixed background, Cby knockout mice demonstrate a partially penetrant phenotype that includes early lethality and runtness. Backcrossing Cby mutant mice to C57 BL/6 strain for at least five generations increased the penetrance of the phenotype. Less than 20% of Cby null mice survive to adulthood; the rest die by postnatal day 30 after 2–3 days of losing weight. All Cby−/− mice are small, anemic, and have virtually no subcutaneous fat. No other abnormalities were revealed in mutants upon gross anatomical examination, though ongoing analysis suggests hematopoietic changes. To investigate whether β-catenin signaling was altered, we used a luciferase reporter assay in primary mouse embryonic fibroblasts and found an upregulation of luciferase activity in Cby−/− and Cby+/− cells relative to wild type cells. Similar upregulation of an independent β-catenin-responsive reporter was observed in mouse embryos carrying various combinations of Cby alleles and the Bat–Gal reporter.

136. A Transgenic Approach to Understanding Imprinting Regulation in the PWS Locus. Christopher R. Futtner, Karen A. Johnstone, Camilynn I. Brannan, and James L. Resnick. Univ. of Florida, Gainesville, FL.

Prader Willi (PWS) and Angelman (AS) syndromes are both neuro-developmental disorders arising from the improper expression of oppositely imprinted genes located on human chromosome 15 q11–13. Imprint regulation of this region is under the control of a bi-partite imprinting center consisting of an Angelman imprinting center (AS-IC) located approximately 35 kb upstream of the paternally expressed Snrpn exon 1 and a Prader Willi imprinting center (PWS-IC) located just 5′ to and including Snrpn exon 1. The PWS-IC has been shown to be a positive element promoting expression of a set of genes on the paternal allele, while the AS-IC provides suppression of the PWS-IC on the maternal allele, thereby suppressing expression. Both are required for proper establishment and/or maintenance of the imprint in the germ line. In the mouse, both gene order and imprinted expression have been conserved with the syntenic region being located on murine chromosome 7C. While the location of the PWS-IC has been conserved in the mouse, the position of the murine AS-IC remains unknown. We have taken a transgenic approach to locating the AS-IC and further dissecting out components of the PWS- IC. Using a recombineering method, we have created a series of deletions within a BAC containing Snrpn that we previously have shown recapitulates the imprinted expression of the endogenous locus in single copy. Analysis of mice carrying these transgenes will help to elucidate the minimal sequences necessary to confer correct imprinting in the PWS locus.

137. The 5′ Flanking Region of *Gtl2* Controls Imprinting and Expression of the *Gtl2* and *Dlk1* Genes in the Mouse. Ekaterina Steshina, Michael Carr, and Jennifer V. Schmidt. Univ. of Illinois at Chicago, IL.

Genomic imprinting is an epigenetic process that results in the preferential expression of one of the two parental copies of a gene. Although many imprinted genes play important roles in embryonic growth and development, the precise mechanisms by which imprinting occurs are still unknown. We have studied an imprinted locus on mouse chromosome 12, containing the paternally expressed *Dlk1* gene and the maternally expressed *Gtl2* gene. A previously existing mutation, *Gtl2*^{lacZ}, was generated by the insertion of a *lac*Z cassette into the upstream region of *Gtl2*. Paternal inheritance of this insertion resulted in growth retardation, suggesting that the *Gtl2* upstream region may be regulating the expression of imprinted genes involved in growth. To localize potential *Gtl2* upstream regulatory elements, we made a targeted deletion of 2.8 kb of the *Gtl2 5′*

flanking region (*Gtl2Δ5′*), replacing it with a neo cassette. We observed that paternal inheritance of the deletion allele led to a dwarf phenotype, reminiscent of Gtl^{2lacZ}. Molecular analysis revealed that paternal transmission of the *Gtl2Δ5′* allele affects imprinting and expression of both the *Dlk1* and *Gtl2* genes. Unexpectedly, when the *neo* sequence was excised from the *Gtl2Δ5′* allele, the paternal dwarf phenotype was lost. This suggests that the deleted region of the *Gtl2* upstream sequence is not directly involved in regulation of the *Dlk1/Gtl2* locus. Rather, we believe that epigenetic changes to the locus caused by the *neo* and *lac*Z insertions affect other nearby regulatory elements for *Dlk1/Gtl2* imprinting and expression.

138. 14Gso: A Translocation Mutant Mouse with a Disruption of the Beckwith–Wiedemann Syndrome Imprinted Region. Colleen M. Elso, Angela M. Tarver, Xiaochen Lu, and Lisa J. Stubbs. Lawrence Livermore National Laboratory, Livermore, CA 94550.

Beckwith–Weidemann syndrome (BWS) is a condition affecting an estimated 1/15,000 human births. The syndrome involves overgrowth of the fetus, organomegaly, abdominal wall defects and a characteristic range of tumors, most notably the Wilms' tumor of the kidney. It is caused by a range of mutations concentrated in the imprinted region of Hsa11p15 and the syntenic Mmu7F4. Mutations affecting *CDKN1C* have been associated with the disease, however, the majority of cases are associated with loss of imprinting at either the *IGF2/H19* locus or the nearby *KCNQ1OT1* locus or paternal uniparental disomy of the region. The T(7;10)(F4;D)14Gso mouse (14Gso) carries a balanced translocation disrupting *Kcnq1*, physically separating several normally imprinted genes from the influence of the *Kcnq1ot1* associated imprinting control element. We have observed oversized, uncoordinated pups in some crosses, and there are growth differences between offspring inheriting the translocation on the maternal chromosome versus the paternal chromosome. We can study the role of genes in this region in the development of BWS through breeding strategies aimed at elucidating the effects of the loss of imprinting of these genes and the production of uniparental disomies. This work was completed under the auspices of the US DOE, OBER, by the University of California, LLNL under contract number W-7405-Eng-48.

139. Implantation Serine Protease 1 and 2. Lin Tang, Shiying Liu, Colleen O'Sullivan, and Derrick Rancourt. Univ. of Calgary, Calgary, AB T2N4N1, Canada.

Implantation, a critical stage in development and reproduction requires the precise synchronization and reciprocal interactions between the embryo and the uterus. We have identified two novel murine trypsin-like serine proteases, designated as Implantation Serine Protease1 & 2 (ISP1 & 2), which are expressed in the embryo during early embryogenesis, embryo hatching and in the uterus during embryo implantation. ISP1 and 2 are closely linked neighbors within a tryptase cluster on mouse chromosome 17 A3.3. Both ISP1 & 2 fall into the chymotrypsin serine protease family S and likely possess trypsin-like properties. The presence of ISPs in early embryos suggests potential roles of ISPs in early embryogenesis. Abrogation of ISPs functions indicates the involvement of ISPs in embryo hatching and outgrowth in vitro. The presence of ISP1 & 2 in the uterine glandular epithelium from day 4.5 to day 8.5 during implantation and the detection of ISPs protein at the boundary of the invading embryo and the uterus at the implantation site lead to the hypothesis that ISP1 & 2 may be secreted into the uterine fluid to influence the progress of implantation. The uterine expression pattern of ISPs is under the control of progesterone and estrogen on transcriptional and post-transcriptional levels. The observations that ISPs share remarkable similarities in regulatory sequences are closely linked and are coexpressed in the embryo, and the uterus indicates that they may be co-regulated. The loss-of-function analysis is currently on the way to help to unfold roles ISP play in development and reproduction.

140. A Prep-1 Null Mutation is Embryonic Lethal at E7.5. Luis C. Fernandez-Diaz,[1] Maria T. Fiorenza,[2] Nancy A. Jenkins,[3] Neal G. Copeland,[3] and Francesco Blasi[1]. [1]Università Vita Salute San Raffaele, Milan, Italy; [2]IFOM (FIRC Institute of Molecular Oncology), Milan, Italy; [3]National Cancer Institute, Frederick, MD, USA.

The Prep-1 Meinox protein is able to interact with all Pbx proteins, to prevent their export from the nucleus, and to cooperate in DNA binding site selection. We have generated a Prep-1 null mouse by deleting its homeodomain by homologous recombination. The phenotype of the Prep1−/− mutation is embryonic lethality at E7.5. Because of this early death, we have analyzed the expression level of Prep-1 and Pbx1 mRNA during development from the one-cell to the blastocyst stage. We find that both mRNA are maternal messages, are transcribed starting at the 4-cell stage, and are strongly amplified at the blastocyst stage. Importantly, we have analyzed in detail the mutation to exclude a dominant negative expressing the amino terminal region of the protein. First, by immunoblotting analysis, we have not found any other form than wild type Prep-1 protein in heterozygous mice. Moreover, we have cloned and sequenced the mRNA at the site of the insertion of the vector and have found a series of stop codons preceding the LacZ part of the vector. Finally, we have analyzed the size of the LacZ protein and found that it was indistinguishable from the same protein expressed in a cell line. We therefore conclude that the mutation is indeed a null mutation and that Prep-1 is required for early (gastrulation) developmental processes. We hypothesize that the complete absence of Prep-1 protein causes the total absence of all Pbx proteins.

141. Regulatory Mechanisms of H19 DMR at the Heterologous Afp Locus. Qi Rong and Karl Pfeifer. Section on Genomic Imprinting, Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

H19 and Igf2 are imprinted genes on the distal end of mouse chromosome 7. H19 is only expressed from the maternal allele, while Igf2 is expressed from the paternal allele. The 2.4 kb differentially methylated region (DMR) upstream of the H19 gene is necessary for the regulation of H19 and Igf2 imprinted expression. Continued presence of the DMR is required to repress expression of the maternal Igf2 allele and function as an insulator. In contrast, the DMR only needs to be present in the early embryo to establish silencing of the paternal H19 (1). Once silencing is established, the continued presence of the DMR is no longer required. This 2.4 kb DMR was inserted into the alpha-fetoprotein (Afp) locus on chromosome 5 where it acts as a mark of parental origin (2) and also represses Afp expression. A DMR flanked with loxP sites was inserted into the Afp region, and a new mouse model was generated. By using a cre recombinase-based strategy, this DMR can be removed from the Afp locus during early embryogenesis or in differentiated somatic cells. We will thereby determine the temporal requirement of the DMR in initiating and maintaining the repression of Afp. (1) Genes and Development. 14:1186–1195. (2) Molecular and Cellular Biology. 24(9):3588–3595.

142. The Homeodomain Protein Hhex is a Direct Repressor of *Endothelial Cell-Specific Molecule 1 (ESM-1)*. Rong Cong, Xiaobing Jiang, Christine M. Wilson, and Clifford W. Bogue. Yale University School of Medicine, New Haven, Connecticut, USA.

Hhex encodes a homeobox-containing protein that functions as both a transcriptional repressor and activator and is necessary for normal embryonic development. ESM-1 is a cysteine-rich protein expressed in the vascular endothelium and may play a key role in vascular biology. We previously reported that a null mutation of *Hhex* leads to abnormalities in vasculogenesis and cardiac morphogenesis and have focused on identifying the transcriptional targets of Hhex necessary for cardiovascular development. Oligonu-

cleotides microarray analysis shows that *ESM-1* is increased 11.3-fold in $Hhex^{-/-}$ embryos ($P < 0.05$). This increase is confirmed by both qRT-PCR and Northern analysis. Furthermore, we have identified ten potential Hhex binding sites in the genomic sequence of *ESM-1*. Electromobility gel shift assays demonstrate that one of these binding sites binds Hhex protein from endothelial cells. Using luciferase reporter assays, we show that co-transfection of endothelial cells with the *ESM-1* promoter/enhancer that includes this Hhex binding site, along with a Hhex-expressing vector, results in a decrease in *ESM-1* transcriptional activity. Mutation of this Hhex binding site abolishes the repressor activity of Hhex. Moreover, we determined, using chromatin immunoprecipitation, that Hhex directly interacts with *ESM-1* in vivo. Finally, *ESM-1* expression is down-regulated when Hhex is over-expressed in endothelial cells. All these data indicate that Hhex directly represses *ESM-1* in vivo. We speculate that Hhex-mediated repression of *ESM-1* is critical for normal function of the vascular endothelium.

143. CREB Activation in Synergy with BMP2 Signaling Directly Regulates Phox2a Gene Transcription. Chutamas Benjanirut, Maryline Paris, Ronald Hullinger, and Ourania Andrisani. Purdue University, West Lafayette, IN 47907.

Combined BMP2 and cAMP signaling induces the sympathoadrenal lineage in neural crest (NC) cultures by increasing expression of proneural transcription factor Phox2a in a CREB-mediated mechanism. To determine the role of CREB in Phox2a transcription mediated by BMP2 + cAMP-elevating agent IBMX, transient transfections of hPhox2a-reporter constructs were performed in avian NC cells and the murine catecholaminergic CAD cell line. Although BMP2 + IBMX increased endogenous Phox2a expression, the 7.5 kb hPhox2a-reporters were unresponsive. Treatment of NC or CAD cells with histone deacetylase inhibitor trichostatin A and BMP2 + IBMX further increased endogenous Phox2a transcription and prolonged CREB phosphorylation, suggesting Phox2a chromatin remodeling is linked to CREB activation. Chromatin immunoprecipitations employing CREB, CBP, and acetylated-histone4 antibodies identified two functional CRE half-sites, 5.5 kb 5′-upstream in murine Phox2a promoter, which are evolutionarily conserved in the human Phox2a promoter. Accordingly, we conclude that CREB is a direct transactivator of Phox2a gene transcription. In addition, proximal to these CRE half-sites are E-box and CCAAT binding sites. Since E-box sites bind bHLH proteins like ASH1, known to be induced in NC cells by BMP2, we propose that this composite *cis*-acting element comprised of CRE, E-box, and CCAAT binding sites mediates the synergistic effect of BMP2 + IBMX on Phox2a gene transcription.

144. Phenotypic and Epigenetic Analysis of SUZ12 Deficient Mice. Stormy J. Chamberlain and Terry Magnuson. Univ. of North Carolina-Chapel Hill, Chapel Hill, NC 27599.

EED, EZH2, and SUZ12 act together in dynamic Polycomb group (PcG) complexes to maintain transcriptionally repressive chromatin states through replication and mitosis. In addition to their role in the maintenance of expression patterns of homeotic genes, these complexes also act upon other genes to maintain cellular differentiation in such processes as development, hematopoiesis, and tumorigenesis. Mice homozygous for a null mutation in the *Eed* gene die between 7.5 and 9.5 dpc, displaying defects in gastrulation. *Eed* mutants also show marked defects in imprinted X-inactivation and autosomal imprinting. We sought to determine whether similar defects occur in mice homozygous for a null mutation in the *Suz12* gene. We reason that, if SUZ12 and EED only act in complexes together during early embryonic development, then their phenotypes should be similar. Here, we generate mice that are deficient for SUZ12 using a gene trap ES cell line and compare the phenotypes of *Eed*, *Suz12*, and *Eed/Suz12* double mutants. We also analyze the X inactivation and autosomal imprinting phenotypes in *Suz12* mutant embryos.

145. Chromatin Remodeling on Promoters of Late Muscle Marker Genes During Myogenesis. Yasuyuki Ohkawa, Concetta G. Marfella, and Anthony N. Imbalzano. Univ. of Massachusetts Med School, MA 01655.

The SWI/SNF family of ATP-dependent chromatin remodeling enzymes is essential for skeletal muscle differentiation. At early stages of myogenesis, MyoD recruits SWI/SNF to the myogenin promoter to induce gene expression. However, in later stages of myogenesis, it remains unclear how chromatin remodeling on late marker genes is regulated. In order to analyze chromatin remodeling on skeletal muscle genes, we established a convenient restriction enzyme accessibility assay. Here, we show that the expression of late marker genes, such as muscle creatine kinase (MCK) and desmin, requires SWI/SNF-mediated chromatin remodeling during myogenesis. Chromatin immunoprecipitation assays (ChIP) revealed that Myogenin and MEF2 are recruited to these promoters at the same time that Brg1 is recruited. To evaluate the function of these factors in the recruitment of SWI/SNF, we introduced Myogenin and MEF2D into fibroblasts that inducibly express dominant negative Brg1. Surprisingly, introduction of both Myogenin and MEF2D induced not only Brg1 recruitment onto late marker genes but also transcription of late marker genes, additionally, coexpression of Myogenin and MEF2D resulted in SWI/SNF-dependent morphological myotube formation which does not occur upon introduction of MyoD. Collectively, these data indicate the importance of SWI/SNF chromatin remodeling enzymes in both early and late gene activation events and suggest that early and late muscle specific genes are regulated by different mechanisms.

146. Identifying Gene Targets of Myogenin in Embryonic and Post-Natal Skeletal Muscle. Eric Meadows, Judy Davie, Jennifer R. Knapp, and William H. Klein. UT MD Anderson Cancer Center.

Skeletal muscle development is a complex biological process that is, in part, regulated by the expression of Myogenic Regulatory Factors. The various stages of muscle development rely on the activity of these transcription factors for commitment, proliferation, differentiation, and fusion. Myogenin is one of these muscle specific transcription factors involved in differentiation and fusion of embryonic myoblasts and adult satellite (stem) cells into myotubes. Knockout of myogenin during embryonic development results in lethality upon birth due to the loss of properly differentiated and fused muscle fibers. Myogenin's role in adult tissue is less well defined. When myogenin expression is conditionally knocked out after embryonic muscle development, it has been shown to be nonessential for adult skeletal muscle development. In order to better understand the process of muscle development and regeneration as a whole, establishing myogenin's role during embryonic and adult skeletal muscle development is required. One aspect of understanding myogenin function would be to identify the genes regulated by myogenin. To accomplish this, microarrays will be performed using satellite cells and isolated muscle tissue. The resulting expression levels of various genes expressed when myogenin is present will then be compared to samples where myogenin has been deleted. Learning about myogenin's function at the cellular and molecular level, distinguishing it from the other muscle transcription factors, and defining its precise role within the muscle development pathway are essential to understanding skeletal muscle differentiation and fusion.

147. Long-Range Regulation of Hoxa13 in Limb Development. Jessica A. Lehoczky, Melissa E. Williams, and Jeffrey W. Innis. University of Michigan, Ann Arbor, MI 48109.

The posterior HoxA and HoxD genes are essential in appendicular development. Prior studies have demonstrated that a 'distal limb enhancer', remotely located upstream of the HoxD complex, is required

to drive embryonic autopod expression of the posterior Hox genes as well as the two additional non-Hox genes in the region: Evx2 and Lnp. Our work demonstrates a similar mode of regulation for Hoxa13 and four upstream genes: Evx1, Hibadh, Tax1bp, and Jaz1. These genes all show embryonic (E11.5–E13.5) distal limb and genital bud expression, suggesting the existence of an enhancer influencing the expression of a domain of genes. Sequence analysis between homologous human and mouse genomic sequence upstream of Hoxa13 revealed a remote 2.25 kb conserved non-coding sequence (mmA13CNS) within the fourth intron of the Hibadh gene. mmA13CNS shares a common 131 bp core identity within a conserved non-coding sequence upstream of Hoxd13, which is located within the previously identified 'distal limb enhancer' critical region. To test the function of this conserved sequence, we created mmA13CNS-Hsp86-*lac*Z transgenic mice. mmA13CNS directed a wide range of tissue expression including the central nervous system, developing olfactory epithelium, limb, and genital bud. Limb and genital bud expression directed by mmA13CNS is not identical to the patterns exhibited by the upstream Hoxa13 genes, suggesting that mmA13CNS is not sufficient to fully recapitulate their expression in those tissues. The Evx1- and 2-like central nervous system expression observed in these mice suggests that the long-range regulatory element(s) for the Hox cluster existed prior to the cluster duplication.

148. Pulmonary Expression and Transcriptional Regulation of α7 Nicotinic Acetylcholine Receptors by TTF-1 and Egr-1 During Development. Paul R. Reynolds and John R. Hoidal. Univ. of Utah, SLC, UT 84132.

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels formed by five homologous subunits that are involved in processes including signal transduction, proliferation, and apoptosis. The developmental role of these receptors, however, is unclear. In the present investigation, α7 nAChR expression was assessed by immunohistochemistry in mouse lungs from embryonic day (E)13.5 to postnatal day (PN)20. Transcriptional regulation of α7 was assessed by transfection of murine bronchiolar epithelial cells with a luciferase reporter containing 1.1 kb of the mouse α7 promoter, TTF-1, and Egr-1. α7 was initially detected at E13.5 in mesenchymal cells and in the epithelium of the primitive tubules at E15.5. From E18.5 to PN1, α7 was expressed in conducting airway and saccule epithelium. From PN10 to 20, α7 was observed in the peripheral epithelium and on the luminal membranes of bronchiolar epithelial cells. Starting at E15.5, type II epithelial cells expressed both proSP-C and α7. From E18.5, Clara cells in the bronchiolar epithelium co-expressed Clara cell secretory protein and α7. TTF-1 activated α7 transcription in vitro by binding specific TTF-1 response elements. Conversely, Egr-1 inhibited α7 expression. Temporal–spatial α7 expression supports the concept that these receptors function during normal lung morphogenesis. These data also support a model whereby α7 is induced by the essential pulmonary transcription factor, TTF-1, and suppressed by an early repressor, Egr-1, during pulmonary development. Supported by HL72903.

149. Characterization of OTK18 Regulation on Neuronal Survival. Stephanie L. Bogers and Kimberly A. Carlson. Univ. of Nebraska at Kearney, Kearney, NE 68849-1140.

Neurotoxins are agents that induce cell death or apoptosis; neurotrophins are agents that help protect the neuron from cell death or injury and aid in growth. Human OTK18 is a novel protein that has anti-retroviral activities that serve to down-regulates HIV-1 (human immunodeficiency virus type one) replication in macrophages and is found up-regulated in severe HIV-1 encephalitic brain tissue. The proposed experiment examines the effects OTK18 has on neuronal survival after neurotoxic insult. The hypothesis is that rat cortical neurons transfected with OTK18 will be protected from the toxic properties of a neurotoxin such as glutamate. If this hypothesis is true, the number of apoptotic neurons and neurotoxins levels will be decreased when neurons are transfected with OTK18, as compared to controls. Alternatively, OTK18-transfected cells could lead to increased neurotoxicity, suggesting a regulatory mechanism that suppresses neurotrophins rather than neurotoxins. The procedures used in this study included transfecting fetal brain mixed cell cultures from Long–Evans rat fetuses (gestation ~17 days) and measuring the level of neuronal death using an MTT assay. At 48 and 72 h post-transfection, the cells were cultured with 50–500 μM glutamate to induce toxicity or left untreated as controls. The protein and mRNA levels of the neurotrophin-three (NT3; a neurotophrin) and tumor necrosis factor alpha (TNF-α; a neurotoxin) are being analyzed by ELISA and RT-PCR. The results from this study will provide insight into a possible mechanism of OTK18 regulation of differential neuronal survival in relationship to severe HIV-1 infection and disease.

150. Functions of Brn3b Transcription Factor in Mouse Retinal Ganglion Cell Development. Ling Pan, Zhiyong Yang, and Lin Gan. Center for Aging and Developmental Biology, University of Rochester, Rochester, NY 14642.

POU-domain Brn3 transcription factors play essential roles in sensory neuron differentiation. Previous studies have shown that targeted deletion of each of the three POU-domain Brn3 factors in mice leads to the developmental failure and apoptosis of a unique set of sensory neurons in retina, dorsal root ganglia, trigeminal ganglia, and inner ear. The specific defects associated with the removal of each Brn3 gene closely reflect their characteristic spatiotemporal expression patterns. Nevertheless, it remains elusive whether Brn3 factors are functionally equivalent and act through a common molecular mechanism to regulate the development and survival of these sensory neurons. By knocking-in Brn3a (Brn3aki) into the Brn3b locus, we showed here that Brn3aki could functionally restore the normal differentiation, survival, and axonal guidance of retinal ganglion cells (RGCs) in the absence of Brn3b. Moreover, Brn3aki fully reinstated the early developmental expression profiles of Brn3b downstream target genes in retina. These results indicate that Brn3 factors are functionally equivalent to each other and that their tissue-specific roles in neurogenesis are determined by their discrete spatiotemporal expression patterns.

151. TGFβ and Wnt Regulation of Cell Proliferation and Extracellular Matrix Synthesis in Murine Embryonic Maxillary Mesenchymal Cells. Dennis R. Warner[1] and Henry S. Smith[2]. [1]Birth Defects Center, Department of Molecular, Cellular, & Craniofacial Biology, University of Louisville; [2]Department of Pediatrics, University of Louisville, Louisville, KY 40292.

The signaling pathways activated by TGFβ and Wnt govern essential processes during embryonic development. Previous studies from this laboratory, including yeast two-hybrid and reporter assays, identified a physical and functional interaction between these two pathways in murine embryonic orofacial tissue and in murine embryonic maxillary mesenchyme (MEMM) cells. Smad 3 binds Dishevelled-1 (Dvl-1) in co-immunoprecipitation assays, and receptor-mediated activation of Smad 3 increased the level of binding to Dvl-1. TGFβ and Wnt-3a each reduced the rate of MEMM cell proliferation—an effect that was enhanced when the two factors were combined. Gene expression array analysis of a panel of growth regulatory genes indicated that this synergism may be controlled at the level of cell cycle gene expression. Moreover, array analysis of a panel of extracellular matrix (ECM) and associated protein genes revealed regulation of key ECM genes by Wnt-3a and also demonstrated co-regulation of ECM genes by both TGFβ and Wnt-3a in MEMM cells. These results demonstrate that signaling through the Wnt canonical pathway leads to similar patterns of cell cycle and ECM gene expression as TGFβ and that TGFβ and Wnt may cooperate to regulate these two processes in orofacial development. This work was supported

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152. Pax6 DRR is Required to Coordinate the Interaction of Widely Spaced Regulatory Elements. Jiha Kim and James D. Lauderdale. Univ. of Georgia, Athens, GA 30602.

Pax6, a member of the paired-family of transcription factors, is required for development of the eye, central nervous system, and endocrine pancreas. Whereas heterozygous mutations in Pax6 cause defects in eye development, homozygous mutations result in loss of eyes, nose, forebrain structures, and endocrine pancreas. Thus, Pax6 function is critically dependent on induction of proper expression levels during embryonic development. Despite intensive study, the regulatory mechanisms governing Pax6 transcription are only partially understood. Recent work by our laboratory and others indicates that a 3′ downstream regulatory region (DRR) is required to coordinate the interaction of widely spaced regulatory elements. To test this, we have taken a transgenic approach using bacterial artificial chromosomes, which will allow us to study regulatory regions in a native context in vivo without disrupting the expression of the endogenous gene. We show that a BAC encompassing the Pax6 transcription unit and a cluster of evolutionarily conserved, non-coding sequences located ~100 Kb 3′ to the coding region is expressed in a native-like Pax6 expression pattern in all regions of the developing mouse embryo, except the diencephalon. With respect to the eye, this result defines an eye regulatory region within the Pax6 DRR and supports its primacy in controlling Pax6 transcription during oculogenesis. We also identified an isoform of the Pax6 protein lacking the paired domain which is expressed in the developing optic vesicle and appears to be downregulated as the optic vesicle differentiates. Lastly, we identified a population of Pax6+ migratory cells in the developing olfactory bulb.

153. Analysis of Pax6 Regulation Using a Comparative Approach. Anirban Majumder and James D. Lauderdale. Univ. of Georgia, Athens, GA 30605.

Pax6, a highly conserved member of the paired-family of transcription factors, is crucial for development of the eye, central nervous system, and the endocrine pancreas. Pax6 function critically depends on induction of proper expression levels during development. To understand the mechanisms governing Pax6 induction, it is necessary to define the regulatory elements that direct expression of Pax6 and determine how these control elements interact with each other in their native chromosomal context. We are analyzing Pax6 regulation using a comparative approach involving mice and zebrafish. Zebrafish have two Pax6 genes, Pax6a and Pax6b, which arose as a result of a genome duplication. These two genes are expressed in both overlapping and discrete domains in the developing embryo. Because the expression patterns of the two zebrafish genes, taken together, are similar to that of the single Pax6 gene in mouse, it is likely that at least some Pax6 regulatory elements are partitioned between Pax6a and Pax6b. Although this partitioning should be evident at the genomic level, a more detailed expression analysis is required to interpret the genomic data. We are analyzing the differences and commonalities in the expression patterns of the Pax6 genes in zebrafish at four developmental time points and referencing back to mouse. This approach is expected to provide an index of potential tissue specific regulatory elements that can be tested in a native-like genomic environment using our Pax6-BAC transgenic approach.

154. T-box Transcription Factor Specificity in the Zebrafish Mesoderm. Aaron T. Garnett, Tina M. Han, Michael B. Eisen, and Sharon L. Amacher. University of California, Berkeley.

T-box proteins are a large family of transcription factors important in many aspects of embryonic patterning. Different T-box proteins have widely varying effects on gene expression, but the source of this diversity is not clear since they have very similar DNA binding preferences. In zebrafish, the T-box genes *no tail (ntl)*/*Brachyury*, *spadetail (spt)*/*VegT*, and *tbx6* are expressed in partially overlapping patterns in the presumptive mesoderm and mutants lacking *spt*, *ntl*, or both gene functions have abnormalities in the development of mesodermal structures. We are studying how these three factors are able to recognize the proper *cis*-regulatory elements and activate transcription of the appropriate target genes. To do this, we are performing in vitro DNA binding selection assays (SELEX assays) with bacterially produced proteins to determine if there are differences in DNA binding preference among the three factors. Our binding data suggest that the binding site for Tbx6 differs somewhat from previously published T-box factor binding sites. We are then using data from SELEX experiments to identify potential regulatory regions in non-coding DNA near genes that we have identified as putative direct T-box targets through microarray analysis. Our progress towards these goals, as well as functional tests of putative regulatory elements, will be presented.

155. DNA Methylation Reprogramming During Embryonic Development in Zebrafish. Amy B. MacKay,[1] Aziz A. Mhanni,[2] and Patrick H. Krone[1]. [1]Univ. of Saskatchewan, Saskatoon, SK, Canada; [2]Univ. of Manitoba, Winnipeg, MB, Canada.

DNA methylation of CpG-rich sequences leads to the repression of gene expression and the formation of compact chromatin conformations. The appropriate establishment of DNA methylation patterns is essential for proper embryonic development. In mammalian models, DNA methylation is removed and re-established during gametogenesis and early embryonic development. Although DNA methylation reprogramming is well known in mammalian model systems, little is known about DNA methylation reprogramming in non-mammalian vertebrates. Several studies have examined DNA methylation reprogramming during early embryonic development in zebrafish, *Danio rerio*, using methylation-sensitive restriction enzyme digests and Southern blot analysis, but have arrived at contradictory conclusions (Mhanni and McGowan, 2004, *Dev Genes Evol* 214: 412; Martin et al., 1999, *Dev Biol* 206:189; Macleod et al., 1999 *Nat Genet* 23:139). Using immunohistochemistry and Southwestern dot blot analysis, we present the first direct evidence of DNA methylation reprogramming in a non-mammalian vertebrate, the zebrafish. After fertilization, the zebrafish genome is fully demethylated by 1.5 h post fertilization (hpf) and remains hypomethylated until the first evidence of DNA methylation occurs at 3.0 hpf. The amount of methylation increases from 4.0 hpf until the genome is heavily methylated at 6.0 hpf. Our study reveals a pattern of DNA methylation reprogramming that is conserved between non-mammalian and mammalian vertebrates and suggests its importance to embryonic development.

156. Regulating GAP-43 Expression in the Developing and Regenerating Nervous System: A Comparative Genomics Approach. Ava J. Udvadia. Univ. Wisconsin-Milwaukee.

Development and regeneration of the vertebrate nervous system are dependent on the capacity of neurons to extend axons and to establish connections with the appropriate postsynaptic targets. While there are many similarities between axon growth during development and regeneration, it has been shown that some requirements for axon growth during regeneration are distinct from those involved in developmental axon growth. The goal of this research is to identify gene regulatory elements that respond to signaling pathways regulating axon growth in the regenerating nervous system. Genes encoding neuronal growth-associated proteins (nGAPs) are likely targets of axon growth regulatory pathways given the tight correlation between nGAP gene expression and periods of

axon growth. A prototypical nGAP is GAP-43, a membrane-associated protein that is enriched in axonal growth cones and is active in axon growth and guidance. In order to define gene promoter elements that are targets of axon growth regulatory signaling pathways during both development and regeneration, I have created a series of GFP reporter constructs containing various deletions of a 3.7 kb GAP-43 promoter isolated from Fugu rubripes. These constructs are being analyzed in transient and stable transgenic zebrafish. Thus far, stable transgenic lines have been established using reporter constructs containing the 3.7 kb promoter fragment and a 708 bp promoter fragment from the Fugu GAP-43 gene. Experiments are currently underway to characterize these lines for transgene expression in the developing, maturing, and regenerating nervous system.

157. Genomic Organization and Functional Analysis of the Zebrafish Methyl-Cytosine Binding Protein 2 (MeCP2) Gene. Louise E. Coverdale, Christopher Martyniuk, Vance Trudeau, and Cristofre C. Martin. Department of Biology, Center for Advanced Research In Environmental Genomics, Univ. of Ottawa, Ottawa, Ontario, Canada.

Epigenetic gene repression occurs as the result of the interactions between DNA and a number of proteins, including methyl-cytosine binding protein 2 (MeCP2). We have isolated a 1680 bp *MeCP2* cDNA from zebrafish that shows deduced amino acid identity with *Xenopus* and mammalian MeCP2α protein sequences. The zebrafish *MeCP2* gene was mapped to linkage group 8 using the LN54 radiation hybrid cell panel. The genomic organization of the zebrafish *MeCP2* and mammalian *MeCP2α* are highly similar. However, it appears that zebrafish do not possess an *MeCP2β* isoform, as is found in humans and mice. Relatively high levels of expression of *MeCP2* mRNA are found in embryos at 1 to 4 h post fertilization (hpf), after 24 hpf, and in adult brain and eyes. Whole mount in situ hybridization was performed on embryos and revealed ubiquitous *MeCP2* expression during early zebrafish development. At 24 and 48 hpf, the highest levels of expression were found in the epiphysis, midbrain, ventricular zone, and the otic vesicles. In adult zebrafish, *MeCP2*-expressing cells were found throughout the brain. Regions that are enriched in *MeCP2* mRNA include the pallium layer of the telencephalon, the preoptic area, the periventricular gray zone, lobus caudalis, and the vagus lobes. In the cerebellum, high expression was found in the Purkinje and Golgi type 2 cells. We are presently conducting functional analysis by microinjection of an antisense morpholino targeted to *MeCP2* mRNA and Western blotting.

158. Role of Histone Deacetylase 3 in Zebrafish Heart Development and Growth. Sigalit Zchut,[1] Robbert Creton,[2] and Lazaros Kochilas[1]. [1]Rhode Island Hospital, Providence, RI 02905; [2]Brown University, Providence RI 02912.

Histone deacetylases (HDACs) are a family of conserved proteins that regulate transcription by changing chromatin architecture resulting in transcription repression. Preliminary results obtained in our laboratory show that blocking HDACs activity with either trichostatin A (TSA) or Scriptaid (both are HDAC inhibitors) results in cardiac abnormality as well as developmental delay and growth retardation. We hypothesize that one or more members of the HDAC family have important roles in heart development and growth. It is accepted that almost all HDACs from Class I and II are equally sensitive to HDAC inhibitors, and therefore, our first goal was to identify more specifically which members of the HDAC family are expressed in the developing zebrafish embryo and study their expression pattern by in situ hybridization. Towards this goal, we cloned the zebrafish HDAC 3, 5, 6, 8, 9, and 10 and performed in situ hybridizations. The results we obtained so far suggest that HDAC3 is expressed throughout the embryo between 15 to 20 somite stages. In situ hybridization of the other HDACs is carried on. Our second goal is to learn about its role in heart development and growth. We knocked down HDAC3 expression using antisense morpholino oligos. Morphological analysis suggests that the injected embryos develop normally until 48 h. However, after 48 h post fertilization, the injected embryos display cardiac hypoplasia, similarly to the TSA-treated embryos. Since zebrafish heart development is completed by 48 h, we suggest that HDAC3 is involved in heart growth. More detailed molecular analysis is in process.

159. Kaiso/p120-Catenin and TCF/Beta-Catenin Complexes Coordinately Regulate Canonical Wnt Gene Targets. Jae-il Park,[1] Jon P. Lyons,[1] Hong Ji,[1] Thi Nguyen,[1] Michelle C. Barton,[1] Tom Deroo,[2] Kris Vleminckx,[2] and Pierre D. McCrea[1]. [1]Univ. of Texas M.D. Anderson Cancer Center, GSBS, Houston, TX 77030; [2]Ghent University, B-9052 Ghent, Belgium.

β-catenin-dependent or canonical Wnt signals are fundamental to animal development and tumor progression. Employing *Xenopus laevis*, we report that Kaiso, a BTB/POZ zinc-finger family member, directly represses canonical Wnt gene targets (*Siamois*, *c-Fos*, *Cyclin-D1*, and *c-Myc*) in conjunction with TCF/LEF (hereafter TCF). Depletion or dominant-negative inhibition of xKaiso resulted in *Siamois* de-repression. Conversely, xKaiso over-expression resulted in additional *Siamois* repression, likely through recruitment of N-CoR and associated chromatin modifications. Conceptually analogous to β-catenin relief of TCF repressive activity, we further demonstrate that p120-catenin acts to relieve Kaiso-mediated repression of *Siamois*. We find that Kaiso and TCF are co-associated, that pronounced *Siamois* expression follows the combined de-repression of Kaiso and TCF, and that co-precipitation of β-catenin with the Siamois promoter is enhanced upon Kaiso depletion. Functional interdependencies are further indicated by the capacity of Kaiso to suppress β-catenin-induced axis duplication and by TCF-3 to rescue Kaiso depletion phenotypes. These studies point to the convergence of p120-catenin/Kaiso and β-catenin/TCF signaling pathways in vertebrate development and possibly carcinogenesis.

160. Early Wnt Signaling and Later BMP Signaling Regulate Geminin Expression in the Presumptive Neurectoderm. Jennifer J. Taylor and Kristen L. Kroll. Washington Univ. St. Louis, MO 63110.

How do intercellular signals specify neural and epidermal fates in vertebrate ectoderm during gastrulation? Bone Morphogenetic Protein (BMP) antagonists secreted by the organizer can specify dorsal ectoderm to a neural fate. Fibroblast growth factor (FGF) signaling was also shown to promote neural fate. Previous studies, however, have not defined molecular mechanisms by which these or other intercellular signaling pathways regulate gene expression in early neurectoderm. Geminin is among the earliest markers of neural ectoderm at the onset of gastrulation in *Xenopus laevis*. We used transgenic *Xenopus* embryos to define two geminin 5′ regulatory elements that are necessary and sufficient to drive neural-specific expression during gastrulation. Each *cis*-domain contains Tcf binding motifs, which mediate Wnt signaling. Mutation of these Tcf binding motifs abolished neural-specific reporter expression in early gastrulae, suggesting that Wnt signaling is required prior to early gastrulation for neural-specific geminin expression. Each *cis*-domain also contains canonical binding motifs for Vent homeodomain proteins, transcriptional repressors that mediate BMP signaling. Mutation of both Vent sites resulted in neural-specific reporter expression in early gastrulae, but ubiquitous expression in mid-gastrulae. Thus, modulation of BMP signaling is not required for neural-specific expression prior to gastrulation, but it is essential to restrict geminin to presumptive neurectoderm during gastrulation. These data reveal distinct mechanisms by which BMP and Wnt signaling influence neural versus epidermal cell fate determination during early development.

161. Regulation of *SoxB1* Genes in Neural Induction in *Xenopus laevis*. Crystal Rogers and Elena Casey. Georgetown University, Washington, DC 20057.

The SoxB1 genes, Sox2 and Sox3 are expressed in the presumptive neural ectoderm and developing nervous system of *Xenopus laevis*. They are required for neural development and both are repressed by BMP. The neural default model states that ectoderm forms neural tissue in the absence of BMP signaling. Because of the spatio-temporal expression of Sox2 and Sox3 and their response to BMP, they are likely to be targets genes of BMP repression required for neural induction. Here, we report our studies to identify the molecular mechanisms that drive the expression of early neural genes. Animal cap assays were performed in the presence of protein synthesis inhibitor cycloheximide to determine if de novo proteins synthesis is required for the inhibition of *Sox2* and *Sox3*. Deletion analysis of 1.5 kb of the *Sox3* promoter in transgenic *X. laevis* embryos has been used to identify important cis-modules. The important protein–DNA interactions of these modules are being identified using gel mobility shift assays. These results along with current experiments suggest that *Sox2* and *Sox3* have distinct modes of regulation even though they have exceptionally similar expression patterns.

162. Regulation of Ath5 Gene Expression in the Vertebrate Neural Retina. Minde I. Hanson, David A. Hutcheson, and Monica L. Vetter. University of Utah.

In many vertebrate species, the basic helix–loop–helix (bHLH) transcription factor Ath5 is tightly associated with both the initiation of neurogenesis in the retina and the genesis of retinal ganglion cells. Proper temporal and spatial control of Ath5 expression is essential for normal retinal development. To better understand how Ath5 expression is regulated, we are studying the *Xenopus* Ath5 (Xath5) cis-regulatory region to identify elements that are required for retinal-specific expression. We have recently identified a proximal and a distal enhancer, each sufficient to drive retinal specific expression in transgenic *Xenopus* embryos. bHLH factors are capable of both auto- and crossregulation and we have shown that the proximal Xath5 enhancer requires bHLH factor binding sites (E-boxes) and bHLH factor activity in order to drive retinal-specific transgene expression. However, our studies suggest that the distal enhancer does not require bHLH activity, indicating that alternate regulatory mechanisms exist. We are currently interested in understanding how bHLH and non-bHLH regulatory mechanisms confer spatial and temporal Xath5 expression patterns. A possible model is that one enhancer initiates expression of Xath5 whereas a second enhancer maintains expression at later stages of development. We are performing a detailed analysis of temporal and spatial expression patterns to elucidate differences in gene expression regulated by the two enhancers. Our preliminary studies indicate that the two enhancers regulate different temporal aspects of Xath5 expression suggesting they may function at different developmental time points.

163. Identification of Functionally Conserved Regulatory Regions Upstream of Vertebrate Optx2 Genes. Michael Zuber,[1] Matt Theisen,[2] and Andrea Viczian[1]. [1]Departments of Ophthalmology and Biochemistry & Molecular Biology, SUNY Upstate Medical University; [2]Department of Biomedical & Chemical Engineering, Syracuse University, Syracuse, NY.

Optx2 is a member of the sine oculis-six family of homeobox containing transcription factors and is expressed in the eye field of vertebrate organisms during early eye development. Optx2, which has intrinsic repressor activity, induces cell proliferation in retinal stem/progenitor cells of the eye field and is therefore important in determining eye size. Optx2 has been highly conserved among vertebrate organisms; therefore, we have used cross-species sequence comparisons to identify conserved elements in the *Optx2* promoter. Both computer analysis and transgenesis were used to identify these elements. Computer analysis helped narrow the location of possible upstream regulatory regions. Transgenic frogs were generated, using an EGFP reporter gene, to test the activity of predicted regulatory elements in vivo. Preliminary results suggest that Insulin-like Growth Factor 1 (IGF1) acts through Optx2 to regulate eye size. IGF1 stimulates *Optx2* expression and regulates eye size in an Optx2-dependent manner. A better understanding of the upstream regulatory regions of the *Optx2* gene is necessary to investigate the potential link between the IGF1, Optx2 and the signaling cascade regulating vertebrate eye size.

164. Characterization of *alpha globin* Genes in *Xenopus tropicalis*. Daniel D. Roche and Richard M. Harland. University of California, Berkeley, CA 94720.

Alpha- and beta-globin gene expression is spatially and temporally restricted; this provides an excellent opportunity to study developmental regulation of these genes. It is previously reported that the *alpha- and beta-globin* genes are linked in *Xenopus tropicalis*. Two distinct *X. tropicalis* genomic libraries were screened and several BACs were identified which constitute two unlinked globin loci, an alpha globin and beta globin. In addition to showing that the *alpha- and beta-globin* genes are not linked, a larger than predicted number of genes were identified. Seven *alpha larval* genes were recognized by homology to a previously identified *X. tropicalis alpha globin* gene. In situ and RT-PCR were used to investigate the timing and domain of expression for each gene. Although all of the genes appear to code for fully functional proteins, not all of the genes are transcribed. DNase hypersensitivity assays were performed to identify changes in chromatin architecture. Promoter comparison of transcription factor binding sites indicates a set of conserved sites required for proper expression of the *alpha larval* genes. This characterization of endogenous gene expression is important for evo–devo comparisons of globin evolution and is essential for studies of regulatory elements in *X. tropicalis*.

165. Identification of Chicken *odd-skipped related 1 (osr1)* Regulatory Elements. Alexandra V. Petrova and Thomas M. Schultheiss. Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA 02215.

Osr1 (*odd skipped related 1*) is a member of the *odd* gene family defined by a highly conserved set of zinc finger domains. The founding member of this family, the *odd-skipped* gene was identified during a genetic screen in *Drosophila*. This pair-rule gene plays a vital role in segmentation of the fly, and seems to function as a repressor. Homologues of the *odd* family have been identified in *C. elegans*, chicken, mouse and human. In the chicken embryo osr1 is the earliest known marker for the intermediate mesoderm (IM), being expressed as early as stage 5. The IM is located adjacent to the somites in the embryo and gives rise to all vertebrate kidney tissues. As development progresses the expression pattern of *osr1* is maintained in undifferentiated IM but is downregulated in differentiating kidney tissues. In addition *osr1* expression is observed in the developing limbs and palate. Despite its seeming importance in vertebrate development, nothing is known about the transcriptional regulation of *osr1* or its function beyond its putative role as a transcription factor. The aim of this study is to elucidate the molecular mechanisms underlying *osr1* regulation in the IM of the chicken. We are analyzing the genomic region surrounding the *osr1* gene for enhancer activity by employing embryo electroporation. The goal is to be able to identify the regulatory regions that control the timing and location of *osr1* expression.

166. Genomic Analysis of Placode Formation. Kathryn L. McCabe, Andrea Manzo, Laura S. Gammill, and Marianne Bronner-Fraser. California Institute of Technology, Pasadena, CA 91125.

The peripheral nervous system is derived from two embryonic cell populations: the cranial neural placodes and the neural crest. Cranial neural placodes are thickened epithelium that give rise to the paired sense organs and some of the cranial sensory ganglia. To better understand the processes involved in placode formation, we performed a subtractive library screen to find new candidates involved in the formation of placodes as well as additional markers for these cell types. Of the 84 genes analyzed in St. 10 chicken embryos by in situ hybridization, 52 were found in one or more placodes, including the olfactory, trigeminal, and otic placodes. Many of these genes were in placodes and neural crest, which is consistent with their shared characteristics. The expression of several of the genes were assayed at St. 15 and were found to be maintained in several placodes, and were found in the newly forming epibranchial placodes. Although placodes are induced by different tissue interactions and molecules, the results from this screen indicate that the placodes utilize similar genetic machinery to accomplish specification and formation.

167. Transcription Regulation of *Drosophila* Neuroblast Temporal Determinants. Thomas Brody, Alexander Kuzin, and Ward F. Odenwald. National Institutes of Health, Bethesda, MD 20892.

During *Drosophila* CNS neuroblast (NB) lineage development, a transcription factor temporal network regulates NB temporal identity and transitions in gene expression, generating in part a changing repertoire of functionally diverse cells (for review see Brody and Odenwald, Development 129: 3763–70, 2002). After initial expression of Hunchback and Kruppel, NBs transiently express the POU homeodomain proteins Pdm-1 and Pdm-2 and then down-regulate these two proteins and up-regulate the zinc finger transcription factor Castor (Cas). We have shown that a 5.3-kb upstream sequence of *pdm-1* is sufficient to drive a reporter in a pattern similar to endogenous *pdm-1* (Kambadur et al., Genes Dev. 12: 246–60, 1998). We have also shown that the bHLH transcription factor Tap/Biparous is repressed downstream of *cas*. To increase our understanding of temporal regulation of *Drosophila* NB determinants, we have taken multiple approaches for in silico analysis of flanking sequences of four late determinants (*pdm-1*, *pdm-1*, *cas* and *tap*) in four *Drosophila* subgroups, and find that these contain blocks of conserved sequences that may represent enhancer elements. In particular, unique combinations of bHLH binding sites are present in flanking sequences of each of the genes. In many cases, analysis reveals that bHLH sites with conserved core and adjacent sequences predominate in these flanking sequences. We will describe our bioinformatics analysis of flanking sequences of these genes and will discuss how this analysis elucidates the basis for temporal gene regulation.

168. Analysis of Nerfin-1 Transcriptional and Post-Transcriptional Regulation. Alexander Kuzin, Thomas Brody, and Ward Odenwald. Neural Cell-Fate Determinants Section, NINDS, NIH, Bethesda, MD 20892-3706.

Nerfin-1 belongs to a conserved subfamily of Zn-finger transcription factors. Our characterization of loss- and gain-of-function mutants reveals that *nerfin-1* is required for interneuron axon guidance (Dev. Biol. 277: 347–65, 2005). During embryonic CNS development, *nerfin-1* mRNA is detected in all early delaminating neuroblasts, many GMCs and transiently in most, if not all, nascent neurons. However, the nuclear Nerfin-1 protein is detected only in neural precursor cells that undergo a single final division to generate neurons (the MP neuroblasts and GMCs) and is transiently expressed in nascent neurons. Nerfin-1 is also expressed in a subset of neuronal precursor cells of the PNS. To better understand the regulation of *nerfin-1* expression we have begun to characterize its cis-regulatory elements. An 11-kb fragment containing 6 kb of upstream sequence servers to completely rescue *nerfin-1* expression. Bioinformatics has revealed blocks of conserved sequences in the upstream region in 5 different *Drosophila* subspecies. Currently we are making constructs fused to GFP to determine which upstream fragments regulate *nerfin-1* CNS and PNS expression. *nerfin-1* 3′ UTR contains multiple predicted binding sites for miRNAs. Because of the discrepancy between *nerfin-1* mRNA and protein synthesis, the large number of predicted mRNA binding sites, and the length of the *nerfin-1* 3′UTR (1.5 kb), *nerfin-1* is prime candidate for post-transcriptional regulation. We are preparing a rescue fragment that lacks the 3′ UTR in order to insert and test the effects of various miRNA sites on *nerfin-1* expression.

169. Dpp Expression and Function in the Posterior Spiracle: Direct Activation by Combinatorial Activity of the Dpp and Wg Pathways and Independence from Dpp Function in Dorsal–Ventral Patterning. Stuart J. Newfeld and Norma T. Takaesu. School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501.

Utilizing a reporter gene, we previously showed that combinatorial signaling by Wg and Dpp activates dpp expression in the posterior dorsal ectoderm and that this aspect of dpp expression is associated with posterior spiracle development. We have recently identified a candidate enhancer within this reporter that may mediate combinatorial regulation of dpp posterior spiracle expression. The enhancer contains two consensus binding sites for dTCF and two sites for Mad and all four sites are highly conserved. We evaluated the regulation of this enhancer utilizing gel shift studies. These experiments showed that dTCF binds to its predicted sites and similar studies with Mad will be discussed. We evaluated the function of this enhancer by creating a unique dpp mutant allele with the enhancer deleted. Several viability and cuticle studies of this allele in a number of dpp null and Haploinsufficient backgrounds showed that the enhancer is required for posterior spiracle development but not for proper dorsal–ventral patterning. These results suggest that in dpp null embryos, posterior spiracle defects (e.g., missing or misshapen Filzkorper) are genetically separable from dorsal–ventral patterning defects, a departure from current thinking.

170. Regulation of Snail Transcription: a Chromatin Structure Approach. Matthew B. Palmer,[1] Jeremy M. Boss,[1] and Paul A. Wade[2]. [1]Emory University, Atlanta, GA 30322; [2]NIEHS, Research Triangle Park, NC 27709.

The transcriptional repressor Snail is operative in a variety of developmental contexts, including gastrulation, neural crest specification, and formation of heart valves and hair follicles. A property of Snail expression common to all of the above developmental situations is strict spatial and temporal expression boundaries. While the regulatory code governing Snail expression in *Drosophila* has been described, our understanding of how the expression of mammalian Snail is orchestrated remains fragmentary. We are using transformed human cell lines as a model system to analyze local chromatin architecture at the Snail locus in both the active and silent states. In addition to constitutive nuclease sensitivity over the core promoter, we have discovered a distal site whose nuclease hypersensitivity correlates with Snail transcription levels. Functional analysis of this sequence element has identified strong transcriptional activity in Snail expressing cells. Phylogenetic comparative analysis demonstrates that this element is evolutionarily conserved, supporting the hypothesis that this element plays an important regulatory role in Snail transcription. Current studies are investigating the mechanism by which this element influences transcription at the Snail locus as well as the identity of transcription factors bound at the element in the active state. These studies

will ultimately contribute to our understanding of regulatory mechanisms utilized to establish appropriate spatial and temporal control of Snail transcription in development and disease.

171. Morpholino Oligonucleotide Knock-down of Slug Reveals a Number of Potential Downstream Target Genes. Lisa A. Taneyhill, Edward G. Coles, and Marianne Bronner-Fraser. California Institute of Technology, Division of Biology, Pasadena, CA 91125, USA.

The neural crest is a population of migratory cells that arises from the dorsal neural tube during neurulation. The induction and migration of the neural crest rely upon the activity of Slug, a protein that plays a key role in regulating the epithelial-to-mesenchymal transition that characterizes neural crest cell delamination from the dorsal neural tube. Slug, a member of the Snail superfamily of transcriptional repressors, functions as an important regulator of the neural crest through its ability to modulate the activity of other genes whose expression is important during crest development. We have applied the technique of morpholino antisense oligonucleotide knock-down to examine effects of Slug on potential downstream target gene expression in avian embryos at various time periods. In particular, we have assessed the resulting response of several genes previously known to be involved in the induction and migration processes (FoxD3, rhoB, rhoA, N-cadherin, Msx1, BMP-4, Pax3) by quantitative PCR (QPCR). We have found that the expression of many genes is unaltered by the knock-down of Slug after long-term incubation in the presence of the Slug morpholino. However, short-term incubation (4 h) with the Slug morpholino allows us to observe both increases (FoxD3) and decreases (rhoB) in gene expression. These data demonstrate the potential utility of morpholino antisense technology in identifying veritable targets of key regulatory molecules at multiple time points during chick embryonic development.

172. The Mechanism of Maternal mRNA Localization in the Early *Drosophila* Embryo. Jennifer L. Semotok and Howard D. Lipshitz. Department of Mol. & Med. Genetics, University of Toronto, Prog. in Dev. Biol., HSC, Toronto, Canada.

During *Drosophila* development, mRNA localization is utilized to achieve cellular asymmetries to determine the body axes and to specify regional cell fates. "Degradation-protection"-based mRNA localization occurs during early embryogenesis and acts to localize initially ubiquitous mRNAs by targeting them for degradation in the bulk cytoplasm while protecting them from the degradation machinery in the posterior pole plasm (future germ line). Recent work has shown that Smaug, an RNA-binding protein, is both necessary and sufficient to trigger maternal mRNA decay in the bulk cytoplasm resulting in the posterior localization of mRNA (Curr Biol. 2005, 15:284–94). Smaug's genetic and physical interaction with components of the CCR4/POP2/NOT deadenylase complex suggests that Smaug acts to recruit the deadenylase complex to specific mRNAs resulting in deadenylation and subsequent decay. Our current work is focused on identifying the cis-element(s) that targets mRNA for Smaug-mediated decay. Preliminary data suggest that an abundant model maternal mRNA, *Hsp83* mRNA, contains a major instability element within its coding region and an auxiliary element in its 3′UTR. The position of these *cis*-elements is reminiscent of mammalian *c-fos* and *c-myc* mRNA instability which require translation through the coding region instability determinant to trigger deadenylation and decay. We are presently defining these cis-elements, determining whether Smaug binds directly or indirectly to one of the elements, and examining the role of translation in Hsp83 mRNA localization.

173. Characterization of the *C. elegans* Hypoxia-Inducible Factor and its Regulators. Jo Anne Powell-Coffman, Chuan Shen, Sara McMaken, and Kelly Gillette. Iowa State University, Ames IA.

During development, homeostasis, or disease states, cellular oxygen levels are often insufficient to meet physiological demands, and this condition is termed hypoxia. Mammalian cells respond to hypoxia by implementing changes in gene expression to increase anaerobic energy production, protect cells from stress, regulate cell survival, and increase local angiogenesis. The requisite changes in gene expression are largely controlled by the hypoxia inducible factor 1 transcription factor. The *C. elegans* hif-1 gene is orthologous to the mammalian hypoxia inducible factor alpha units, and *C. elegans* has proven to be a powerful genetic model system for deciphering hypoxia signaling and response. Stability of the *C. elegans* HIF-1 protein is regulated by the evolutionarily conserved EGL-9/VHL-1 pathway. When oxygen levels are sufficiently high, the EGL-9 enzyme hydroxylates a specific proline residue in HIF-1. This promotes binding of HIF-1 to the VHL-1 E3 ligase, and HIF-1 is targeted for degradation. Other cellular signals are known to converge on HIF-1, but the mechanisms by which they interact to control hypoxia response are not fully understood. Using whole-genome microarrays, we identified 110 genes that exhibited a > 2-fold difference in mRNA expression in hypoxic conditions. Sixty-three of these gene expression changes required hif-1 function. The central goal of our current studies is to identify novel regulators of HIF-1 and to characterize the complexes and networks that control this critical transcription factor. Towards these goals, we have employed genetic strategies to identify genes that control HIF-1 function.

174. The Prep-1 Interactome and a Novel Inhibitor of Prep-Pbx Activity, p160 Myb-Binding-Protein. Victor M. Diaz Cortes,[1] Angela Bachi,[1] and Francesco Blasi[2]. [1]University of Vita Salute San Raffaele, DIBIT, Milan, Italy; [2]University Vita Salute San Raffale and IFOM (FIRC Institute of Molecular Oncology), Milan, Italy.

The Prep-Pbx dimers play an important role in development. We have started a proteomic approach to identify the molecular interactors of the Prep-Pbx dimer by cloning a modified version of Prep-1, Prep-1-TAP which allows the Tandem Affinity Purification of the protein and associated partners. Prep-1-TAP contains a C-t tag with a Calmodulin Binding Peptide (CBP), two Protein A (ProtA) modules and a TEV cleavage sequence between each domain. We have first verified the functionality of Prep-1-TAP expressed in vivo concluding that the protein is indistinguishable from wild type. Nuclear and cytoplasmic extracts containing Prep-1-TAP has been affinity purified in two non denaturing steps: (1) binding to an IgG matrix and elution with the TEV protease; (2) purification with calmodulin-coated beads in the presence of calcium. The final highly purified eluate has been resolved by SDS-PAGE, silver stained, and proteins identified by MALDI-TOF/MS. The purified complex contained, in addition to Prep-1 also Pbx-1 and Pbx-2, confirming the specificity of the purification, and other proteins, many involved in transcription, like RNA-Pol-II. We now report data on one specific protein, p160MBP (Myb-Binding Protein). We have verified the interaction by co-immunoprecipitation and immunofluorescence co-localization. We show that p160MBP inhibit the binding of Prep-Pbx dimers to DNA and decrease transcription from a HoxB1 enhancer-luciferase construct. We conclude therefore that we have identified a novel, strong, interactor/inhibitor of Prep-1.

175. Functionality of JcDNV-Derived Somatic Transformation Vectors in Insects and the Role of Viral Enhancer Sequences. Paul D. Shirk,[1] Richard B. Furlong,[1] Jennifer Gillett,[2] and Herve Bossin[3]. [1]USDA ARS CMAVE, Gainesville, Florida; [2]University of Florida, Gainesville, FL; [3]FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria.

Stable somatic transformation of insects following microinjection of syncytial embryos (Royer et al, 2001) or by transfection of cells lines (Bossin et al., 2003) can be achieved by integration of entire plasmids

containing the Junonia coenia lepidopteran densovirus (JcDNV) genome. We assessed effects of sequence modifications including the presence of expression cassettes on the efficiency of JcDNV somatic transformation activities in Lepidoptera and Diptera. Cloning of 3xP3EGFP outside the JcDNV sequence did not affect the somatic transformation rate. Removal of coding sequences for some JcDNV nonstructural proteins or the 3′ inverted terminal repeat (ITR) had no effect on the transformation rate. Removal of 177 bp from the 5′ ITR did not decrease somatic transformation rates. However, removal of a 680-bp region within the 3′ terminus of the nonstructural protein coding sequence eliminated most transcriptional activity directed by the P9 promoter. Addition of the 680-bp DNV-enhancer to JcDNV vectors lacking this sequence restored transcriptional activity. Together with previously published results, these modifications demonstrate that the somatic transformation activity is dependent upon sequences of the 3′ ITR and influenced by sequences internal to the densovirus genome. Bossin et al. (2003) J. Virology. 77: 11060–11071 Royer et al. (2001) Insect Mol. Biol. 10: 275–280. Research supported in part by USDA ARS, Exelixis Inc. & CSREES NRI to PDS.

176. The Zebrafish *kohtalo/trap230* Gene is Required for the Development of the Brain, Neural Crest and Pronephric Kidney. Sungkook Hong,[1] Caroline E. Haldin,[2] Paul E. Ulanch,[2] Nathan D. Lawson,[1] Brant M. Weinstein,[1] Igor B. Dawid,[1] and Neil A. Hukriede[2]. [1]NIH, Bethesda, MD 20892; [2]University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Many mutations that display defects during embryonic development affect molecules which have region or cell type-specific expression. The results presented here stress the fact that gene products with widespread expression and function may nevertheless have regionally specific roles during embryogenesis. We isolated two alleles of a recessive lethal mutation that affects zebrafish development. By positional cloning, we showed that *kohtalo(kto)* is the trap230 homolog. *TRAP230* is a component of the mediator complex, which has a role in the transcription of multiple genes by mediating the interaction of enhancer-binding factors with the basal transcriptional machinery. In *kto* mutants, morphogenesis of the forebrain and midbrain is compromised and the anterior–posterior expansion of the hindbrain is incomplete. In addition, pigment cells of the trunk region fail to migrate and branchial arches do not develop normally in the mutant, suggesting that *trap230* is important in neural crest development. Also, glomerular anlagen of the pronephric kidney are specified in mutant embryos but fail to move towards the midline. Therefore, a common feature of *kto* mutant embryos is a failure of morphogenesis thereby implicating a component of the mediator complex in the control of cell migration in the zebrafish embryo.

177. GEISHA is the Gallus Expressed Sequence Tag In Situ Hybridization Analysis. Diana Darnell, George Bell, Simran Kuar, and Parker Antin. University of Arizona Health Sciences Center, Department of Cell Biology and Anatomy, Tucson, AZ.

Several major vertebrate genomes have been sequenced, and emphasis is shifting to understanding gene function, which remains unknown for approximately half of all genes. A crucial early step is to determine spatial and temporal patterns of gene expression, especially in early embryos in which genes regulating normal development are expressed. Since gene expression analysis in human embryos is ethically untenable, mapping of expression patterns will be accomplished in model organisms such as the chicken. The chicken embryo provides many advantages for large-scale expression screening, including striking early developmental similarities to human, low expense and ready availability. The goal of the GEISHA (gallus est in situ hybridization analysis) project is to create a chicken embryo in situ hybridization expression resource. The objectives are as follows: (1) to use automated high throughput whole mount in situ hybridization analysis to map the location of expressed sequences in chick embryos through 4 days of development; (2) to acquire additional reliable expression data from individual researchers and from published resources (Developmental Dynamics, Developmental Biology and MOD are on board to share information); and 3) to house expression information in a relational database accessible to the research community via the web (http://geisha.biosci.arizona.edu). This will provide a valuable and comprehensive resource for the biomedical research community. The GEISHA project is funded by NIH grant 344080.

178. Genome-Wide Expression Profiling of *Xenopus laevis* metamorphosis programs. Biswajit Das,[1] Liquan Cai,[1] Mark G. Carter,[2] Alexei A. Sharov,[2] Yu-lan Piao,[2] Minoru S. Ko,[2] and Donald D. Brown[1]. [1]Department of Embryology, Carnegie Institution of Washington, 115 W University Pkwy., Baltimore, MD 21210; [2]Developmental Genomics and Aging Section, Laboratory of Genetics, National Institute on Aging, National Institutes of Health, 333 Cassell Drive, Suite 3000, Baltimore, MD 21224.

During *Xenopus laevis* metamorphosis, TH controls genetic programs ranging from complete organ growth such as limb development to cell death in the gills and tail. In addition, most tadpole organs are induced by TH to remodel. These include the intestine, pancreas, heart, liver, and brain. To identify the T3 dependent pathways involved in metamorphosis, we have explored gene expression profiles of some of these programs using an oligonucleotide-based microarray (Agilent Technologies Inc.). The 21564 unique 60-mer oligo spots in the microarray represent about 99% of all the UniGene clusters (Build 48). The expression profile of genes expressed in stage 54 tail was compared with the profiles after 24 h and 48 h of T3 induction. In addition, tails at the climax of metamorphosis (stage 62) were profiled. The significance and reliability of the system is described. The TH-dependent proliferation programs in the hindlimb and brain were also profiled. New pathways involved in the TH-induced tail resorption program and HL and brain proliferation programs are revealed and confirmed by in situ hybridization.

179. A Genetic Map for *Xenopus tropicalis*. Dan Wells,[1] Laura Gutierrez,[1] Zhenkang Xu,[1] Larry Bellot,[1] Ye Yuan,[2] Matthew Hitchens,[2] Amy K. Sater,[1] and Steven E. Scherer[2]. [1]Dept. of Biology and Biochemistry, University of Houston, Houston, TX; [2]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

We are developing a genetic map for *X. tropicalis*, an essential resource for the emergence of *X. tropicalis* as a model system for the genetic analysis of vertebrate development. We are using a bioinformatics-based strategy to identify Simple Sequence Length Polymorphisms (SSLPs). First, unique sequences containing tri- or tetranucleotide repeats are identified within *X. tropicalis* genomic sequence assemblies provided by the Joint Genome Institute. These sequences are then tested for size polymorphisms between Nigerian and Ivory Coast strains. We have identified over 1000 SSLPs; the SSLP marker database is currently available online at http://tropmap.biology.uh.edu. We have also generated a mapping cross DNA panel with over 500 sibling F2 individuals from an original cross of inbred Nigerian (male) and Ivory Coast (female) strain grandparents. This mapping cross DNA panel represents over 1000 informative meioses, providing a theoretical limit of resolution of 0.1 cm. A preliminary map of 1000 SSLP markers against a subset of the mapping cross DNA panel representing 192 F2 individuals should be available by summer 2005. Our goal is to map 5000 SSLP markers against the mapping cross DNA panel, yielding an invaluable aid for positional cloning of genes identified by mutation. We would like to thank Vijay Dontharaju, Shiran Pasternak, Peter Blythe, and Keelan Hamilton who also contributed to this work.

180. The Mouse Atlas of Gene Expression: SAGE Analysis of Preimplantation Stages. Mona K. Wu,[1] Rebecca Cullum,[1] James L. Rupert,[1] Asim Siddiqui,[2] Allen Delaney,[2] Anita Charters,[2] Jaswinder Khattra,[2] Steven Jones,[2] Marco Marra,[2] and Pamela A. Hoodless[1]. [1]Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, Canada; [2]Genome Sciences Centre, B.C. Cancer Agency, Vancouver, Canada.

Our project is designed to generate a comprehensive and quantitative profile of gene expression during mammalian development using the C57BL/6J mouse. Various tissues representing key points in embryogenesis and organogenesis are dissected from embryos, neonates and adults, and 21 base pair Serial Analysis of Gene Expression (longSAGE) libraries are prepared. For this project, SAGE provides three major advantages over conventional methods of gene expression analysis: (1) SAGE libraries provide a quantitative profile of the transcriptome; (2) analysis is not restricted to detecting known genes; and (3) the SAGE database can be expanded as further data become available. We have generated an efficient pipeline for the construction and sequencing of SAGE libraries. As of March 9th, 2005, 93 libraries have been completed. These libraries contain over 11 million SAGE tags. The data from the libraries are available on our website, www.mouseatlas.org, with additional libraries being added when completed. Ultimately, the Atlas project expects to create and analyze 200 SAGE libraries. In addition to the data, software for analysis, including library comparisons and tag-to-gene mapping is available on the website. Here, we present SAGE analysis of the transcriptome of three preimplantation libraries: fertilized egg, morula, and blastocyst. This project is funded by Genome Canada, the BC Cancer Agency and NCI-NIH.

181. SAGE Analysis of Early Mouse Endoderm. Juan Hou,[1] Mona K. Wu,[1] Anita Charters,[2] and Pamela A. Hoodless[1]. [1]Terry Fox laboratory, B.C. Cancer Agency, Vancouver, BC, Canada; [2]Genome Science Center, B.C. Cancer Agency, Vancouver, BC, Canada.

Three primary germ layers (endoderm, mesoderm and ectoderm) give rise to the tissues of the developing embryo. The endoderm forms between the 6th and 7th days of murine embryogenesis. Within 2 days, it undergoes a folding process to become the primitive gut tube, which is divided into foregut, midgut and hindgut regions. During organogenesis, this develops into the gastro-intestinal tract, from which the respiratory tract and digestive organs will bud. For example, the lung, liver, and pancreas originate from the foregut, and the intestine and colon will form from the hindgut. Understanding how these endoderm-derived organs are specified, differentiate, grow, and undergo morphogenesis is key to understanding visceral organ disorders and tissue regeneration, and will ultimately leading to novel approaches for therapeutic control of cell differentiation. As part of the ongoing Genome BC Mouse Atlas of Gene Expression project (www.mouseatlas.org.) in our laboratory, we generated three Serial Analysis of Gene Expression (SAGE) libraries from the early endoderm of murine embryo. Here, we present SAGE analysis and validation of these three libraries: whole definitive endoderm (0–6 somite stage), foregut (8–12 somite stage), hindgut (8–12 somite stage).

182. Microarray Analysis of Transcriptomes in Normal Developing Mouse Autopods and Hoxa13 Knockout Mouse Model of Hand-Foot-Genital Syndrome. Siming Shou[2] and Scott H. Stadler[1]. [1]Shriners Hospital for Children, Research Division, 3101 SW Sam Jackson Park Road, Portland, OR 97239; [2]Oregon Health and Science University, Department of Molecular & Medical Genetics, 3181 SW Sam Jackson Park Road, Portland, OR 97239.

Mutations in Hoxa13 cause Hand-Foot-Genital syndrome (HFGS). To elucidate the transcriptional regulatory networks affected by the loss of Hoxa13 function, twelve independent MOE-430 GeneChip experiments were performed using RNA samples from Hoxa13 mutant and wild-type mouse E12.5 autopods. Transcriptome analysis identified approximately 26179 transcripts expressed in the developing wild-type autopod. Analysis of Hoxa13 mutant and wild-type transcriptomes identified roughly (number of transcripts) to exhibit a greater than two-fold differences in expression when compared to wild-type autopods (t test, $P<0.01$).The altered gene expression patterns were found to associate with the mutant limb genotype, indicating that these transcripts require Hoxa13 function for normal expression in the autopod. Real-time quantitative RTPCR experiments were performed to validate the microarray discoveries. Furthermore, using network analysis programs (Cytoscape 2.0), we found a significant correlation between Hoxa13 and several transcripts (Pearson correlation > 0.7, $P < 01$); suggesting that Hoxa13 and the associated network genes function to coordinate autopod development and play an essential role in HFGS. Microarray gene expression databases of developing mouse wild-type autopods (E12.5) have been established and will be publicly accessible: (http://www.shcc.org/mouselimbdb) to serve the research community.

183. Expression of Wnt5a Gene in Mouse Embryonic Orofacial Development. Jing Xiao,[1] En-xin Zhu,[1] Noriyuki Nagai,[2] Hitoshi Nagatsuka,[2] and Changgong Li[3]. [1]Dalian Medical University, Dalian 116027, China; [2]Okayama University, Okayama City 700-8525, Japan; [3]University of Southern California, CA 90033.

Wnt5a, a signaling molecule of the Wnt family, has been shown to underlie the outgrowth of the vertebrate limb and proximal–distal (P–D) morphogenesis of the lung. To date, nothing is known about the functional role of Wnt5a in outgrowth components of the first branchial arch including maxillary (secondary palate), mandible and anterior portion of tongue. In our current research, by whole-mount in situ hybridization analysis of mouse embryos, we have detected high levels of Wnt5a mRNA in outgrowing maxillary, mandible and tongue of embryonic day 12.5 (E12.5). Immuno-histochemical staining of E13.5 and E16.5 mouse head frontal sections showed Wnt5a protein is located in the cytoplasm of epithelial and mesenchymal components of maxillary, mandible, tooth germ, palate and tongue. In the process of secondary palate growth and fusion from E12.5 to E16.5, high levels of Wnt5a were detected in the palate shelves and tongue by RT-PCR analysis. Serial frontal sections were made from E14.5, E16.5 and Post-natal 1 day (PN1) Wnt5a functional gene knockout mice and their morphology was determined. At E14.5 Wnt5a knockout mice showed delayed P-D outgrowth of secondary palate. At E16.5, the tongue is still at an elevated position and the secondary palate failed to fuse completely with posterior portion of palate shelves keeping in a vertical direction. At PN1 Wnt5a knockout mice showed cleft palate with or without cleft lip (CL/P). Our data suggest that Wnt5a participates in mouse embryonic orofacial development.

184. The Mouse Hair Growth Cycle as a Model for Studying Cyclic Biological Processes. Kevin K. Lin, Darya Chudova, Padhraic Smyth, and Bogi Andersen. Departments of Biological Chemistry, Medicine and Computer Sciences, Univ. of California, Irvine, CA 92697.

Understanding the regulation of hair follicle cycling is of great interest because aberrant regulation of hair cycle control genes is responsible for several types of abnormal hair loss and skin cancers. To gain insight into the nature of the hair growth cycle, we performed a comprehensive gene expression profiling of mRNAs isolated from the mouse back skin at representative time points during the first synchronous hair cycle and asynchronous cycles. We found that known hair cycle-related genes have significantly higher replicate variance during the asynchronous cycles in comparison to the synchronous cycle, and thus we developed a statistical algorithm to identify hair cycle-associated gene. We developed a mixture

model clustering algorithm for replicated measurements to cluster the time-course gene expression profile data. Distinct profile clusters contained significant overrepresentation of genes that belong to the same Gene Ontology category and genetic/physical interaction network, providing insights into the regulation of hair follicle cycling. The list of hair cycle-associated genes was then refined by systematic comparison of cluster membership between expression profiles of the first and second hair cycles. We identified many genes not previously recognized to be hair cycle-associated and confirmed their temporal and spatial expression patterns using qr-PCR and in situ hybridization. The same bioinformatics approach can be applied to other time-course gene expression studies of complex tissues in identifying expression changes associated with a cyclic process of interest.

185. Withdrawn.

186. Genetic Pathways that Regulate Early Development of the Mouse Embryo and Neural Tube Closure. Irene E. Zohn,[2] Andrew Pollock,[2] Kathryn V. Anderson,[1] and Lee Niswander[2]. [1]Developmental Biology Program, Sloan-Kettering Institute, New York, NY 10021; [2]Howard Hughes Medical Institute, Department of Pediatrics, Section of Developmental Biology, University of Colorado Health Sciences Center, Aurora, CO 80045.

Disruption of multiple developmental pathways in the mouse can result in a failure to close the neural tube, thus making neural tube closure an extremely sensitive readout of a wide variety of defects in early mouse development. From our ENU mutagenesis screens, we have identified a series of novel mouse mutants that display specific and unique developmental defects, all of which result in a failure of neural tube closure. The droopy eye (dey) mutant mouse line is described here. dey mutant embryos exhibit a range of phenotypes including exencephaly, spina bifida, misshapen eyes and gastrulation defects, and carry a hypomorphic mutation in a novel p38MAPK-interacting protein (p38IP). Interestingly, p38IP interacts with p38MAPK in a yeast 2-hybrid assay, and phospho-p38MAPK levels are decreased in dey mutant embryos, suggesting that p38IP is required for p38MAPK activation in vivo. Mutant embryos harboring a more severe mutation (p38IPgenetrap) exhibit completely penetrant defects in mesoderm development. Remarkably, at gastrula stages, p38IPgenetrap mutant embryos resemble FGF8 mutant embryos and display defects in mesoderm migration, however, they express mesoderm markers. Intriguingly, FGF8 is thought to act through multiple downstream pathways resulting in both induction and migration of mesoderm. The similarity between p38IPgenetrap and FGF8 mutant embryos suggests that FGF8-stimulated cell migration requires p38MAPK activation.

187. Overexpression of Human Sclerostin (Sost) in Mice Impairs Limb Development. Nicole M. Collette,[1] Jessie Chang,[1] Richard M. Harland,[2] and Gabriela G. Loots[1]. [1]Genome Biology Division, Lawrence Livermore National Laboratory, Livermore, CA, USA; [2]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA.

Sost is a negative regulator of bone formation involved in the pathogenesis of two rare skeletal dysplasia characterized by generalized increased bone density: sclerosteosis and van Buchem disease. Using overexpression studies in human BAC transgenic mice we have shown that *sost* plays a role in bone metabolism by causing osteopenia in the axial and appendicular skeleton. In addition, overexpression of human *sost* causes moderate to severe limb developmental defects in a dosage-dependent loss of distal skeletal elements, while the patterning and development of proximal elements are unaffected. Morphological changes can be observed as early as E10.5, prior to bone condensation which initiates ~E14.5. This suggests that very early molecular interactions influence the pattering of distal limb elements, and elevated levels of *sost* perturb the normal sequence of events. We are investigating the mechanism by which *sost* interferes with proper limb development, and what signaling pathway (Fgf, BMP and Wnt) sost is most likely to modulate. We have coupled microarray expression analysis performed on E10.5 human *sost*-transgenic mouse forelimbs with in situ hybridization on whole-mount embryos to elucidate the molecular pathways that participate in this phenotype and to address the role of *sost* during limb development. This work has been performed under the auspices of the U.S. Department of Energy, University of California, Lawrence Livermore National Laboratory Contract No. W-7405-Eng-48.

188. Identification of Genes with Oscillatory Expression in Mouse Somitogenesis Using a Synchronized Human Stem Cell Model. Dilusha A. William,[1] Biagio Saitta,[2] Vladimir Markov,[2] Dorian A. Hall,[1] Joshua D. Gibson,[1] Mizuho S. Mimoto,[1] Eric F. Rappaport,[1] and Kenro Kusumi[1]. [1]The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA 19104; [2]The Coriell Institute for Medical Research, Camden, NJ 08103.

Oscillatory expression of genes in the notch and wnt pathways regulates the segmentation clock. Synchronized mouse C2C12 cells display oscillatory expression of the notch pathway gene *Hes1*. To develop in vitro models for identifying genes with oscillatory expression, we used human mesenchymal stem cells (MSCs) that have a differentiation capacity similar to paraxial mesodermal cells, in addition to mouse C2C12 cell cultures. We synchronized these cell culture models and carried out microarray and quantitative PCR analysis to identify novel oscillatory genes. Human *HES1* exhibited cyclical expression in MSCs with a periodicity of 5.5 h, confirmed by quantitative PCR, while mouse *Hes1* displayed cycling with a periodicity of 2 h in C2C12 cells. Additional candidate genes in the notch, wnt, and other signaling pathways were identified that display oscillatory expression in MSCs. Selected candidate genes were characterized using in situ hybridization to examine expression in the presomitic mesoderm of mouse embryos. Thus, screening for oscillatory expression using in vitro cell culture models has yielded a number of candidate genes to characterize further as components of the segmentation clock. (Supported by NIH grant AR050687 and a Hitchings-Elion Fellowship of the Burroughs Wellcome Fund.)

189. Molecular Dissection of the *C. elegans* DTC Leader and Niche Cell Functions. Dana Byrd,[1] Daniel Hesselson,[2] and Judith Kimble[1]. [1]HHMI and Dept. of Biochemistry, University of Wisconsin-Madison; [2]Dept. of Genetics, University of Wisconsin-Madison, Madison, WI 53706.

Distal tip cells (DTCs) serve at least two critical roles during development of the *C. elegans* hermaphrodite gonad. They function as migratory leader cells to regulate morphogenesis of the U-shaped gonad arms and as niche cells to regulate germline proliferation. Ablation of the DTC early in development results in a block in the migration/expansion of the gonad tube into its normal U shape, and ablation of the DTC at any time point results in the loss of germ cell proliferation. At a molecular level, these functions are separable. Mutations in genes encoding guidance molecules, such as *unc-5* (Netrin receptor), *unc-6* (Netrin), and *unc-40* (DCC/Netrin receptor), result in the failure of the DTCs to lead the migration of the gonad arms to their normal positions, yet do not abolish germline proliferation. By contrast, in mutants with defects in germline proliferation, such as *glp-1* (Notch), the gonad still assumes its normal U shape. We are taking a microarray approach to

determine the gene expression profiles of the DTC during development to further explore the molecular basis of its leader and niche cell functions. DTC transcripts will be enriched by immunoprecipitation of Flag-tagged PAB-1 [poly(A) binding protein] with bound RNAs (Roy et al., 2002). We then test the functions of genes that are expressed in DTCs by cell-specific RNAi. Our progress will be described.

190. Identification of Factors Mediating Cell Competition in *Drosophila melanogaster*. Claire de la Cova, Meng-Ping Tu, and Laura A. Johnston. Genetics and Development, Columbia University, New York, NY 10032.

Cell competition, in which cell–cell interactions result in elimination of "losing" cells and survival of "winning" cells, contributes to the precise control of cell number within growing organs of both vertebrates and *Drosophila melanogaster*. We use the wing imaginal disc of *Drosophila* as an in vivo model system to study cell competition during organ growth. In *Drosophila*, *Minute* mutant cells, which are deficient in a ribosomal protein and grow slowly, are competitively eliminated from the growing wing when in the presence of wildtype cells. Recently, we identified the growth regulator *dmyc*, a homolog of the *c-myc* proto-oncogene, as a factor that induces cell competition. In mosaic wing imaginal discs, cells deficient for *dmyc* grow very slowly and are eliminated. Conversely, cells expressing higher levels of *dmyc* out-compete wildtype cells, ultimately leading to apoptotic death of their wildtype neighbors. Our goal is to identify the cellular processes and molecular factors mediating such competitive cell interactions. To this end, we are isolating cell populations from different competitive contexts induced in vivo in the growing wing, including neighboring *dmyc*-expressing and wildtype cells, and neighboring *Minute* mutant and wildtype cells. We are then employing a gene expression-based approach to examine these different types of "winning" and "losing" cells, with the hope of obtaining a molecular signature of cell competition. Identification of competition-specific expression changes in *Drosophila* may uncover previously unknown cues that regulate cell growth, proliferation, and survival in growing organs.

191. Control of Growth in *Drosophila* Head by Elbow and No ocelli. Carlos M. Luque[2] and Marco Milán[1]. [1]Institució Catalana de Recerca i Estudis Avançats (ICREA); [2]Institut de Recerca Biomèdica, Parc Científic de Barcelona, Spain, EU.

The *Drosophila* head is a complex organ with a precise architecture. Most of it is occupied by the compound eyes and the antennae. The presumptive eye and antennal tissues of the fly, referred to as the eye-antennal imaginal discs, are allocated late in embryogenesis as two bilateral groups of approximately 20 cells. Before the second instar larval stage cellular growth and proliferation are continuous. Several signaling pathways have been shown to regulate cell cycle progression and affect cell numbers in eye-antennal development. Notch signaling at the dorso-ventral boundary promotes cell proliferation in the undifferentiated cells. JAK/STAT pathway is known to regulate cell cycle progression in the first mitotic wave, while EGFR pathway regulates G2/M progression in the second. Cell death also contributes to precise regulation of retinal cell number. The elbow (el) and no ocelli (noc) genes are located in close proximity on chromosome 2L and encode closely related zinc finger proteins. We present evidence that Elbow and Noc proteins are expressed in the presumptive cells of eye-antennal imaginal disc, where they are required to repress the proliferation of cells in the head capsule. Double mutant clones induced in the eye-antennal disc are able to produce extensive outgrowth of tissue in a very specific region of the head, close to the dorso-ventral boundary. The outgrowth is formed mostly by mutant tissue and expresses Notch at high levels. This suggests that Elbow and Noc role in growth control in the head could be achieved by local regulation of the Notch signalling pathway.

192. Frizzled-10 is Required for the Expansion of the Dorsal Neural Tube During Chick Development. Ann N. Easton,[1] Roeben N. Munji,[1] Lisa M. Galli,[1] Rowena Mae O. Suriben,[1] Michael R. Stark,[2] and Laura W. Burrus[1]. [1]San Francisco State University, San Francisco, CA 94132; [2]Brigham Young University, Provo, UT 84602.

Wnt proteins are a family of secreted signaling factors that govern diverse roles in embryonic development. While Wnt-1 and Wnt-3a have been implicated in both the regulation of cell proliferation and fate specification of neural and neural crest cell lineages within the developing vertebrate neural tube, the specific Frizzled receptors that mediate Wnt-1/3a signaling are unknown. Analysis of the expression patterns of frizzled homologues in human, chick, zebrafish and *Xenopus* has identified *frizzled-10* as a candidate neural Wnt receptor. We have used whole mount in situ hybridization to analyze the expression pattern of chick *frizzled-10* and have confirmed its presence in the dorsal neural tube, where it overlaps with *Wnt-1* and *Wnt-3a*. These data led us to hypothesize that chick Frizzled-10 may mediate Wnt-1/3a signaling in the neural tube. To test this hypothesis, we utilized in ovo electroporations of chick embryos with anti-sense morpholinos to inhibit translation of endogenous Frizzled-10. Morpholino-electroporated embryos were analyzed for the level of proliferation by quantifying antiphosphohistone-H3 positive cells. Consistent with known roles of Wnt-1 and Wnt-3a, our results suggest that Frizzled-10 is required for proliferation. We are currently testing the effects of *frizzled-10* morpholinos on cell fate specification in the dorsal neural tube. We are also using pull down assays to test for direct interaction of Frizzled-10 with Wnt-1 and Wnt-3a.

193. lbx1 Promotes Myoblast Proliferation Through Down-Regulation of *myoD*. Benjamin L. Martin and Richard M. Harland. Univ. of California, Berkeley.

Lbx1 has been shown to be involved in hypaxial myoblast migration in the mouse. We have further investigated the function of lbx1 using loss and gain of function approaches in the frog *Xenopus laevis*. While myoblast migratory defects are observed in antisense MO injected embryos targeting *lbx1*, this may be a secondary defect caused by a lack of myoblast cell proliferation. Over-expression of *lbx1* mRNA results in enlarged somites, but a lack of differentiated muscle. The enlarged somites also display an increase in cell proliferation. On the other hand, loss of *lbx1* function using antisense MOs results in a decrease of myoblast proliferation in the hypaxial domain. We have linked the control of cell proliferation to a strong down-regulation of *myoD* expression in gain of function experiments. Furthermore, co-injection of *myoD* mRNA with *lbx1* mRNA rescues the over-proliferation phenotype observed when *lbx1* is injected alone. The results indicate that a primary function of *lbx1* in hypaxial muscle development is to repress *myoD*, allowing myoblasts to proliferate before the eventual onset of terminal differentiation.

194. Efflux Transporters at the Crossroads of Development and the Environment. Amro Hamdoun and David Epel. Hopkins Marine Station, Stanford University, Oceanview Boulevard, Pacific Grove, CA 93950, USA.

We have recently found that one consequence of the membrane reorganization at fertilization is an increase in efflux transporter activity. This increase in activity does not involve de novo synthesis of efflux transporters; rather pre-existing proteins in the egg are delivered to the membrane on microfilaments. One result of the post-fertilization increase in efflux transporter activity is exclusion of hydrophobic xenobiotic molecules from the embryo. The role of this xenobiotic exclusion is to protect the embryo from toxicants in the environment. Another role promotes progression through the cell cycle, with anaphase the mitotic stage most dependent on this efflux transporter activity. A probable mechanism for this developmental effect of the transporters is action through the efflux of

endogenous signaling molecules. Likely, candidates are the lipid-derived eicosanoids which are structurally related to efflux transporter substrates and which could be produced by a lipoxygenase activity present in the egg following fertilization. Using GCMS, we are screening extracts from sea urchin eggs and embryos for the expression of these molecules after fertilization and during mitosis. We have cloned three of the sea urchin transporters present at fertilization and will express the corresponding proteins in insect cells so that we can test individual transporters for interaction with the candidate eicosanoid molecules.

195. Multiple Roles for Snail in Mesoderm Development of the Sea Urchin Embryo. Shu-Yu Wu and David R. McClay. Department of Biology, Duke University, Durham, NC 27708, USA.

At the onset of gastrulation in sea urchin embryos, primary mesenchyme cells (PMCs), which are derived from micromeres, ingress into blastocoel and become the larval skeleton. Another population of cells, the secondary mesenchyme cells (SMCs) form at the tip of archenteron and become non-skeletogenic mesoderm. The *Snail* gene has been shown to play important roles in the formation of mesoderm, in both vertebrates and invertebrates. Sea urchin *snail* was cloned and characterized from *Lytechinus variegatus*. The expression pattern of *LvSna* is dynamic throughout early sea urchin development, and is expressed in different populations of mesodermal cells, including both PMCs and SMCs. Functional knockdown of *LvSna* with morpholino-substituted antisense oligonucleotides results in a block/delay of PMC ingression, loss/reduction of pigment cell differentiation, and stunted arm rods in pluteus larva. Ectopic/overexpression studies by injecting eggs with *LvSna* mRNA produces several developmental defects in sea urchin embryos, including mesenchymal extrusion, and disorganization of skeleton formation. Furthermore, several transcription factors connect *snail* to the sea urchin mesodermal gene regulatory network (GRN). These data suggest that *LvSna* functions as a key regulator of mesoderm formation, especially the epithelial–mesenchymal transition (EMT) process, which has been implicated to be the ancestral function of *Snail* gene in both proterostomes and deuterostomes, in the early development of sea urchin embryos.

196. Differential Protein Turnover, Negative Autoregulation, and Transcriptional Repression Precisely Regulate SoxB1 Levels in the Early Sea Urchin Embryo. Lynne M. Angerer,[1] Laurel A. Newman,[2] and Robert C. Angerer[1]. [1]NIDCR/NIH, Bethesda, MD 20892; [2]University Rochester, Rochester, NY 14634.

SoxB1 antagonizes β-catenin's transcriptional function in endomesoderm development in early sea urchin embryos. Tight regulation of SoxB1 is required for specification of these cell fates. We identified multiple mechanisms that downregulate SoxB1 in endomesoderm progenitors. The asymmetric 4th cleavage and repression of *SoxB1* transcription from the blastula to pluteus stage decrease SoxB1 levels in vegetal blastomeres. In addition, monitoring levels of SoxB1-GFP revealed robust β-catenin-dependent SoxB1 turnover specifically in blastula endomesoderm. This turnover is mediated by different β-catenin-dependent pathways in different vegetal blastomere tiers. SoxB1 degradation in macromere-derived cells requires both sequences in its C-terminal half and nuclear entry, whereas in the micromere lineages, it requires neither. In micromeres, nuclear β-catenin activates transcription of pmar-1, which can generate a signal(s) leading to loss of SoxB1 in nearby blastomeres [Oliveri et al., 2003; Dev. Biol. 258, 32–48]. This loss is likely to result from SoxB1 degradation, since SoxB1-GFP is reduced in embryos that mis-express pmar-1. Finally, SoxB1 is negatively auto-regulated in ectoderm, as *SoxB1* mRNA levels rise 10-fold in blastulae lacking SoxB1 protein as a result of morpholino injection. All these results strongly support our model that early cell fate specification requires precise regulation of SoxB1 in each tier of blastomeres along the AV axis of the sea urchin embryo.

197. Dishevelled Regulates Nuclear β-Catenin During Germ Layer Segregation in the Cnidarian, *Nematostella vectensis*. Shalika Kumburegama and Athula Wikramanayake. Department of Zool., University of Hawaii at Manoa, Honolulu, HI 96822.

Eggs of cnidarians, including the anthozoan *N. vectensis* do not have the animal-vegetal (A/V) axis seen in most bilaterian animals, and initiation of pattern formation in cnidarians is poorly understood. In early *N. vectensis* embryos, β-catenin is nuclearized in the future gastrodermis and it specifies endoderm. Understanding how β-catenin is nuclearized in endoderm will provide insight into pattern formation in cnidarian embryos. Elucidating these mechanisms in cnidarians may also provide insight into the evolution of the A/V axis. A key regulator of nuclear β-catenin in bilaterian embryos is the phosphoprotein Dishevelled (Dsh). To determine if Dsh is required for the activation of β-catenin, a dominant negative form of Dsh (Dsh-DIX) was expressed in *N. vectensis* embryos by mRNA injection. Embryos expressing Dsh-DIX failed to nuclearize β-catenin and failed to gastrulate. These results suggested that there was selective activation of Dsh in the endoderm precursor cells. To determine if there was selective accumulation of Dsh in these precursor cells, zygotes were injected with Dsh:GFP mRNA. Our results showed that Dsh:GFP becomes localized to one half of the embryo beginning at the 16-cell stage and to the future endoderm by the early gastrula stage. These results suggest a mechanism where degradation of Dsh in the ectoderm precursor cells leads to the selective activation of β-catenin in the endoderm, and suggests an important role for Dsh in the evolution of polarities in animal eggs. Preliminary studies to elucidate the mechanisms that regulate Dsh localization to the endoderm will be discussed.

198. *C. elegans* Non-Muscle Myosin Regulates Apicobasal Par-3 Distribution and Blastocoel Size. Jeremy Nance,[1] Kathryn Good,[2] Ryan Cinalli,[1] Kim Wachter,[2] and James R. Priess[2]. [1]Skirball Institute, NYU School of Medicine, New York, NY 10016; [2]Fred Hutchinson Cancer Research Center and HHMI, Seattle, WA 98109.

During the four-cell stage of *C. elegans* development, cells polarize along the embryonic inner-outer, or apicobasal, axis. Apicobasal polarity is important for cellular asymmetries in adhesion that appear during blastocoel formation and cytoskeletal asymmetries that form during gastrulation. PAR-3 is required for these asymmetries and is itself asymmetrically localized. During the early four-cell stage PAR-3 is present around the entire cortex, but by the end of the four-cell stage PAR-3 disappears from regions of cell contact (basal and lateral surfaces) and becomes restricted to caps centered at the apex of the contact-free (apical) surface. Foci of cortical non-muscle myosin (NMY-2) in early embryonic cells move away from regions of cell contact and become enriched in apical caps similar to those of PAR proteins. We have identified a mutation in *nmy-2* that alters cell adhesion, producing an enlarged blastocoel. When myosin activity is reduced, PAR-3 still becomes restricted to the apical surface but fails to form apical caps. We are currently testing the model that myosin movements condense PAR proteins into an apical cap that regulates adhesion at the opposite, basal surface to control blastocoel size.

199. Secondary Axis Formation by Transplantation of D-Quadrant Micromeres in the *Tubifex* Embryo. Ayaki Nakamoto[1] and Takashi Shimizu[2]. [1]Dept. of MCB, Univ. of Arizona; [2]Division of Biological Sciences, Graduate School of Science, Hokkaido University.

In the embryos which undergo spiral cleavage (e.g., mollusks and annelids), it has been known that one cell at four cell stage, the so-called D quadrant, is important for axis formation. In oligochaete annelids, classic cell ablation experiments showed that D-quadrant micromeres play a

pivotal role in embryonic axis formation: when the second (2d) and fourth (4d) micromeres of the D quadrant were ablated at 24 cell stage, the embryos developed into a ball of endoderm covered with an epithelial sheet of ectoderm (Penners, 1926). So far, however, it has not been demonstrated directly that D-quadrants indeed have potency for axis formation. To address this, we carried out a series of D-quadrant micromere transplantation experiments in the oligochaete annelid *Tubifex tubifex*. Isolated micromeres of the D-quadrant were transplanted to the ventral region of a host embryo from which D-quadrant had been ablated. The reconstituted embryos developed distinct head and tail. This suggests that D-quadrant micromeres (2d and 4d) are sufficient for organizing an anteroposterior axis. Furthermore, we transplanted isolated D-quadrant micromeres to the ventral region of an intact host embryo and found that the reconstituted embryos formed duplicated tails and/or heads. Cell lineage analysis suggests that both the ectoderm and mesoderm of the secondary axis were derived from the transplanted micromeres, while the endodermal tissues along the secondary axis were derived from the host embryo. This confirms that the progeny of the transplanted D quadrant micromeres has the capacity to organize an embryonic axis.

200. Regulating mRNA Distribution in the Early Ilyanassa Embryo. Jessica Wandelt, James Cooley, Michelle LaGioia, Tom Oberg, and Lisa M. Nagy. University of Arizona, Tucson, AZ 85721.

The anterior–posterior axis of the mud snail, *Ilyanassa obsoleta* is determined through a series of asymmetric, determinative cleavages. We know that the mRNAs for several patterning genes are asymmetrically inherited into specific daughter cells through a novel centrosome-mediated localization mechanism. mRNAs to be inherited by the next daughter cell 'load' onto the interphase centrosome of the mother cell. During mitosis, the mRNAs localize to a discrete region of the animal cortex that is solely inherited by the animal daughter cell. We are currently investigating the regulation of centrosome-mediated mRNA sorting during Ilyanassa cleavage. Treatment of embryos with Actinomycin D disrupts the correct localization of mRNAs, suggesting that transcriptional regulation is at least partly involved in the process. To understand the mechanisms involved in the discrete localization of the sorted mRNAs throughout the cell cycle, we are characterizing additional components involved in localization. We have found a commercially available protein kinase c (PKC) antibody that recognizes an antigen(s) whose localization mimics that of 'sorted' mRNAs. We are currently characterizing the target protein, its localization pattern and function.

201. Early Embryonic Patterning in the Amphipod Crustacean, *Parhyale hawaiensis*. Melinda S. Modrell,[1] Alivia L. Price,[2] Roberta Hannibal,[1] and Nipam H. Patel[3]. [1]University of California-Berkeley; [2]University of Chicago; [3]HHMI.

The amphipod crustacean, *Parhyale*, displays total (holoblastic) cleavage during early embryogenesis, and we hope to determine how tightly cell lineage and cell fate are coupled in this species. In *Parhyale*, the first two divisions are radial, holoblastic, and slightly asymmetrical. The third division is highly asymmetric and produces an embryo that consists of four macromeres and four micromeres. Lineage studies performed at the eight cell stage demonstrate that each cell normally contributes to only one of each of the germ layers (Gerberding et al., 2002). However, it is not known if these cells are specified or determined at this stage. Cell ablations are a traditional method for addressing how lineage is coupled to cell fate, and using this approach at the eight cell stage we find that there is regulation, but this only occurs between lineage restricted equivalency groups that give rise to the germline, mesoderm and ectoderm. The molecular mechanisms involved in setting up the asymmetric cell fate pattern in *Parhyale* are unknown, therefore we have taken a candidate gene approach to address this question. Partioning-defective genes (Par) establish cell polarity in a number of cellular contexts in a variety of species, thus representing a highly conserved mechanism for this process. We have cloned a *Parhyale* ortholog of *Par6* and have generated an antibody to detect the corresponding protein to examine its potential role in regulating cell fate decisions. Additional ablation experiments, blastomere isolations, and mis-expression/ knockdown experiments should further reveal the mechanisms behind this process in *Parhyale*.

202. Analysis of *hedgehog* Signaling During Segmentation in *Tribolium casteneum*. Laila Farzana and Susan J. Brown. Kansas State University, Manhattan, KS 66506.

In *Drosophila*, the maintenance of the parasegmental boundaries and formation of segmental grooves depend on the interactions between the segment polarity genes. Comparative expression analysis between Tribolium and *Drosophila* suggests that the roles of wingless and engrailed in segment boundary formation are conserved in both short germ and long germ modes of segmentation. However, there have been no studies of segment polarity genes in the Hedgehog signaling pathway in Tribolium castaneum to confirm this observation. Here, we report the analysis of *hedgehog* (*hh*), *smoothened* (*smo*), *patched* (*ptc*) and *cubitus-interruptus* (*ci*) genes in Tribolium. Our data provide evidence that the expression pattern of these genes is well conserved between *Drosophila* and Tribolium, suggesting their probable role as segment polarity genes in the latter. To investigate the role of these genes during segmentation, we analyzed knockdown phenotypes generated through parental RNAi. Depletion of positive regulators (*hh*, *smo* and *ci*) of the pathway resulted in similar cuticular phenotypes. The most severely effected embryos are small spheres with highly reduced heads and little or no evidence of segmental groove formation. On the other hand, *ptc* RNAi resulted in embryos with enlarged, misshapen head and thoracic appendages. We used Engrailed (En) staining to follow segmentation during RNAi. In the absence of the positive regulators, early embryogenesis and elongation are mostly normal, and defects are detected during germband retraction. Our results indicate that the hedgehog signaling pathway genes play a conserved roll as segment polarity genes in short germ insects such as Tribolium.

203. Identification of a New Variant (TH-B) of Tumorhead, a Cell Proliferation Activator from *Xenopus*. Edwin E. Traverso, Min-Soon Cho, Chuan Fen Wu, and Laurence D. Etkin. University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

Tumorhead (TH) is a gene from *Xenopus laevis* identified as a positive regulator of cell proliferation during the development of the ectodermal germ layer. TH overexpression results in increased cell proliferation within the ectoderm, causing an expansion of the neural plate. Conversely, loss of TH function results in inhibition of proliferation of ectodermal cells. TH's effect on proliferation is associated with differentiation in the nervous system as embryos with altered levels of TH protein do not express neural differentiation markers. To date, the molecular mechanism by which TH affects cell proliferation during embryogenesis is unknown. We have used the yeast two-hybrid system to identify protein partners of TH that could lead us to define the mechanism or pathway through which TH functions. Using this assay we have identified several putative TH interacting proteins. One of these TH potential protein partners is a new variant of TH called TH-B. The sequence for TH-B was found to be 85% identical at the amino acid level to the previously determined TH sequence, namely TH-A. Further characterization of this variant using RT-PCR indicates that it is expressed throughout development until at least the tadpole stage. TH-B was shown to be expressed ubiquitously in embryos at different developmental stages ranging from early cleavage stages until the tailbud stage. The identification of TH-B as a TH-A binding protein suggests that these molecules may function as a multimer during embryogenesis. We are currently studying if TH-B is involved in the regulation of cell proliferation as is TH-A.

204. Analysis of *Sox3* Function in Early Neurogenesis of *Xenopus laevis*. Naoe Harafuji and Elena M. Casey. Georgetown University, Washington, DC 20057.

SoxB genes encode HMG-box transcription factors which are involved in early neural development. In vertebrates, *SoxB1* genes also play a role in the maintenance of neural stem cells. In *Xenopus laevis*, *Sox2* and *3* are expressed in neural ectoderm prior to gastrulation and in the developing brain and neural tube. Sox2 has been demonstrated to expand and induce neural markers, however, the molecular mechanisms, partner proteins, and target genes of both Sox2 and Sox3 during neurogenesis are still unknown. Here we perform a detailed analysis of Sox3 function in *X. laevis* during early neural development.

205. FGF8 Spliceforms Differ in Activity and are Involved in Both Mesoderm and Posterior Neural Formation in *Xenopus laevis*. Russell B. Fletcher and Richard M. Harland. University of California-Berkeley.

In *Xenopus*, early formation and patterning of the neuroectoderm involves the activity of BMP antagonists, Wnts, retinoids, and FGFs. Ultimately, the neuroectoderm will give rise to the architecturally and functionally distinct regions of the nervous system (forebrain, midbrain, hindbrain, spinal cord). Specification of these different domains occurs early in development, and FGF signaling plays an important role. There are two FGF8 spliceforms in *Xenopus laevis*, FGF8a and FGF8b, which yield mature proteins that differ by only an 11 amino acid cassette-yet, FGF8a and FGF8b have very different activities. We demonstrate that FGF8 is necessary for proper mesoderm formation and that FGF8b is the predominant FGF8 spliceform involved in this process. Additionally, FGF8a can act as a caudalizing factor in neural patterning. Using loss of function approaches, we show that both FGF8 spliceforms, and even the individual spliceform FGF8a, are necessary for formation of posterior neural tissue. Alternative splicing is a powerful mechanism for attaining specific responses in developmental patterning.

206. *Xenopus* Gut Macroglobulin-1, a Novel alpha-2 Macroglobulin, is Expressed in a Restricted Dorsal Domain of the Developing Primitive Gut Tube. Liliam L. Pineda Salgado, Eileen Craig, Rebecca Blank, and Daniel S. Kessler. University of Pennsylvania, PA 19104.

Establishment of the functional digestive system is dependent on developmental patterning of the primitive gut, an undifferentiated tube of endoderm that forms with the completion of gastrulation. Using a degenerate PCR strategy, we isolated *Xenopus* Gut Macroglobulin-1 (XGMG-1), a member of the alpha-2 macroglobulin gene family (A2M). XGMG-1 has 56–60% amino acid similarity with *Xenopus*, mouse, rat and human A2Ms, suggesting that it is a new family member. XGMG-1 mRNA is first detected at the end of gastrulation and expression persists through the feeding tadpole stage. XGMG-1 is initially expressed in a broad region of the archenteron roof. With the completion of neurulation, expression is restricted to a narrow dorsal region of the gut endoderm adjacent to the notochord and extending along the entire anterior–posterior axis. In the developing tailbud, XGMG-1 expression extends around the posterior tip of the notochord into the ventral floor plate domain of the posterior neural tube. Analysis of isolated gut tubes during gut coiling stages indicates dorsal expression of XGMG-1, followed by strong expression in the embryonic liver. Taken together, the ability of A2M proteins to bind embryonic inducers and the presence of XGMG-1 in cells adjacent to the notochord suggests that XGMG-1 may modulate the activity of or cellular response to signals secreted by the notochord, thus regulating the dorsal–ventral patterning and/or differentiation of the primitive gut tube. Loss-of-function experiments using morpholino oligos against XGMG-1 are currently being pursued.

207. Notch and Shh Signaling in Dorsal Midline Patterning in *Xenopus laevis*. Sara M. Peyrot,[1] Yassi Hafezi,[1] John B. Wallingford,[2] and Richard M. Harland[1]. [1]University of California, Berkeley, CA 94720; [2]University of Texas, Austin, TX 78712.

The floorplate of the vertebrate neural tube is responsible for dorsoventral patterning of the spinal cord and for commissural axon guidance. While experiments in other model systems have implicated Shh-dependent and Shh-independent mechanisms of floorplate specification, the genesis of the floorplate in amphibians has not been extensively investigated. We show here that Notch signaling plays an interesting role in cell fate decisions in the dorsal midline of *Xenopus laevis*. Notch activation in the midline not only influenced dorsoventral patterning of the neural tube but surprisingly expanded floorplate markers anteriorly, coincident with loss of the ventral midline of the forebrain. These results suggest that Notch signaling may posteriorize cells of the ventral forebrain by activating a floorplate developmental program. However, several markers of the prechordal plate, mesendoderm that underlies the ventral forebrain and may be important for its induction, are also perturbed upon Notch activation in the midline. Since Notch activation can result in Shh upregulation in the midline, we are presently investigating whether the effect of Notch signaling on floorplate and ventral forebrain specification is mediated by Shh signaling. To investigate more thoroughly the relationship between Notch and Shh in midline patterning, we have undertaken a variety of methods to activate and block these pathways, alone and in combination, in *Xenopus* embryos. Though both Shh and Notch play a role in floorplate development in zebrafish, a role for Notch signaling in anteroposterior patterning of the midline is novel.

208. BMP Antagonists are Required in Spemann's Organizer for Dorsal Patterning. Mustafa K. Khokha, Joanna Yeh, Timothy C. Grammer, and Richard M. Harland. UC-Berkeley, Berkeley, CA 94720.

Spemann's Organizer can induce dorsal embryonic cell fates (brain, muscle, and notochord), but the requirement for Organizer signals in normal development has been uncertain. At least five BMP antagonists are specifically expressed in the Organizer, and these can mimic aspects of Organizer function. Here, we use morpholino oligonucleotides to reduce Organizer gene function in *Xenopus tropicalis* and show that reducing the BMP antagonists chordin, noggin, and follistatin results in a catastrophic failure of dorsal development and an expansion of ventral and posterior fates. In addition, we show that signals necessary for neural specification are required during gastrulation and that patterning of the early gastrula mesoderm is dependent on BMP signaling. We conclude that BMP antagonists are the required signal from Spemann's Organizer to specify dorsal fates and are necessary for the formation of the neural plate and dorsal mesoderm. Finally, *X. tropicalis* appears to be an ideal amphibian model system for loss of function studies, and we show that genetic analysis is feasible in this system.

209. *Xenopus* Protein-*O*-Fucosyltransferase 1 is Important for Posterior Patterning. Chang-Yeol Yeo, Hye Lin Lee, and Yeon-Jin Kim. Dept. Life Sciences and Center for Cell Signaling Research, Ewha Womans Univ., Seoul 120-750, Korea.

EGF-CFC factors play essential roles during early vertebrate development, particularly in the regulation of Nodal signaling. EGF-CFC factors act as co-receptors of Nodal-related ligands. Recent study indicates that EGF-CFC factors are *O*-fucosylated and *O*-fucosylation of EGF-CFC factors is

important for their ability to facilitate Nodal signaling. This modification is likely catalyzed by protein-*O*-fucosyltransferases. We isolated a cDNA that encodes putative *Xenopus laevis protein-O-fucosyltransferase 1 (XPOFUT1)*. *XPOFUT1* is expressed maternally and zygotically, and its expression increases until tailbud stages and then gradually decreases. In animal cap assays, overexpression of XPOFUT1 has different effects on Smad2 phosphorylation induced by various activin-like signaling molecules. To analyze the function of *XPOFUT1*, we examined the effects of a morpholino antisense oligonucleotide (MO) against *XPOFUT1*. Injection of *XPOFUT1* MO caused posterior truncation in tadpoles. Our results suggest that *XPOFUT1* may regulate posterior patterning by modulating the function of a subset of activin-like molecules.

210. Maternal XTcf1 and XTcf4 Have Distinct Functions and are Required for Axial Development of the *Xenopus* Embryo. Henrietta J. Standley[1] and Janet Heasman[2]. [1]Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK; [2]Cincinnati Children's Hospital Research Foundation, Cincinnati, OH 45229, USA.

Lymphoid enhancer factor/T-cell factor (Lef/Tcf) transcription factors interact with the Wnt pathway effector β-catenin, which is required for axis formation in the early *Xenopus* embryo. Three XLef/XTcf proteins, XTcf1, XTcf3 and XTcf4, are synthesised maternally and are therefore present in the egg prior to fertilisation. We have used an antisense approach to deplete maternal XTcf1 and XTcf4 from *Xenopus* oocytes in order to study their function. XTcf1-depleted embryos are anteriorised, with a shortened anteroposterior axis and enlarged anterior endoderm. XTcf1-depleted gastrulae show ectopic ventral expression of chordin and other genes ordinarily restricted to the dorsal side of the embryo, suggesting that XTcf1 normally represses these genes in ventral cells. In contrast, XTcf4-depleted embryos are ventralised and show reduced expression of dorsally expressed genes during gastrulation, indicating that XTcf4 functions as a transcriptional activator. Maternal XTcf1 and XTcf4 are therefore both required for normal development, and have different functions distinct from that of maternal XTcf3.

211. Groucho Regulation of Nodal Signaling in the *Xenopus* Embryo. Aaron B. Steiner, Sergey Yaklichkin, and Daniel S. Kessler. University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Nodal ligands, members of the TGF-β family of signaling molecules, are required in the vegetal hemisphere of the *Xenopus* embryo to induce both endoderm and mesoderm, and later are expressed in the Spemann Organizer where they play a vital role in patterning dorsal mesoderm and the anterior–posterior axis (Jones et al., 1995). Inappropriate expression of Nodal in the animal pole of the embryo results in a conversion of ectodermal tissues to dorsal mesoderm (Jones et al., 1995). However, mechanisms by which Nodal signals are excluded from the animal pole remain uncharacterized. While testing the effects of the Groucho family corepressor Grg4 on mesoderm induction, we found that Grg4 can block the ability of the Nodal ligand Xnr1 to induce mesoderm. Grg4 also blocked autoregulation of Nodal ligands by Xnr1 in this assay. Interestingly, injection of Grg5, a naturally occurring dominant negative inhibitor of Grg4, resulted in mesoderm induction in the animal cap, implying that relief of Groucho-dependent repression is sufficient to allow activation of the mesodermal program. Further studies identified an interaction between Grg4 and the forkhead-box family transcription factor FAST-1 (FoxH1), a key component of the Nodal-dependent transcriptional response. Consistent with an interaction between Groucho corepressors and FAST-1, Grg4 and Grg5 inhibited or enhanced transcription from a FAST-1-dependent luciferase reporter, respectively. The data suggest a model in which interaction between FoxH1 and Grg4 helps maintain Nodal genes and their targets in a transcriptionally inactive state in the animal pole.

212. Temporal Analysis of the Early BMP Functions: Distinct Anti-Organizer and Mesoderm Patterning Phases. Abraham Fainsod, Karen Marom, Vered Levy, and Graciela Pillemer. Hebrew University, Jerusalem 91120, Israel.

BMP signaling performs multiple important roles during early embryogenesis. Signaling through the BMP pathway is mediated by different BMP ligands expressed in partially overlapping temporal and spatial patterns. Assignment of different BMP-dependent activities to the individual ligands has relied on the patterns of expression of the various BMP genes. The BMP pathway was activated or blocked at different developmental stages using glucocorticoid-controlled Smad proteins. Early activation of the BMP-specific, Smad1 and Smad5, revealed that BMP-dependent suppression of Spemann's organizer formation in *Xenopus* embryos, can only take place prior to the onset of gastrulation. Blocking BMP signaling with the inhibitory Smad6 results in dorsalized embryos or secondary axis induction, only when activated up-to early gastrula stages. BMP2 efficiently represses organizer-specific transcription from the midblastula transition onwards while BMP4 is unable to prevent the early activation of organizer-specific genes. Manipulation of the BMP pathway during mid/late gastrula affects mesodermal patterning with no external phenotypic effects. These observations suggest that the malformations resulting from inhibition or promotion of organizer formation, ventralized or dorsalized, respectively, are the result of a very early BMP function, through its antagonism of organizer formation. This function is apparently fulfilled by BMP2 and only at its latest phase by BMP4. Subsequently, BMP functions in the patterning of the mesoderm with no apparent phenotypic effects.

213. Microarray Dissection of Spemann–Mangold Organizer Function. Andrew L. Hufton and Julie C. Baker. Stanford University, Stanford, CA 94305, USA.

Studies of the *Xenopus* Spemann–Mangold organizer have laid the foundation for our understanding of the conserved signaling pathways that pattern vertebrate embryos during gastrulation. From these studies, BMP and Wnt inhibition have been implicated as the two major activities of the organizer. To study the genome-wide expression changes that these two activities can induce during gastrulation, we have employed Affymetrix oligonucleotide arrays, thereby providing a new view of the molecular identity of organizer tissues. We used the BMP inhibitor Noggin and the canonical Wnt inhibitor Dkk-1 to induce different aspects of organizer function in gastrulating ventral embryo tissue, and compared their expression to endogenous dorsal and ventral tissue. Five different conditions at both and early gastrula were analyzed with stringent statistical criteria to select genes whose expression was genuinely altered in organizer tissues. After hierarchical clustering of these genes, known gastrula patterning regulators were highly enriched in certain clusters, and these genes were divided into distinct functional sub-groups. We then used these data to predict expression patterns for 39 unknown genes. Of the 24 genes that showed specific patterns by in situ hybridization, 23 fit our predictions, supporting the success of our functional enrichment strategy. Furthermore, we examine the implications of our results for the design of future *Xenopus* microarray experiments.

214. Establishing Frog Genetics and the Identification of Mutants in *Xenopus tropicalis*. Timothy C. Grammer, Mustafa K. Khokha, Maura A. Lane, Kenston Lam, and Richard M. Harland. [1]Molecular and Cell Biology, UC, Berkeley; [2]Center for Integrated Genomics, UC, Berkeley; [3]Department of Pediatrics, UCSF School of Medicine.

Xenopus tropicalis offers the potential for genetic analysis in an amphibian. In order to take full advantage of this potential, we have been developing frog genetic resources, including inbreeding strains of frogs for ongoing forward and reverse genetic screens. While inbreeding a

population of Nigerian frogs, we identified three mutations in the genetic background of this strain. These mutations are all recessive embryonic lethals. We show that multigenerational mutant analysis is feasible and demonstrate that mutations can be identified, propagated, and readily characterized using hybrid, dihybrid, and even trihybrid crosses. In addition, we are optimizing conditions to raise frogs rapidly and present our protocols for *X. tropicalis* husbandry. We find that males mature faster than females (currently 3 months versus 6 months to sexual maturity). Here we document our progress in developing *Xenopus tropicalis* as a genetic model organism and demonstrate the utility of the frog to study the genetics of early vertebrate development.

215. Ecsox17 Expression in the Early Embryo of a Direct Developing Frog. Srikanth Singamsetty, Sean R. Williamson, and Richard P. Elinson. Duquesne University, Pittsburgh, PA 15282.

The transcription factor *Xsox17*, expressed throughout the cells in vegetal region of the model frog *Xenopus laevis*, is necessary for the development of the definitive gut. In *Eleutherodactylus coqui*, a direct developing frog with 3.5 mm egg, the yolky vegetal cells get internalized during gastrulation. Some of these nutrient-rich cells might not contribute to the definitive gut. To test this, we cloned the *E. coqui* ortholog of *Xsox17*, *Ecsox17*. The sequence codes for a 380aa protein that shows an overall identity of 73% and an identity of 82% to the HMG domain of *Xsox17α1*. *Ecsox17* shows 53% and 43% nucleotide identity to *Xsox17α2* and *Xsox17β*, respectively. RT-PCR indicates that *Ecsox17* expression starts at gastrula stage (TS2), remains high until the tail bud stage (TS5) and continues at lower levels until hatching of the froglet (TS15). Whole mount in situ hybridization of the early gastrula shows the expression of *Ecsox17* animal to the dorsal lip. In addition, expression extends laterally preceding lateral lip formation. At mid-gastrula, a stage when the lip surrounds the embryo completely, the entire blastoporal lip is stained, and this lip staining persists until the end of gastrulation. At no stage of gastrulation do the yolk-rich vegetal cells show staining in *E. coqui*. In contrast, both *Xsox17α* and *Xsox17β* are expressed throughout the vegetal region of the *X. laevis* gastrula. The lack of *Ecsox17* expression in the large yolk-rich cells suggests their function to be in nutrition rather than in gut formation. Future experiments will include section in situ and RT-PCR on dissected early embryos.

216. Zebrafish KLF4 is Essential for Hatching and Haematopoiesis. Melissa R. Gardiner,[1] Dave F. Daggett,[2] Sharon L. Amacher,[2] Peter D. Currie,[3] Leonard I. Zon,[4] and Andrew C. Perkins[1]. [1]Institute for Molecular Biosciences, Queensland University, Australia; [2]Department of Molecular and Cellular Biology, University of California, Berkeley, California; [3]Victor Chang Cardiac Research Institute, Sydney, Australia; [4]Children's Hospital, Harvard Medical School, Boston.

Gene knockout studies of Krüppel-like factors (KLFs) in mice have shown essential roles in organogenesis. Little is known of the genetic and biochemical pathways involving KLFs. A screen for KLF family members in zebrafish identified many KLFs. One of these, zKLF4, the homologue of *neptune*, a *Xenopus laevis* KLF, is one of the earliest markers of lateral plate mesoderm, marking the earliest appearance of blood and vascular tissue. zKLF4 is initially expressed in ectodermal cells fated to become the polster (which gives rise to the hatching gland) but from 20 hpf is restricted to the intermediate cell mass (ICM), the site of embryonic haematopoiesis. Using morpholinos to knockdown expression of zKLF4, we demonstrate that it plays essential roles in differentiation of mesodermal cells into hatching gland and embryonic red blood cells. From as early as 80% epiboly, we see a complete absence of expression of the hatching gland gene (hgg1) in zKLF4 morphants. Interestingly, zKLF4 itself is still expressed in the polster, indicating that the polster as a distinct tissue is specified correctly, but terminal differentiation into the hatching gland is defective. xKLF4 studies suggest a role in red blood cell development by regulation of GATA-1 expression. Our zKLF4 morphants show a dramatic down-regulation of GATA-1 and embryonic globin expression indicating a critical role for zKLF4 in haematopoiesis.

217. Novel Dvl2 Interacting Protein Crip2 Regulates Notochord Cell Intercalation of Zebrafish Embryo. Yasuyuki Kida, Takayuki Sato, and Toshihiko Ogura. IDAC, University of Tohoku, Japan.

The Wnt/PCP signaling pathway plays crucial roles in vertebrate development. Among several molecules identified to play important roles in this pathway, Dvl2 is one of essential components regulating convergent and extension (CE) movement. Here, we report that zebrafish Crip2, which is expressed strongly in developing notochord and heart, interacts directly with Dvl2 to regulate CE movement. We used antisense morpholino oligonucleotides to knockdown Crip2 activity. In such Crip2-knockdown embryos, CE movement of notochord cells was severely impaired, resulting in short body length with additional abnormalities in heart. Abrogation of Crip2 activity enhanced phenotypes induced by both Dvl2- and Daam1-knockdown embryos. This indicates that Crip2 is a component of the Dvl2/Daam1 signal transduction cascade. Based on these observations, we analyzed molecular interactions between Dvl2/Daam1 and Crip2, and found that Crip2/Dvl2/Daam1 form a ternary complex, which is an essential component of cell intercalation of notochord and control of morphogenetic cell movement during zebrafish gastrulation.

218. Morpholino Knockdown Uncovers a Requirement for FoxH1/Schmalspur for Normal Morphogenesis and Survival During Gastrulation. Wuhong Pei and Benjamin Feldman. Medical Genetics Branch, National Human Genome Research Institute, Bethesda, MD 20892.

We have reassessed the function of zebrafish FoxH1/Schmalspur using antisense morpholinos (MOs). FoxH1 is a co-transcription factor recruited by activated Smad2/Smad4 and Smad3/Smad4 downstream of several TGFβs, including Nodals. FoxH1's transcriptional targets include agonists (i.e., Nodals) and antagonists (i.e., Lefties) of Nodal signaling. We find injecting either of two non-overlapping MOs blocking translation of *foxH1* causes a developmental delay and a block in epiboly followed by lethal rupture of the yolk cell. This phenotype is more severe than – and qualitatively different from – that of embryos maternally and zygotically homozygous for the sur^{m768} mutation. Associated with the developmental delay, FoxH1 depletion compromises cell proliferation but not cell viability. The delayed development of FoxH1-depleted embryos is also measurable as an increase in cell size and a retarded increase in the G1 cell fraction. By monitoring movements of photo-labeled cells, we have further observed that FoxH1 depletion disrupts internalization, a key gastrulation movement. Perturbations in Nodal signaling fail to modulate the FoxH1-depleted phenotype, indicating that FoxH1 functions independently of Nodal signaling. To identify downstream targets of FoxH1, we performed microarray experiments on RNA from early- and later-stage FoxH1-depleted embryos, revealing 14 diverse down-regulated targets in early stage embryos and 21 down-regulated targets in later-stage embryos. Our ongoing research to validate candidate target genes, including epistasis studies, will be presented.

219. FoxD3 Regulation of Mesoderm Induction in the Zebrafish Embryo. Lisa L. Chang and Daniel S. Kessler. University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Nodal ligands, members of the TGFβ superfamily, are required for germ layer induction and patterning in the vertebrate embryo. During gastrulation of the zebrafish, the expression domain of the Nodal-related genes, Cyclops and Squint, overlaps that of FoxD3 in the shield, suggesting a possible role

for FoxD3 in mesodermal development. Overexpression of FoxD3, a member of the forkhead class of transcription factors, resulted in expansion of dorsal mesoderm during shield stage as shown by ectopic expression of No tail, Goosecoid and Chordin. Dorsally derived tissues were also affected by FoxD3 overexpression during early somitogenesis. We found expanded head and trunk tissues and, in some cases, duplication of the body axis. Knockdown of FoxD3 resulted in ventralized embryos, a phenotype shared with Nodal pathway mutants (Cyc; Sqt and MZoep mutants). Ongoing experiments will determine which tissues are affected by FoxD3 knockdown, as well as assess Nodal expression after overexpression or knockdown of FoxD3. Finally, we will employ zebrafish Nodal pathway mutants to determine the functional interaction of FoxD3 with the Nodal pathway. Results so far suggest that dorsal mesoderm induction is regulated, at least in part, by FoxD3. We hypothesize that FoxD3 is regulating Nodal expression in the zebrafish shield by inhibiting a Nodal repressor, therefore indirectly inducing Nodal expression and mesoderm development.

220. Twisted Gastrulation Promotes BMP Signaling in Zebrafish DV Axial Patterning. Shawn C. Little and Mary C. Mullins. University of Pennsylvania, Philadelphia, PA 19104.

In vertebrates and invertebrates, the Bone Morphogenetic Protein (BMP) signaling pathway patterns cell fates along the dorsoventral (DV) axis. In vertebrates, BMP signaling specifies ventral cell fates, whereas restriction of BMP signaling by extracellular antagonists allows specification of dorsal fates. One such antagonist is Chordin, which is itself regulated by Tolloid, a metalloprotease that cleaves Chordin into fragments with reduced anti-BMP activity. The conserved extracellular factor Twisted gastrulation (Tsg) can either promote or antagonize BMP signaling when overexpressed at different levels in *Xenopus* embryos. A model of Tsg function proposes that the activity of Tsg depends on the status of Chordin: in the presence of full length Chordin, Tsg acts to antagonize BMP signaling, whereas Tsg can promote BMP signaling by inactivating Chordin fragments produced by Tolloid. To investigate the role of Tsg in early DV patterning, we performed morpholino (MO) knockdown studies of *tsg1* in zebrafish. We found that loss of *tsg1* results in a moderate dorsalization of the embryonic axis, suggesting that Tsg1 promotes ventral cell fates. Knockdown of *tsg1* combined with mutations in *tolloid* or *bmp2b* enhanced dorsalization, supporting a role for Tsg1 in specifying ventral cell fates as a BMP agonist. Moreover, loss of *tsg1* partially suppressed the ventralized phenotypes of *chordin* and *ogon*. Our results support a model in which endogenous Tsg1 promotes BMP signaling, and thus ventral cell fates, during DV axial patterning by a mechanism that does not rely exclusively on the presence of Chordin or Tolloid-generated Chordin fragments.

221. Temporal Progression of BMP Signaling in Dorsoventral Patterning of the Early Zebrafish Embryo. Jennifer A. Tucker and Mary C. Mullins. Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

A bone morphogenetic protein (BMP) signaling pathway patterns the dorsoventral axis of the vertebrate embryo. Ventral regions exposed to the highest BMP activity levels contribute to ventral-posterior structures of the tail, whereas dorsal regions exposed to the lowest levels form neural ectodermal tissues. This BMP activity gradient may function as a morphogen, with different positions within the gradient determining distinct cell fates. Such models imply that cell fates along the DV axis are determined simultaneously. However, examination of zebrafish dorsalized BMP pathway mutants suggests that BMPs act progressively over time to pattern DV tissues in the head, trunk, and tail. To examine the temporal activity of the BMP gradient, we generated a heat-shock inducible transgenic line expressing the BMP antagonist Chordin. We inhibited BMP activity at various timepoints prior to, during, and after gastrulation, and examined the extent of dorsalization of head, trunk, and tail tissues. In complementary studies with a heat-shock inducible BMP type I receptor (*alk8*) transgene, we examined the temporal requirement of this receptor to rescue the *laf/ alk8* dorsalized mutant. Our data suggest that there are at least two time periods of BMP activity: gastrula stages to pattern head and trunk, and early somitogenesis to pattern the tail. Furthermore, we have evidence that BMP signaling acts progressively during gastrulation in patterning head and trunk tissues, supporting a model in which BMP activity acts progressively to specify cell types along the DV axis.

222. BMP Patterning Activities can be Defined in Zebrafish According to their Dependence on Wnt8. Arne C. Lekven, Marie-Christine Ramel, Gerri R. Buckles, and Kevin D. Baker. Texas A&M University, College Station, TX 77843.

Many vertebrate mesoderm fates are established according to position in the dorsoventral (D/V) axis. While much attention has focused on the role of BMP signaling in D/V patterning of the vertebrate mesoderm, much less is understood regarding the role of Wnt8 and its epistatic relationship to BMP signaling in this process. In zebrafish, BMP-dependent D/V patterning is mediated by several different ligands and downstream effectors. We have examined the epistatic relationship of Wnt8 and BMP signaling pathways in patterning the mesoderm through a combination of loss of function and overexpression assays in zebrafish. We have found that, while BMP signaling is not necessary for wnt8 expression, Wnt8 signaling is necessary for the maintenance of bmp2b expression in the embryonic margin. This suggests that Wnt8 may lie upstream of BMP2b activity in patterning the mesoderm. In agreement with this hypothesis, we find that ectopic BMP2b, Alk8, the type I receptor that mediates BMP2b function, and Smad1, the mediator of late zygotic BMP signaling, are all able to ventralize wnt8 mutants while the maternal BMP ligand Radar and the maternal Smad5 are not. Thus, Wnt8-dependence distinguishes BMP activities that perform different functions during zebrafish gastrulation.

223. Dlx3b/4b Act to Prolong Otic Competence by Restricting BMP Signals from the Neural Plate. Robert Esterberg and Andreas Fritz. Emory University, Atlanta, GA 30322.

Otic placodes develop from an ectodermal thickening that lies adjacent to the neural plate. Members of the Dlx family of transcription factors, *dlx3b/4b*, are expressed throughout the nonneural ectoderm bordering the neural plate beginning in midgastrulation. Their expression is progressively restricted during somitogenesis to the otic and olfactory placodes. This expression pattern suggests a role in providing competence to the otic placode. Previous studies have demonstrated that Fgf3/8 are required for proper ear development. We show that loss of Dlx3b/4b blocks ear development by reducing expression of the four Fgf receptors throughout the embryo. This widespread loss of Fgf receptor expression is caused by a transient misexpression of *bmp2b/4/7* that returns to normal during mid to late somitogenesis. Overexpression of *bmp2b/4* or loss of function of *chordin* is sufficient to reduce Fgf receptor expression and provide a similar pattern of expression to *dlx3b/4b* compromised embryos. Our results provide evidence that *dlx3b/4b* function to provide extended competence to otic cells, and restrict BMPs from dorsal structures that would normally adopt a neural fate.

224. The Role of Insulin-Like Growth Factor-2 (IGF-2) in Zebrafish Embryonic Development. Karena McCarthy,[1] Catherine Glynn,[1] Lori Hartnett,[1] Catherine Nolan,[2] and Lucy Byrnes[1]. [1]National University of Ireland, Galway, Ireland; [2]University College Dublin, Ireland.

The IGF system is essential for normal embryonic growth and development. A novel role for the IGF system in neural induction has been described

in *Xenopus* where IGF-2 favours neural induction by inhibiting BMP signaling[1]. We have shown a functional role for the IGF system in dorso-anterior embryonic development in zebrafish[2]. Here, we examine the role of IGF-2 in zebrafish embryonic development by injecting *IGF-2* mRNA into embryos at the 1 cell stage. Over-expression of *IGF-2* resulted in a dorsalised phenotype with expansion of anterior structures at the expense of trunk and tail structures. Somites lost their characteristic V-shape appearing as flattened discs. Several embryos had a single eye-field. Embryos also displayed lack of circulating blood, disrupted heart formation and pericardial oedema. In situ hybridization of *IGF-2*-injected embryos revealed a disruption of eye marker, *rx3*, and an expansion of neural marker *otx2*, with rhombomere marker *krox20* unaffected. Expression of the shield marker, *chordin* was increased ventrally and *goosecoid* was expanded ventrally but reduced dorsally. The somite marker, *her1*, displayed a weak, asymmetrical pattern and *myoD* expression had broadened but was reduced along the anterior–posterior axis. We conclude that IGF-2 is involved in dorso-anterior development in zebrafish. Altered expression of shield markers suggests that IGF-2 also plays a role in the establishment (and/or) function of the embryonic shield. [1]Pera et al., 2001, Dev. Cell 1:655–665. [2]Eivers et al., 2004, Int. J. Dev.Biol. 48: 1131–1140. Funds: IRCSET & Enterprise Ireland.

225. *Zic2* Function in the Embryonic Zebrafish Forebrain. Nicholas A. Sanek,[1] Molly K. Nyholm,[2] and Yevgenya Grinblat[2]. [1]Genetics Training Program; [2]Departments of Anatomy and Zoology; [3]University of Wisconsin, Madison.

Our laboratory studies the roles of the zic family of zinc finger transcription factors in the developing zebrafish. *Zic2* mutations in humans lead to holoprosencephaly (HPE), a common group of birth defects typified by aberrant forebrain development. Mechanisms underlying HPE are not well understood; therefore, we chose to dissect *zic2* function in the zebrafish forebrain. Expression of *zic2* in the zebrafish forebrain is remarkably similar to that in higher vertebrates and includes the dorsal telencephalon, thalamus, and optic stalk. We are asking if zebrafish *zic2* plays a role in regionalization, neurogenesis, and cell cycle control using anti-sense morpholinos. *Zic2* seems to be particularly important for correct expression of *dlx2* (a marker of dopaminergic neuronal precursors in the ventral thalamus) and *isl1* (a marker of primary neurons derived from the *dlx2* domain). To better define the morphant defect, neurogenesis is being characterized using both immunohistochemistry and live analysis in transgenic lines. Cell cycle control is being addressed by examining cell proliferation (pH3 and BRDU) and cell death (TUNEL). A new direction of our research addresses potential functional interactions between Zics and shh pathway components, which are strongly implicated in HPE. In summary, *zic2* appears to be critical for correct regional patterning and neurogenesis in the zebrafish diencephalon. These data will lend new insights into genetic pathways controlling morphogenesis and neuronal differentiation in the forebrain, and may contribute to a zebrafish model of HPE.

226. Characterization of zic1 Expression in Early Chick Development. Lisa Sun Rhodes, Robert VanTreese, and Christa Merzdorf. Montana State University, Bozeman.

Zic transcription factors are regulators of early events in neural and neural crest development. Zic family members regulate transcription of neural and neural crest-specific genes and are expressed in the cells of the dorsal neural tube and the premigratory neural crest. In order to study whether Zic genes play a role in neural crest migration, we have begun to characterize zic1 expression in early chick development. During neurulation, in situ hybridization showed zic1 expression in the neural folds and in the dorsal neural tube. Shortly after the somites epitheliarized, zic1 was expressed in the dorsomedial tips of the somites. Later in somite development, zic1 was expressed in the medial dermomyotome and in the dorsal part of the sclerotome. Throughout these stages, zic1 staining was more intense in the posterior regions of the somites than in the anterior parts. As the dorsal root ganglia (DRG) formed, zic1 was expressed at high levels in the sclerotome surrounding the DRG. zic1 in situ hybridization combined with immunofluorescence for the migratory neural crest epitope HNK-1 showed that cells expressing zic1 did not show HNK-1 immunofluorescence, suggesting that neural crest cells do not express zic1 at any stage in their migration. Similarly, condensing DRG were labeled with HNK-1 but not with zic1. Double immunofluorescence with an antibody against Zic proteins combined with the HNK-1 antibody confirmed that migratory neural crest cells do not express the zic1 gene. These data suggest that zic1 plays a role in somite development and we are currently defining zic1 expression in the dermomyotome in more detail using somite-specific markers.

227. Defining Organ-Position Along the Main Axis of the Gut. Elke Bayha and Anne Grapin-Botton. ISREC, Chemin des Boveresses 155, CH-1066 Epalinges s/Lausanne, Switzerland.

During embryonic development, the epithelium of the gut and other organs derive from the endoderm. Our earlier studies have shown that the lateral plate mesoderm patterns the endoderm in a posterior-dominant fashion analogous to the patterning of the neural tube (Kumar, 2003). Four different classes of molecules have been implicated in regionalizing the nervous system: retinoic acid (RA), Fgfs, Wnts, and BMPs. Like Fgfs and Wnts, RA is considered to be a posteriorizing factor most represented in posterior regions. We are investigating how the RA signaling pathway controls endoderm patterning in vivo using predominantly the chick model system. By adding exogenous RA, we show that RA signaling affects the branchial arch region but does not affect more posterior areas during somitogenesis. During gastrulation, our preliminary results suggest that RA targets a broader domain from the head to the trunk as reported by others in zebrafish and *Xenopus* (Stafford, 2002; Chen, 2004; Stafford, 2004). The time frame and location of RA pathway activity along the AP axis is similar to its activity in the nervous system. Pathway inhibition by RA antagonists is being performed to address where along the axis and when the pathway is required. In addition, our preliminary experiments using dominant-active and negative retinoic acid receptors in *Xenopus* suggest that RA acts directly in the endoderm. In future, we will test how Fgfs cooperate with the RA pathway during gastrulation, when their functions overlap largely. Nodes of interaction have already been reported in the neurectoderm.

228. FGF-Signaling is Necessary for Establishing Gut Tube Domains Along the Anterior-Posterior Axis in Vivo. James M. Wells,[1] Jessica Dessimoz,[2] Robert Opoka,[1] Jennifer Kordich,[1] Aaron M. Zorn,[1] and Anne Grapin-Botton[2]. [1]Cincinnati Children's Hospital Research Foundation, OH, USA; [2]ISREC, Lausanne, Switzerland.

Within 2 days of gastrulation, the endoderm germ layer is transformed from unspecified group of cells into a patterned gut tube. Here, we report that FGF4-mediated signaling is required for establishing gut tube domains along the A–P axis in vivo, a process that is evolutionarily conserved in vertebrates. At gastrulation stages in *Xenopus*, chick and mouse, FGF4 is expressed in the posterior mesoderm adjacent to the presumptive midgut and hindgut endoderm. When anterior endoderm is exposed to recombinant FGF4 protein, midgut and hindgut domain markers are shifted anteriorly and anterior endoderm gene expression and foregut morphogenesis are inhibited. FGF-mediated endoderm patterning is in a cell autonomous manner and not via a secondary induction from mesoderm. Loss-of-function studies demonstrate that FGF-signaling is necessary for the establishment and maintenance of midgut and hindgut gene expression boundaries and for repressing foregut gene expression. We have evidence that FGF4 patterns endoderm by 2 mechanisms; by direct specification of cell fate and by acting as a chemoattractant to regulate endoderm cell migration along the A–P axis. Lastly, we have identified an endoderm-specific, FGF4-responsive gene encoding FGF binding protein 1 (FGFbp1)

that binds to and enhances FGF4 signaling activity. Two possible mechanisms by which FGFbp1 regulates FGF4 activity during patterning, enhancing FGF4 diffusion and signaling range or by regulating ligand receptor interactions, will be discussed.

229. Cloning and Characterization of a New Chick Hairy/Enhancer of Split Homologue. Raquel P. Andrade, Sara Santos, and Isabel Palmeirim. Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal.

Throughout the Animal Kingdom, embryonic development undergoes strict spatial-temporal control. Each step of the process must occur at the right time and place. Time control is particularly crucial during embryo segmentation processes, where the number of generated segments, as well as the time of formation of each segment, is extraordinarily constant and specific for each species. Somitogenesis is the process through which the vertebrate presomitic mesoderm is segmented along its anterior–posterior axis into round-shaped masses of epithelial cells, named somites. In the chick embryo, a new pair of somites is formed every 90 min. This clock-like precision is dictated by the somitogenesis molecular clock. The first molecular evidence for an intrinsic clock operating in the avian presomitic cells was obtained in 1997, with the discovery of the dynamic expression pattern of the chick hairy1 gene (Palmeirim et al., 1997, Cell 91:639–648). Several other genes have been found to exhibit cyclic gene expression in vertebrate presomitic mesoderms, presenting an oscillation period corresponding to the time required to form a pair of somites (Rida et al., 2004, Dev. Biol. 265:2–22). Similarly to hairy1, many of these genes belong to the Hairy/Enhancer of split family of transcription factors. We have cloned a new chick Hairy/Enhancer of split homologue and characterized its expression pattern throughout embryonic development. The involvement of this gene in somitogenesis was evaluated and will be discussed.

230. Temporal Control of Limb Bud Development: Evidence of a Molecular Clock. Susana Pascoal,[1] Claudia Carvalho,[2] and Isabel Palmeirim[1]. [1]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; [2]Centro de Biologia do Desenvolvimento, Instituto Gulbenkian de Ciõncia, Oeiras, Portugal.

The early chick limb bud grows out as a tongue-shaped mass consisting of mesenchyme enveloped in ectoderm. It has been postulated that limb mesenchymal cells are assigned positional values in a three-dimensional coordinate system, depending on the time cells spend in a distal undifferentiated (progress) zone. How cells measure time, however, remains unknown. We analyzed the expression pattern of the hairy2 gene (known to be a part of the molecular clock controlling somitogenesis) during limb bud development. We experimentally identified a dynamic and cyclic temporal variation in the expression of hairy2 at the level of the progress zone (proximal–distal variation). This work clearly establishes hairy2 cyclic expression periodicity and its functional relevance in limb bud development. Our results suggest for the first time that the molecular clock described for somitogenesis is also providing positional information to limb cells.

231. Terra is a LeftRight Asymmetry Gene Crucial to Maintaining Bilateral Symmetry of the Segmentation Clock. Saúde Leonor,[1] Raquel Lourenço,[1] Alexandre Gonçalves,[1] and Isabel Palmeirim[2]. [1]Centro de Biologia do Desenvolvimento, Instituto Gulbenkian de Ciência, Oeiras, Portugal; [2]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal.

The vertebrate body is not symmetric and the distinction between the right and the left sides of vertebrates is regulated by the so-called left–right asymmetry pathway. However, many vertebrate body structures are essentially symmetric. Somites are transient embryonic structures bilaterally located on both sides of the axial organs. These give rise to symmetrical body structures, such as vertebrae, ribs and skeletal muscles. The formation of somites is linked to an evolutionarily conserved molecular clock which presents a strict bilateral symmetry. We identified the chick terra gene that encodes a protein with a zinc finger-like DNA binding motif and studied its pattern of expression. Terra presents a left-sided expression pattern at very early developmental stages, reminiscent of left–right asymmetric genes. Additionally, terra is expressed in the anterior presomitic mesoderm and in differentiating somites. This work describes a novel left–right asymmetry gene that, when knocked-down, perturbed both embryo left–right patterning and the bilateral symmetry of the segmentation clock. These results clearly demonstrate that there is a molecular link between these two processes and identifies terra as a gene that plays a key role in their intersection.

232. A Microarray-Based Approach of Somitogenesis. Mary-Lee Dequeant, Earl Glynn, Matthias Wahl, Jie Chen, Galina Glazko, Arcady Mushegian, and Olivier Pourquie. Stowers Institute For Medical Research.

The vertebrate body plan is characterized by a segmented organization which is first established during embryogenesis through somitogenesis. This process is associated with a molecular oscillator, the segmentation clock, whose periodicity matches that of somitogenesis process. Molecular evidence for the existence of this clock in species ranging from zebrafish to chick and mouse has been obtained on the basis of a periodic expression in the presomitic mesoderm (PSM) of several genes, called cyclic genes, which are linked to the Notch and Wnt pathways. We have undertaken a microarray approach to identify new cyclic genes by analyzing transcriptional profiles of mouse caudal PSM undergoing the segmentation clock oscillations. Due to the limiting amount of starting RNA (50 ng), our protocol included two rounds of amplification controlled for its reproducibility and quality. We performed a Fourier analysis to identify periodic profiles: as a validation of the method, known cyclic genes ranked among the most significant periodic genes detected. By hierarchically clustering the expression profiles, we identified three clusters: the first one contained cyclic genes related to Notch signaling, the second one contained cyclic genes related to Wnt signaling, the third cluster was composed of genes not reported so far as cyclic. The most significant candidate cyclic genes are being validated by in situ hybridization. Further characterization by Gene Ontology will potentially link other signaling pathways to the process and therefore provide a more comprehensive view of the molecular network underlying the Segmentation clock regulation.

233. *ichabod* is Required for Forebrain Formation in the Mouse Embryo. Xin Zhou[1] and Kathryn Anderson[2]. [1]Weill Graduate School of Medical Sciences, Cornell University, 445 E69th Street New York, NY 10021; [2]Memorial Sloan Kettering Cancer Institute, 1275 York Avenue, New York, NY 10021.

To identify novel genes that control mammalian embryonic development, we have been undertaking a systematic ENU mutagenesis screen for recessive mutations that alter development of the mouse embryo. Here, we describe one mutant identified in the screen, *ichabod*. The *ichabod* homozygous mutants exhibit severe forebrain truncation and they die at approximately e11.5. The mutation has been mapped to a 2.1-Mb interval on chromosome 5. Although the mutants have less brain tissue than wild type starting from head fold stage, the anterior–posterior neural axis is properly patterned as shown by the presence of forebrain and midbrain markers like *Bf1*, *En2* and *Fgf8*. The initiation of the forebrain is also normal as shown by early forebrain markers *Six3* and *Hesx1*. We have

observed neither substantial increase in cell apoptosis nor significant reduction in cell proliferation until after e9.5, suggesting that abnormal apoptosis or proliferation rate is not the primary cause for the forebrain truncation phenotype. As the formation of head region of mouse embryos depends on signals from the anterior visceral endoderm (AVE), the axial mesendoderm and the anterior definitive endoderm (ADE), we have examined marker gene expression of these embryonic structures. Our current data suggest that AVE and ADE are properly patterned in *ichabod* mutants while there may be a defect in the axial mesendoderm. We are currently investigating the role of the axial mesendoderm in head formation of *ichabod* mutants.

234. Genetic Dissection of Midbrain and Anterior Hindbrain Development. Mark Zervas and Alexandra Joyner. Developmental Genetics Program, Skirball Institute, NYU School of Medicine, New York, NY 10016.

The midbrain (Mb) and anterior hindbrain (aHb) arise from the mesencephalon (mes) and rhombomere1 (r1), respectively. *Otx2* and *Wnt1* are expressed in the mes, *Gbx2* and *Fgf8* in r1, while *En1* encompasses both regions. The isthmic organizer (IsO), located between mes and r1, patterns adjacent mes/r1 through Fgf8. Collectively, these events result in an intricately patterned aHb that is adjacent to, but segregated from the Mb. Using $Wnt1^{sw/sw}$ (*Wnt1* point mutation) and *Gbx2* conditional knockout (cko) mice we are investigating the cascade of events underpinning Mb/aHb development. *Wnt1*, *Otx2*, *Hoxa2a* are induced correctly at E8.5 in $Wnt1^{sw/sw}$ mice, but by E9.5 clusters of *Otx2* and *Wnt1* expressing cells are ectopically located in r1 while *Gbx2*, *Fgf8*, and *En1* are downregulated. Adult $Wnt1^{sw/sw}$ mice have a loss of posterior Mb, ventral Mb/aHb nuclei, and a cerebellum (Cb) that apparently consists of only hemispheres (lateral Cb). $Wnt1^{sw/+}$ mice also display a subtle phenotype. In contrast, *Gbx2* cko mice do not have a ventral phenotype, but do display an aberrant Cb. Genetic inducible fate mapping of mes-derived cells shows that lineage restriction is disrupted in $Wnt1^{sw/+}$ mice resulting in mes/r1 cell intermingling and altered mes/r1 gene expression. Fate mapping the Cb hemispheres on $Wnt1^{sw/sw}$ mice suggests that loss of the vermis is not because of a transformation of vermis (medial tissue) to hemisphere. Collectively, these data suggest that while *Gbx2* is a dorsal r1 determinant, *Wnt1* maintains the posterior mes lineage restriction boundary that positions and maintains the IsO, which patterns and refines the Mb/aHb in a temporal manner.

235. Shifting Boundaries of Retinoic Acid Activity Control Hindbrain Segmental Gene Expression. Ioan Ovidiu Sirbu,[1] Lionel Gresh,[2] Jacqueline Barra,[2] and Gregg Duester[1]. [1]The Burnham Institute, La Jolla, CA 92037, USA; [2]Institute Pasteur, Paris, France.

Retinoic acid (RA) generated by Raldh2 in paraxial mesoderm is required for specification of the posterior hindbrain including restriction of Hoxb1 expression to presumptive rhombomere 4 (r4). Hoxb1 expression requires 3′ and 5′ RA response elements for widespread induction up to r4 and for r3/r5 repression, but RA has previously been detected only from r5–r8, and vHnf1 is required for repression of Hoxb1 posterior to r4 in zebrafish. Here, we demonstrate in mouse embryos that an RA signal initially travels from the paraxial mesoderm to r3 forming a boundary next to the r2 expression domain of Cyp26a1 encoding an RA-degrading enzyme. After Hoxb1 induction, the RA boundary quickly shifts to r4/r5 coincident with induction of Cyp26c1 in r4. A functional role for Cyp26c1 in RA degradation was established through examination of RA-treated embryos. Analysis of Raldh2−/− and vHnf1−/− embryos supports a direct role for RA in Hoxb1 induction up to r4 and repression in r3/r5, as well as an indirect role for RA in Hoxb1 repression posterior to r4 via RA induction of vHnf1 up to the r4/r5 boundary. Our findings suggest that Raldh2 and Cyp26 generate shifting boundaries of RA activity, such that r3–r4 receives a short pulse of RA and r5–r8 receives a long pulse of RA. These two pulses of RA activity function to establish expression of Hoxb1 and vHnf1 on opposite sides of the r4/r5 boundary.

236. The Role of BMP Antagonism by the Organizer in Mammalian Neural Induction. Yu-Ping Yang and John Klingensmith. Department of Cell Biology, Duke University Medical Center, Durham, NC 27710.

The Spemann organizer in amphibians gives rise to axial mesendodermal structures (AME) and is capable of inducing neural tissues; this is thought to occur largely via the antagonism of Bone Morphogenetic Protein (BMP) signaling by the organizer. We are examining the role of BMP signaling and the mouse organizer in mammalian neural induction. We find that forebrain specification and maintenance are promoted by signals from AME, where the BMP antagonists are expressed. Ectopic BMP represses forebrain gene expression in explants, while wild-type anterior AME is capable of relieving this repression if cultured together. Surprisingly, mouse *Foxa2* mutant embryos lack an organizer, yet still undergo neural induction. This neural patterning, though without normal forebrain formation, occurs in the absence of axial expression of organizing factors, such as the BMP antagonists Chordin and Noggin. We found that the onset of forebrain gene expression is not detected in most *Foxa2* mutants. Several genes expressed in anterior visceral endoderm (AVE), also thought to promote forebrain development, are not expressed or abnormally localized in mutant embryos. Further, BMP signaling is found ectopically elevated in the distal tip of E7.25 *Foxa2* mutants, in all germ layers in the vicinity of the prospective forebrain, and later in the anterior neural epithelium. We conclude that the forebrain truncation phenotype in *Foxa2* mutants is likely due to inhibitory effects of increased BMP signaling on forebrain gene expression. Collectively, our results suggest that BMP signaling inhibits both the induction and maintenance of forebrain development in mouse embryos.

237. Neural Tube Patterning and the Role of BMP2. Trisha Castranio and Yuji Mishina. Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709.

Neural tube defects continue to perplex the scientific community. The developing neural tube requires gene expression that supports dorsal–ventral patterning within the neural tube as well as proliferation and differentiation. BMPs have been shown to play a role in neural tube development particularly as dorsalizing factors. BMP2, -4, -7 expression patterns in the surface ectoderm near the dorsal portion of the neural tube is well established. Since Zhang, et al. reported that BMP2 homozygous null embryos die before neurulation begins, we explored the possibility that Bmp2 could then play a role in the developing neural tube in this next stage of development. Analysis of BMP2 chimeras generated from BMP2 null ES cells and wild type blastocysts revealed that embryos display both open and closed neural tube defects, ranging from complete failure to close to disorganized growth of neural tissue. Exclusion of BMP2 null ES cells from the dorsal portion of the neural tube did not always prevent defects. Together with the morphological and molecular analysis of heterozygous and homozygous null embryos, these results indicate that BMP2 plays a critical role in normal neural tube patterning and closure.

238. A Balance of FGF, BMP and Wnt Signalling Positions the Sensory Placode Territory in the Head. Andrea Streit, Anna Litsiou, and Hanson Sven. Dept. Craniofacial Development, King's College London, London, UK.

The sensory nervous system in the vertebrate head arises from two different cell populations: neural crest and placodal cells. In contrast, in the trunk it originates from neural crest only. How do placode precursors

become restricted exclusively to the head and how do multipotent ectodermal cells make the decision to become placodes or neural crest? At neural plate stages, future placode cells are confined to a narrow band in the head ectoderm, the pre-placodal region (PPR). Here, we identify the heart mesoderm as the source of PPR inducing signals, reinforced by factors from the neural plate. We show that several independent signals are needed: attenuation of BMP and Wnt is required for PPR formation. Together with activation of the FGF pathway, BMP and Wnt antagonists can induce the PPR in naive ectoderm. We also show that Wnt signalling plays a critical role in restricting placode formation to the head. Finally, we demonstrate that the decision of multipotent cells to become placode or neural crest precursors is mediated by Wnts: activation of the Wnt pathway promotes the generation of neural crest at the expense of placodes. This mechanism explains how placode territory becomes confined to the head and how neural crest and placode fates diversify.

239. Hydrodynamic Basis of Nodal Flow: De Novo Formation of LeftRight Asymmetry in Mouse Development. Shigenori Nonaka,[1] Satoko Yoshiba,[2] Daisuke Watanabe,[3] Shingo Ikeuchi,[2] Tomonobu Goto,[4] Wallace F. Marshall,[1] and Hiroshi Hamada[2]. [1]UCSF, San Francisco, CA 94143-2200; [2]Osaka University, Osaka 565-0871, Japan; [3]Kitasato University, Kanagawa 228-8555, Japan; [4]Tottori University, Tottori 680-8552, Japan.

In the gastrulating mouse embryo, hundreds of monocilia on the ventral side on the node generate leftward flow of extraembryonic fluid (nodal flow) and the direction of this flow determines left–right (L–R) asymmetry of future development. The mechanism by which the rotational movement of node cilia can generate a unidirectional flow remains unknown. A recent paper describing theoretical fluid dynamics simulations of nodal cilia rotation showed that a linear flow could result if the rotation axis of the cilia has a posterior tilt (Cartwright et al. PNAS 101, 7234–9). However, their computer simulation actually predicted bidirectional flow that seems different from our previous observations. Instead, we propose a model that can produce leftward-directed flow by the same tilt. High-speed video microscopy of the living cilia revealed their motion patterns explainable as conical rotations tilted. Stereography of scanning electron micrographs showed an average 22.6° tilt of the cilia towards the posterior. Finally, fluid dynamics experiments showed that the posteriorly tilted rotations could produce leftward flow of the reasonable intensity. These results explain not only the hydrodynamics of the nodal flow but also the origin of L–R asymmetry in mammalian development. L–R asymmetry is suggested to arise de novo by combining three sources of spatial information: antero-posterior and dorso-ventral axes, and the chirality of ciliary movement.

240. Requirement of the Anterior Visceral Endoderm for the Establishment of Anterior-Posterior Axis and Mesoderm Patterning. Shigeto Miura and Yuji Mishina. Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA.

The anterior visceral endoderm (AVE) is an extraembryonic tissue that is believed to have a role in establishing the authentic primitive streak in mice. To further confirm the notion, we directly ablate the distal visceral endoderm (DVE) of 5 dpc mouse embryo, a future AVE, using a micromanipulator equipped with a glass needle. Embryos are further subjected to whole embryo culture for 2 days to know the consequences of ablation of the DVE. When nearly entire DVE is removed, the AVE does not form and in such embryos posterior genes are expressed proximally. The results show that the AVE has an essential function to establish anterior–posterior axis. Also, our data argue that constriction observed in Lim1 or Otx2 mutant results from dysfunction of the DVE. Shortly after migration begins to anterior side of the embryo, the DVE exhibits a heterogeneity; proximal Lefty1/Dkk1 expressing population and distal Cer1 expressing population. Ablation of distal population of the DVE results in an impaired AVE that does not have an ability to restrict Wnt3 expression to posterior side of embryos. Nonetheless, the AVE still retains antagonistic activity to WNT signaling and the primitive streak is formed in those embryos. However, the expression of Wnt3a and Brachyury is altered. Thus, our data indicate that the AVE is required for normal patterning of the mesoderm.

241. Multiple Roles for BMP Antagonism in Left–Right Asymmetry of Mouse Embryos. Thomas J. Sitzman, Ryan M. Anderson, and John Klingensmith. Duke University Medical Center, Durham, NC 27710.

Bone morphogenetic protein (BMP) and nodal signaling are both necessary to correctly establish the left–right axis in mice. The role of nodal signaling in axis formation is well characterized, while less is known for BMP signaling. Using a genetic model of decreased BMP antagonism, we are examining the function of BMP signaling in mammalian left–right axis establishment. Mice mutant for the BMP antagonists Chordin and Noggin exhibit randomization of the left–right axis and deletion of midline structures. These embryos do not correctly establish asymmetric heart looping, and have the midline deletion syndrome holoprosencephaly. By molecular analysis, we now show randomization of *nodal* expression to the left or right lateral plate mesoderm (LPM) in these mutants. Further, expression of the nodal targets *lefty2* and *pitx2* in LPM is disrupted. To explore the interaction between BMP and nodal signaling, we developed a series of complex mouse mutants lacking alleles of *chordin*, *noggin*, *nodal*, and the nodal target *foxa2*. Molecular analysis of mice homozygous for a null allele of either *chordin* or *noggin* and heterozygous for *foxa2* demonstrate bilateral expression of *nodal* and *pitx2* in the LPM. The midline barrier is also disrupted in these mutants, as determined by expression of *shh* and *lefty1*, possibly allowing left determinants to diffuse across the embryo. This result indicates a role for BMP antagonism in promoting establishment of the midline barrier. In combination, these studies suggest BMP signaling is acting at multiple times and positions in the left–right axis.

242. BMP Signaling is Required in the LPM to Block *Nodal* Expression on the Right Side of the Mouse Embryo. Mark J. Solloway,[1] Milena B. Furtado,[1] Elizabeth J. Robertson,[2] and Richard P. Harvey[1]. [1]Victor Chang Cardiac Res. Inst., Darlinghurst, NSW, Australia 2010; [2]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX2 7BN, UK.

In all vertebrates, a conserved pathway controls the invariant L/R positioning and morphology of the heart and other viscera. Symmetry is initially broken within the node, potentially via nodal flow, and a signal is then transmitted to the lateral plate mesoderm (LPM). However, both the identity and mechanism of signal transduction from the node to the LPM remains unknown. Nodal, a member of the TGF-beta superfamily, is expressed in both of these tissues and is a critical upstream component responsible for initiating L/R axis determination. Controversially, previous studies demonstrate that BMP signaling can regulate *Nodal* either positively or negatively at the level of both the node and LPM. We have used null and conditional mutants of *Smad1*, an effector molecule specific to the BMP pathway, to demonstrate a clear requirement for BMP signaling in the LPM to repress *Nodal*. We demonstrate that in embryos lacking *Smad1* expression of left-side specific genes, including *Nodal*, becomes bilateral while several markers of the midline and node remain unchanged. We also used a Mesp1-CRE mouse line to specifically delete *Smad1* in the anterior mesoderm and thus focus our investigation on the potential role of BMP signaling in the LPM. In these embryos, expression of *Nodal* and a Nodal-responsive *Pitx2-lacZ* transgene becomes bilateral within the heart. We argue that BMP signals are thus required to suppress Nodal signaling both early, within the nascent mesoderm, as well as later in the cardiac mesoderm.

243. Down-Regulation of Geminin by RNAi in the Early Post-Implantation Mouse Embryo. Lisa S. De Boer, Theresa E. Gratsch, and Sue O'Shea. Department of Cell & Developmental Biology, University of Michigan.

The factors that promote neural identity and maintain BMP inhibition during the transition from neural induction to neural differentiation are largely unknown. We have examined the role of the bi-functional geminin molecule in neural induction and differentiation in the mouse embryo using shRNA and gene targeting. Geminin is unique because it both transcriptionally suppresses BMPs, while its C-terminal domain controls DNA replication and axial patterning. Geminin is strongly induced by noggin and chordin, and is ideally placed to both maintain BMP inhibition required for neural induction and to control cell-cycle progression during neural differentiation. When shRNAs to geminin were delivered to pregnant dams, embryos were characterized by expanded endodermal tissues (node and body wall) and by characteristic midbrain defects and axis anomalies on E8.5. QRT-PCR analysis and in situ hybridization was carried out to confirm knock-down and analyze positional markers in both targeted and control embryos. We have also developed null embryos and ES cells for parallel studies. Geminin appears to play a unique role in controlling early neural differentiation and axis patterning in the mouse embryo. NIH 039438.

244. SPC-Independent Nodal Signaling During Early Mouse Embryogenesis. Nadav Ben-Haim, Marcela Guzman-Ayala, and Daniel B. Constam. Developmental Biology Group, Swiss Institute for Experimental Cancer research (ISREC), Chemin des Boveresses 155, CH-1066 Epalinges, Switzerland.

Using the mouse embryo as a model system, our lab seeks to define inductive interactions which act across considerable distances to coordinate the differentiation of multiple cell lineages. Our current work focuses on Nodal, a secreted protein of the TGFβ family, its regulation and how it organizes the early post-implantation stage conceptus. Nodal and related proteins of the TGFβ family are initially synthesized as precursors that undergo endoproteolytic cleavage by secreted proteases of the subtilisin-like proprotein convertases (SPC) to remove an N-terminal, inhibitory pro peptide. Unexpectedly, our recent analysis of mouse embryo explants revealed that a subset of Nodal target genes is induced even by a mutant recombinant precursor devoid of an Spc cleavage site. The present project defines Spc-independent activities of Nodal in vivo, by analyzing a knock-in allele that encodes an Spc-resistant mutant form. Analysis of homozygous mutant embryos indicates that Spc-resistant Nodal is able to transiently maintain a trophoblast stem cell compartment within the extraembryonic ectoderm, and that this is achieved at least in part by inducing Fgf4 expression in the epiblast. This study provides a paradigm for understanding the effect of proteolytic maturation on TGF-β activities in vivo.

245. Cdx2 is Required for Maintenance of the Trophectoderm in the Mouse Blastocyst. Amy Ralston, Dan Strumpf, Yojiro Yamanaka, Kallayanee Chawengsaksophak, and Janet Rossant. Samuel Lunenfeld Research Institute, Toronto, ON M5G 1X5, Canada.

The first structure to form during mouse development is the trophectoderm, an epithelial outer layer that will eventually contribute to the placenta. The trophectoderm differentiates from the inner cell mass during formation of the blastocyst. At this stage, the Caudal-related transcription factor Cdx2 is expressed in the trophectoderm, while inner cell mass cells express the pluripotency-promoting transcription factors Oct4 (Pou5f1) and Nanog. We show that Cdx2 is required for the repression of *Oct4* and *Nanog* in the trophectoderm, making Cdx2 the earliest known determinant of this lineage. However, the initial formation of the trophectoderm does not depend on *Cdx2*, nor does it apparently require repression of *Oct4* or *Nanog*. Loss of *Cdx2* does result in a loss of epithelial polarity and eventual collapse of trophectoderm during late blastocyst stages. These results suggest that Cdx2 is required for the maintenance, but not the initial specification of trophectoderm fate. We examine the fate of *Cdx2* mutant trophectoderm further.

246. Evidence for Positive and Negative Regulation of the Mouse Cdx2 Gene. Cooduvalli S. Shashikant and Wayne C. Wang. The Pennsylvania State University, University Park, PA 16802.

Patterning of the vertebrate anteroposterior axis requires the actions of multiple transcription factors that establish regional domains of gene expression. The vertebrate caudal gene, Cdx2, is required for patterning of posterior structures around late primitive stage embryos. Here, we report the identification of enhancers governing mouse Cdx2 expression in both ectodermal and mesodermal tissues. Our transgenic analysis using reporter genes shows that a 10-kb genomic region of Cdx2 containing the entire coding region, introns and flanking sequences is sufficient in directing reporter gene expression in a temporally and spatially restricted manner consistent with endogenous expression. Deletion analysis reveals the presence of discrete regulatory elements, some of which are remarkably conserved across species. Furthermore, these elements interact in both positive and negative manner in order to generate embryonic expression pattern.

247. Characterization of Cell Potential in the Early Mouse Embryo. Robert O. Stephenson,[1] Frieda H. Chen,[2] Yojiro Yamanaka,[2] and Janet Rossant[1]. [1]University of Toronto and Samuel Lunenfeld Research Institute, Toronto, ON, Canada; [2]Samuel Lunenfeld Research Institute, Toronto, ON, Canada.

By embryonic day 4.5 (E4.5), three cell types are apparent in the mouse embryo. Outer cells make up the trophectoderm (TE-later forms the embryonic portion of the placenta), while a clump of cells encompassed by the TE (the ICM) is made up of the primitive endoderm (PE-later becomes the yolk sac), and the epiblast (EPI-later forms the embryo). The ICM itself was previously believed to be a uniform population of cells prior to E4.5. Recent work from our lab, however, indicates that this may not be the case. Studies of the fates of cells isolated from the early ICM and the expression of EPI and PE markers within the early ICM have shown that it consists of two cell types prior to E4.5. To further investigate the development of the first 3 cell lineages in the mouse, we are generating fluorescent fusions with markers for each of these lineages in their endogenous loci. We are using Cdx2 as a marker of the TE, Nanog for the EPI and Gata-6 for the PE. By using different coloured fluorescent proteins, we will be able to visualize the expression of these markers simultaneously in real time in cultured embryos. Interestingly, recent studies examining early cell fates have indicated that not all cells are equivalent at the 4-cell stage and they may have different developmental potentials. Using antibodies against the markers of cell fate mentioned above, we are also examining the potential of cells isolated from 4-cell embryos cultured in vitro.

248. Role of Daam Genes in the Regulation of Cell Polarity During Mammalian Embryogenesis. Masa-aki Nakaya,[1] Lino Tessarollo,[1] Raymond Habas,[2] Xi He,[3] and Terry P. Yamaguchi[1]. [1]NCI-Frederick/NIH, Frederick, Maryland 21702, USA; [2]University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854, USA; [3]Childrens Hospital, Harvard Medical School, Boston 02115, USA.

The establishment of cell polarity is critical for the development of functional epithelial tissues and for the regulation of cell movements during

development. Regulation of cell polarity in the plane of the tissue orthogonal to the apical–basal axis, i.e., planar cell polarity (PCP), is required for the polarized cell movements of gastrulation known as convergent extension (CE) movements. We are interested in understanding how Wnts regulate cell polarity, and how cell polarity directs the highly complex cell movements of embryogenesis. In vertebrates, Daam (Dishevelled (Dvl)-associated activator of morphogenesis) proteins couple Wnt signaling to Rho by binding directly to Dvl and Rho. We have identified two mouse homologues of Daam, mDaam1 and mDaam2. Both genes encode Formin homology proteins, and are expressed throughout embryogenesis. A targeted mutation of mDaam1 reveals that it is required for proper blastocyst formation during preimplantation development, and for the establishment of apical–basal polarity in the trophectoderm. Our current experiments address the potential roles of Daam in the regulation of the actin cytoskeleton and adherens junctions. We will also present genetic evidence that mDaam1 participates in a PCP pathway that is necessary for head formation. Compound mDaam1; Looptail mutants lack heads or display varying degrees of holoprosencephaly including cyclopia. These results suggest that mDaam1 plays important roles in the regulation of both apical–basal and PCP.

249. The Functional Role of Amnionless in Middle Primitive Streak Assembly in Mammalian Gastrulation. Claudia Munoz,[2] Sharon Strope,[1] Roberta Rivi,[1] Thomas Metzger,[2] and Elizabeth Lacy[1]. [1]Developmental Biology, Sloan-Kettering Institute, New York, NY 10021; [2]Molecular Biology, Weill Graduate School/Cornell University, New York, NY 10021.

Mouse Amnionless (Amn) is a transmembrane protein required in the visceral endoderm (VE) for the generation of non-axial trunk mesoderm during gastrulation. Amn is also expressed in kidney proximal tubules (KPT) and intestinal epithelium. Loss of both AMN and Cubilin (CUBN) in humans result in hereditary megaloblastic anemia (MGA) due to vitamin B12 malabsorption, suggesting they function in the same pathway. CUBN is expressed in the same polarized epithelia as AMN. CUBN can also bind AMN in vitro and we demonstrated that Cubn is not properly localized to the cell surface in Amn−/− embryos and chimeric kidneys. The role of Amn in the VE might be to properly localize Cubn to the VE apical membrane, to traffick Cubn and/or a cubn ligand through the VE, or both. A mutation in the cytoplasmic domain of Amn in MGA patients suggests that Amn has an intracellular role separate from localizing Cubn, since Cubn is a membrane associated protein and does not have an intracellular domain. Thus, in order to address the function of Amn in relation to Cubn, we will study the function of the Amn cytoplasmic tail (CT). We will determine whether there is an apical sorting signal, internalization motif, and/or trafficking motif in Amn CT. We will also analyze the localization of Cubn in different Amn CT mutants and how these mutants affect the gastrulation defect of Amn−/− embryos.

250. Disruption of the Mouse La Gene Leads to Failure to Develop Beyond Blastocyst and Prohibits Embryonic Stem Cell Development. Jung-Min Park,[1] Matthew J. Kohn,[1] Monique Bruinsma,[1] Claire Vech,[1] Ipsita Mukherjee,[1] Stacy Fuhrmann,[1] Alex Grinberg,[1] Paul E. Love,[1] Keith E. Latham,[2] Robert V. Intine,[1] Melvin DePamphilis,[1] and Richard J. Maraia[1]. [1]NICHD, NIH, Bethesda, MD 20892; [2]Temple University School of Medicine, Philadelphia, PA 19140.

The human La antigen is a ubiquitous RNA-binding protein that binds to a variety of RNAs, and has been implicated in multiple pathways related to the production of tRNAs, ribosomal proteins and other components of the translational machinery (Kenan and Keene 2004. Nat Struct & Mol Biol, 11:303–05). We created mice carrying a disrupted allele of the La gene and analyzed the offspring from $La^{+/-} \times La^{+/-}$ intercrosses. Nullizygous $La^{-/-}$ offspring were detected at the expected frequency among blastocysts prior to implantation, whereas no nullizygotes were detected after implantation, indicating that La is required for early development. Although living $La^{+/-}$ mice exhibited no overt phenotype, they were obtained at approximately 25% less than expected frequency suggesting a complex requirement for La in a significant fraction of the heterozygotes. Blastocysts derived from $La^{+/-} \times La^{+/-}$ intercrosses yielded a total of twenty-three $La^{+/-}$ and $La^{+/+}$ embryonic stem (ES) cell lines but no $La^{-/-}$ ES cell lines suggesting that the mouse La protein contributes a critical function toward the establishment or survival of ES cells in the laboratory. To increase understanding of the mechanism by which early $La^{-/-}$ embryos fail, the fates of the inner cell mass and trophoblast cells from explanted $La^{-/-}$ blastocyst outgrowths will also be presented.

251. Toll-Like Receptor and Relish Expressions in Human Embryonic Tissue are Age-Dependent. Hsiao-Nan Hao and Paul H. Wooley. Wayne State University.

Toll-like receptor (TLR) is demonstrated as an essential receptor for host against microbial infection not only in *Drosophila* but also in human cells. All TLR signals participated in host immune response are involved to regulate nuclear factor (NF-κB) activity. Relish (Rel) nuclear transcription factors are part of protein complexes with IêB, and regulate NF-κB activation. Although these factors were identified in human tissue, the expression correlation of TLR and Relish in human tissue is not clear. Using different gestational age-specific embryonic human splenic and brain tissues (weeks 9 to 23), we demonstrate that mRNA of TLR2/4 constantly expressed in all brain embryonic tissues, but the level of expression reduced in the tissues collected from 2nd trimester In addition, Rel expression was also detected from brain tissue, but not from 1st trimester brains. In contrast, both TLR2/4 and Rel were detected in the splenic tissue. The expression level in 2nd trimester samples was higher than that of 1st trimester spleens. We also found that expression density of splenic TLR/Rel is stronger than that of brain tissue. The expressions of TLR2/4 were paralleled with Relish expression, and became stronger following the gestational-age increase in the splenic tissues only. The expression alterations of TLR/Rel suggest that the immunity function of hematopoietic or splenic cells against microbial infection become more sufficient than that of early gestational splenic cells. For brain tissue, decreased TLR expression in 2nd trimester samples indicates that human brain may request TLR for its neurogenesis but not for its immunity development.

252. Implantation of Eight-Cell Embryos in Transgenic Mouse Production. Fuming Pan and Juqin Wang. LSU Health Sciences Center, New Orleans, LA 70112.

Transgenic mouse technology has been widely used to create animal models in biomedical research areas for decades. Pronuclear microinjection is still the primary tool adopted to generate transgenic mice. One of the critical steps in transgenic mice production is the surgical implantation of microinjected mouse embryos into the oviduct(s) of a pseudopregnant female mouse. Normally, either 1-cell embryos or overnight-cultured 2-cell embryos are implanted into the oviduct(s) of foster female mice after microinjection. Here, we then cultured microinjected, already developed, 2-cell embryos overnight in KSOM media. There were 88% 2-cell embryos developed into 8-cell embryos from overnight culture. We implanted those 8-cell embryos into the oviducts of foster female mice with a corpulation plug overnight. The percentage of lives born from the 8-cell embryos implantation was 41%. This method serves as a unique rescuer in the case of lacking foster females for 2-cell embryo transfer in transgenic mouse production.

253. EMAGE, Edinburgh Mouse Atlas of Gene Expression. Jeffrey Christiansen, Lorna Richardson, Shanmugasundaram Venkataraman, Peter Stevenson, Nick Burton, Yiya Yang, Richard Baldock, and Duncan Davidson. MRC Human Genetics Unit, Edinburgh EH4 2XU, UK.

EMAGE is a database of spatially mapped gene expression patterns in the developing mouse embryo that we are developing as part of the Edinburgh Mouse Atlas Project (EMAP). All EMAGE data are housed in a standard framework: the EMAP Digital Atlas of Mouse Development which consists of at least one representative 3D digital embryo model at most Theiler stages (TS) as well as a standardised nomenclature for anatomical structures that are present at every Theiler stage of development. As the digital embryo models are 3D objects, it is possible to cut virtual sections in any plane through them to reveal anatomical detail within that plane. Incoming EMAGE data images are mapped spatially into the corresponding regions within the embryo models. At TS07–14, the 3D standard models have anatomical regions defined within them and as such, data spatially mapped into these models is automatically annotated to the corresponding text terms for these structures. This is accompanied by further manual text annotation. Searching the data in EMAGE can be done spatially by defining a region of interest in a particular embryo model, or by using text terms. New developments include identifying groups of co-expressed genes by hierarchical clustering and domain intersection analysis. Free software to search EMAGE can be downloaded from the EMAP website (genex.hgu.mrc.ac.uk). The same software can also be used to prepare private databases for in-lab data management or to prepare electronic submissions to EMAGE. Alternatively, specimens can be sent directly to EMAGE for entry into the public database.

254. The Mouse Gene Expression Database (GXD): Integrated Access to Expression Information for the Laboratory Mouse. D.A. Begley, J.H. Finger, T.F. Hayamizu, D.P. Hill, I.J. McCright, C.M. Smith, M. Ringwald, and The GXD Group. The Jackson Laboratory, Bar Harbor, ME 04609.

The Gene Expression Database (GXD) collects and integrates gene expression information about the developing laboratory mouse. By combining diverse types of expression data, GXD provides information about the expression profiles of transcripts and proteins in different mouse strains and mutants, thus enabling insights into the molecular networks underlying developmental and disease processes. Expression patterns are described using an extensive dictionary of standardized anatomical terms, making it possible to record expression results from assays with differing spatial resolution in a consistent manner. GXD is integrated with the Mouse Genome Database and interconnected with other community resources, to include expression data in a larger biological and analytical context. Data are acquired by curation from the literature, database downloads and electronic laboratory submissions. To facilitate the direct submission of data to GXD we have developed the Gene Expression Notebook (GEN). GEN functions as a laboratory notebook to store and organize expression data, assay details and images for in situ hybridization, immunohistochemistry, RT-PCR and Northern and Western blot experiments. It also allows the researcher to effortlessly submit selected expression data to GXD. Capturing these data will further enhance GXD's utility as a community resource. GEN is available at http://www.informatics.jax.org/mgihome/GXD/GEN/. GXD is accessible through the Mouse Genome Informatics web site at http://www.informatics.jax.org/. GXD is supported by NIH grant HD 33745.

255. Interactions Between the Cell Cycle and Embryonic Patterning in *Arabidopsis* Uncovered by a Mutation in DNA Polymerase Epsilon. Pablo D. Jenik,[1] Rebecca J. Jurkuta,[2] and Kathy Barton[1]. [1]Carnegie Institution of Washington, Stanford, CA 94305; [2]University of Wisconsin-Madison, Madison, WI 53706.

Pattern formation and morphogenesis require that cell division rates and orientations are coordinated with the developmental signals that specify cell fate. We isolated mutations in the TILTED1 locus, which encodes the catalytic subunit of DNA polymerase e of *Arabidopsis thaliana*, and analyzed their effects on cell cycle length and patterning during embryogenesis. A weak mutant allele causes a lengthening of the cell cycle by approximately 30% throughout embryo development, while strong mutant alleles result in early embryonic lethality. The cells and nuclei of the slowly developing embryos are larger than those of wild type embryos. Unlike wild type embryos, which develop at a constant pace, embryos homozygous for the weak allele pause at the late globular stage. The mutant embryos show abnormal patterning of the hypophyseal lineage (quiescent center and columella cells), leading to a displacement of the root pole from its normal position on top of the suspensor. Our results uncover an interaction between the cell cycle and the processes that determine cell fate during plant embryogenesis.

256. Regulation of Dictyostelium Development by Presenilin and GSK3 Signaling. Vanessa C. McMains,[2] Lisa Kreppel,[1] and Alan R. Kimmel[1]. [1]NIDDK/NIH Bethesda, MD 20892; [2]Johns Hopkins University, Baltimore, MD 21218.

Under starving conditions, individual Dictyostelium discoideum cells aggregate to form multicellular organisms that undergo cell-specific differentiation. Terminal development is characterized by the formation of a mass of spore cells supported by stalk cells. Response to secreted cAMP, which is detected by membrane receptors, controls both aggregation and differentiation. Two of these receptors, CAR3 and CAR4, direct prespore or prestalk cell determination by antagonistically regulating GSK3, a serine/threonine protein kinase. We have now shown that Presenilin (PS) signaling also regulates Dictyostelium development. PS has two suggested functions; one as a component of the proteolytic γ-secretase complex that cleaves single pass transmembrane proteins thereby releasing transcription factors, and another to scaffold GSK3. To understand the genetic interactions between cAMP and PS signaling, we have created strains with multiple mutations. Phenotypes of car3/ps- and car4/ps-null mutants suggest that PS promotes prespore cell fate determination. I wish to determine if PS action is through γ-secretase or scaffolding activity via GSK3. I will examine the developmental phenotypes and gene expression patterns of car3- and car4-nulls that possess PS but that lack γ-secretase activity. These data will clarify how PS2 controls cell fate specification.

257. Hedgehog Maintains Groove Cell Fate During *Drosophila* Embryogenesis. Stephane Vincent and Jeff Axelrod. Stanford University.

The segmental grooves of the *Drosophila* embryo form at the site where epidermal cells express the pair rule transcription factor odd skipped (odd). These cells are located immediately posterior to the engrailed (en) stripe of cells that organizes the segment by secreting the hedgehog (hh) signaling molecule. It was thought previously that the organizer induces groove formation. Here, we show that the organizer cannot direct groove formation in absence of odd cells, indicating an indirect function for hh signaling. We show that hh is essential to maintain odd cell fate after stage 11 and that it acts as a short range signal to narrow the width of the odd stripes to a single row of cells. Conversely, over-activation of hh signaling results in the appearance of additional rows of groove cells. Both Ptc loss of function and hh over-expression lead to the formation of five rows of odd cells, all of which express groove markers such as Crumbs and aPKC, and accumulate Enabled in a groove-cell specific manner. This suggests that all the odd expressing cells present at stage 11 keep their fate through embryogenesis if they receive hh signal, and that cells that do not express odd at stage 11 are not competent to become groove cells. Our data show that hh acts as a fate

maintenance factor during groove formation rather than a morphogen that generates new cell fate. We propose that the maintenance of cell lineage is a critical function of the organizer in the control of embryonic patterning.

258. Evidence for Maternal Contribution of Med and End Function to their Zygotic Roles in Endoderm Specification in *C. elegans*. Morris F. Maduro,[1] Gina Broitman-Maduro,[1] Jiangwen Zhu,[2] Jan Sumerel,[2] and Joel H. Rothman[2]. [1]Biology Department, UC Riverside, CA 92508; [2]Department of MCDB, UC Santa Barbara, CA 93106.

At the 8-cell stage of *C. elegans* development, the genes *med-1* and *med-2*, encoding a redundant pair of divergent GATA factors, specify the MS (mesoderm) and E (endoderm) precursors. RNAi depletion of *med-1,2* function results in a penetrant absence of MS- and E-derived tissues. In E, *med-1,2* activate another pair of GATA factor-encoding genes, *end-1,3*, which specify endoderm. Reporters and in situ hybridization of the *meds* and *ends* show embryonic expression. Thus, our model for mesendoderm specification has implied a strictly zygotic role for these genes, consistent with genetic data and the finding that they are zygotic targets of maternal transcription factors, including SKN-1 and POP-1, a transducer of the endoderm-inducing Wnt/MAPK/Src pathway.

We now report multiple lines of evidence strongly suggesting that a maternal contribution of the *meds* and *ends* supplements their zygotic functions. First, in situ hybridization detects germline *med* and *end* mRNAs. Second, a MED-1 antibody detects a germline epitope. Third, an *end*-3 mutant, and strains double-mutant for deletions removing *med-1* and *med-2*, display strong maternal rescue of the endoderm defect. As *med* and *end* mRNAs are not found in early embryos, their protein products may be supplied to developing oocytes. We propose that both maternal and zygotic function of the *med* and *end* genes contribute to endoderm specification, raising the unexpected possibility that much of the embryonic mesendoderm gene regulatory network may be mobilized in the developing germline.

259. Elucidating the Function and Expression of Target Genes of the *C. elegans* Mesendoderm-Specifying Genes *MED-1* and *MED-2*. Gina Broitman-Maduro, Wendy W. Hung, Tan-Hui Lin, and Morris F. Maduro. University of California, Riverside, CA 92521.

Our lab is interested in elucidating the network of genes that direct mesendoderm development in *C. elegans*. At the 8-cell stage, the pair of transcription factors encoded by *med-1,2* activates a regulatory gene cascade in the MS and E cells that specifies these cells as mesodermal and endodermal precursors, respectively. Recently, we reported the identification of the MED binding site and used its sequence to identify another 19 candidate target genes from the *C. elegans* genome. Many of these show mesendodermal expression, suggesting they are bona fide MED targets. One of our current goals is to characterize the function and expression of two identified MED-1 target genes, *hlh*-25 and *tbx*-35, which are activated specifically in the MS cell. By manipulating promoter::GFP reporters of these genes, we hope to understand how the Wnt pathway (which makes E different from MS) collaborates with MED-1 to activate these genes specifically in MS. In particular, we wish to identify what cis-acting signals direct expression to MS versus E.

We have also begun to expand our understanding of the evolution of the mesendoderm gene network by using an in vitro genomic selection/amplification approach to confirm targets in *C. elegans* and search for targets of MED homologs in the related nematode *C. briggsae*.

Results of the promoter analysis and in vitro selection experiments will be presented.

260. RhoA Triggers Endoderm Invagination During Sea Urchin Gastrulation. Wendy S. Beane,[1] Jeffrey M. Gross,[2] and David R. McClay.[1] [1]Duke University, Durham, NC; [2]Harvard University, Cambridge, MA.

The morphology of cell movements during sea urchin gastrulation has been studied in detail, and the urchin gene regulatory network outlines the transcriptional and signaling events involved in endoderm specification. However, while the processes of specification and morphogenesis have received much attention, few details link cell specification with directed cell movements. The Rho family of GTPases mediate actin cytoskeletal rearrangements downstream of extracellular signaling, making them excellent candidates for such a link. Therefore, the role of *Lytechinus variegatus* RhoA during gastrulation was examined. Dominant negative LvRhoA inhibits endoderm invagination, while activated LvRhoA (actRhoA) causes precocious onset of invagination. Pharmacological inhibition reveals that Rho activity is required just prior to invagination, while chimeric assays suggest this activity is required only in endomesodermal cells. Although data from other systems suggest RhoA regulates convergent extension (CE), in the sea urchin precociously invaginating embryos wait to undergo CE alongside controls. Additionally, actRhoA rescues both endoderm invagination and differentiation following Brachyury inhibition. Together, these data suggest RhoA activity is essential for initiation of endoderm invagination during gastrulation in the urchin embryo. ActRhoA induces a single precocious invagination only in those tissues which normally produce invaginations, thus prior endodermal specification is necessary for RhoA function. Having determined LvRhoA can direct gastrulation movements, its ability to connect those movements to specification state is currently being investigated.

261. New Components of Endoderm Specification Including Bmp4 and SnoN. Julie Baker, Eric Chiao, Jeff Leonard, and Kari Dickinson. Department of Genetics, Stanford University, Stanford, CA 94062.

A cross-species functional screen for components of endoderm specification has identified 8 new members of this pathway, including the uncharacterized molecules Hspa8, Kif22, Cdh1, and Tcfeb and the known molecules Otx2 and Foxa2. Some of these molecules can induce both the endodermal markers endodermin and Sox17b, while others can only induce endodermin, suggesting they act downstream of Sox17b. Further characterization of these genes has given us an insight into the endoderm pathway downstream of Sox17b as well as a separate branch point regulated by Bmp4. Here we show that Foxa2, Otx2, Habp2 and Tcfeb are downstream of Sox17b and can directly induce endodermin from *Xenopus* ectoderm. Furthermore, we test whether Cdh1, a known regulator of the TGFb pathway identified in this screen, induced endoderm by eliminating SnoN, thus allowing the expression of Bmp4 downstream targets. Although Bmp4 has not been implicated in endoderm formation to date, we show that it, as well as its downstream components, is necessary and sufficient to induce endoderm in *Xenopus* embryos. Furthermore, we demonstrate that the ability of Bmp4 to induce endoderm is antagonized by SnoN. Taken together, as a result of a large-scale expression screen, we have added to the general knowledge of the endoderm specification pathway and continue to pursue the mechanistic role each of these molecules play.

262. The Role of *Xenopus* Tbx2, T-Box Transcription Factor, For Lateral Plate Mesoderm Formation. Gun-sik Cho and Jin-Kwan Han. Department of Life sciences, Pohang University of Science and Technology, San 31, Hyoja-Dong, Nam-Gu, Pohang 790-784, Republic of Korea (south).

Xenopus Tbx2 (XTbx2) gene, a member of the T-box transcription factor family, is expressed in the lateral plate mesoderm and pronephric region. To investigate the function of XTbx2 gene during embryonic development, we performed loss-of-function experiments using the morpholino antisense oligo. Injection of XTbx2-MO into the ventral-vegetal one blastomere at 8 cell stage decreased expression of Pax2, Pax8 and Lim1, pronephric markers at the pronerhric region, along with disruption of pronephric tubule and duct formation. The expression of

MyoD, paraxial mesoderm marker, was decreased in XTbx2-MO injected embryos and these also have the small size somite. Interestingly, section in situ hybridization shows that XTbx2 is expressed only at the lateral plate mesoderm and XTbx2-MO change the fate of intermediate mesoderm to lateral plate mesoderm. Consequently, somite and pronephros could not develop normally in these XTbx2-MO-injected embryos. From these results, we suggest that XTbx2 is essential for formation of lateral plate mesoderm.

263. An Analysis of Pluripotency of the Dorsal Ectoderm and Mesoderm in *Xenopus*. TaiJuana A. Sylvester,[1] Lisa A. Dali,[2] and Carmen R. Domingo[1]. [1]San Francisco State University, San Francisco, CA; [2]Nasa Ames Research Center, Moffatt Field, CA.

Gastrulation is an embryological event characterized by dramatic cell movements that form the three embryonic germ layers; ectoderm, mesoderm and endoderm. Previous work from our lab revealed that cell fates are not restricted to germ layer derivatives by the end of gastrulation (Domingo and Keller, 2000). Given this observation, we investigated the extent to which ectodermal and mesodermal cells remain pluripotent. We performed cell transplantation experiments in which we grafted labeled cells from either the anterior or posterior ectoderm into the pre-somitic mesoderm of unlabeled embryos. Grafted embryos were cultured to tadpole stages, fixed and analyzed by fluorescent microscopy. We found that cells from the anterior neural ectoderm lose their ability to change fate into muscle by the end of gastrulation, whereas cells from the posterior neural ectoderm remain competent to form muscle tissue until mid-neurulation. Next, we examined the competence of pre-somitic mesoderm cells to adopt a neural fate by grafting labeled pre-somitic cells into the posterior neural ectoderm region of unlabeled embryos. We found that pre-somitic mesoderm cells were able to change their fate and become neural tissue when placed into the neural ectoderm of host embryos undergoing neurulation, but not prior to neurulation. In addition, we found that pre-somitic mesodermal cells lose their ability to adopt a neural fate by the neural plate stage. In conclusion, we find that cells within the posterior neural ectoderm remain more pluripotent than cells in either the anterior ectoderm or in the pre-somitic mesoderm.

264. Hairy-2a Promotes Floor Plate Fates at the Expense of the Notochord, Blocking Involution in the *Xenopus* Organiser. Silvia L. López, María V. Rosato-Siri, Paula G. Franco, Alejandra R. Paganelli, Paula Raimondi, and Andrés E. Carrasco. LEM-IBC. School of Medicine, UBA-CONICET, Buenos Aires, Argentina.

In *Xenopus* embryos, the midline neural plate cells that later become the floor plate (FP) conform the notoplate, which arises from the dorsal non-involuting marginal zone (DNIMZ), a region of ectoderm just above the dorsal involuting marginal zone (DIMZ). The latter contains mesodermal cells that enter through the blastopore during gastrulation, giving rise to the prechordal plate and notochord. The notoplate precursors remain in the ectodermal layer and undergo convergent-extension movements, laying the future FP along the A–P axis. The *Xenopus* organiser contains cells equally potent to develop as notochord or FP. Before mid-gastrula, Notch executes a binary cell-fate switch that favours FP development at the expense of the notochord (López et al., Development 130, 2225–2238, 2003). Now, we found that the Notch-target gene hairy-2a is expressed in an arc within the DNIMZ and in scattered cells in the DIMZ, where delta-1 transcripts are also scattered. Hairy-2a+ cells gradually converge and extend along the A–P axis, forming the notoplate. We propose that Delta-1 is the ligand that triggers the binary cell-fate switch by activating Notch in the surrounding cells within the DIMZ. Notch, in turn, activates hairy-2a, which impedes the involution of cells through the blastopore, promoting their incorporation into the population of notoplate precursors in the DNIMZ.

265. The Function of Growth/Differentiation Factor 11 (Gdf11) in Rostrocaudal Patterning of the Developing Spinal Cord. Jeh-Ping Liu. Univ. of Virginia, Charlottesville, VA 22908.

Subclasses of motor neurons are generated at different rostrocaudal positions along the developing spinal cord. Members of the Hox-c gene family are expressed in spinal motor neurons located at different rostrocaudal positions [1] and one of the functions of these Hox genes is to define the columnar identity of the spinal motor neurons [2]. Using an in vitro assay system, we demonstrated that FGF and Gdf11 signals located at the caudal end of chick embryos have the ability to induce posterior Hox-c protein expression [1]. More recently, FGF was also shown to induce posterior Hox-b gene expression in an in vivo system [3]. To investigate the function of Gdf11 in the rostrocaudal patterning of the spinal cord in vivo, we mis-expressed Gdf11 in the developing spinal cord in chick embryos by in ovo electroporation. We find that ectopic expression of Gdf11 in the neural tissue induces a rostral shift of Hox-c protein expression, accompanied by rostral shifts in the expression of motor neuron columnar and pool markers. Moreover, mis-expression of follistatin, an antagonist of Gdf11, has a converse effect on the expression patterns of the markers examined. These results indicate that like FGF, Gdf11 has a function in patterning caudal neural tissue during development. We are currently investigating the interactions between FGF and Gdf11 signaling pathways, and the neural phenotype of Gdf11 knock out mice [4]. References [1] Liu et al., Neuron 32, 997–1012, 2001. [2] Dasen et al., Nature 425, 926–933, 2003. [3] Bel-Vialar et al., Development 129, 5103–5115, 2002. [4] McPherron et al., Nat. Genet. 22, 260–264, 1999.

266. Pax6 Controls Cell Fates in the Developing Spinal Cord by Regulating Wnt Signaling. Kamana Misra, Eddie Kuang, Shike Li, and Michael P. Matise. UMDNJ, Piscataway, NJ 08854.

Pax6 is a homeodomain containing transcription factor that has been shown to play a central role in cell fate specification in the developing CNS. However, little is known about the downstream mechanisms mediating Pax6 activity. Here, we provide evidence that Pax6 influences cell fates by regulating Wnt signaling in the developing SC. We show that Lbx1, a transcription factor normally restricted to dorsal interneurons, is upregulated specifically in the V2 domain in Pax6 mutants. These mutants also exhibit downregulation of sFRP1&2 in the ventral SC. The s*FRP* genes encode secreted frizzled related proteins that function as antagonists of Wnt signaling. To test whether these factors normally function to repress Lbx1 fates ventrally, we transfected full-length s*FRP* cDNAs into the neural tube of developing chick embryos. Overexpression of both sFRP1&2 inhibited Lbx1. To further explore the mechanisms regulating normal Lbx1 induction, we developed an assay in which co-electroporating Mash1 and Gsh1 could elicit ectopic Lbx1 expression. sFRP2, but not sFRP1 was able to inhibit Lbx1 induction by these factors. Strikingly, in both the wild type and *Pax6* mutants, Lbx1 cells are generated from Mash1+ progenitors, suggesting a possible role for Mash1 in Lbx1 induction. Consistent with this, ectopic Lbx1 expression in the V2 domain was significantly reduced in $Mash1^{-/-}$; $Pax6^{-/-}$ double mutants. We conclude that Pax6 regulates cell fates in the SC by controlling Wnt signaling via sFRPs. Our data suggest that loss of sFRP expression in $Pax6^{-/-}$ mutants leads to up-regulation of Wnt signaling, which in co-operation with Mash1 results in up-regulation of Lbx1.

267. Analysis of the Role of Pax3/7 in Mediating Wnt Signaling in the Chick Dorsal Neural Tube. T.L. Barnes,[1] L.M. Galli,[1] S.R. Knight,[1] A. Kawakami,[2] and L.W. Burrus[1]. [1]Department of Biology, SFSU, SF, CA; [2]University of Tokyo, Japan.

The proper patterning and growth of the developing neural tube (NT) requires signals from dorsal and ventral structures that balance proliferation

with differentiation of specific neural lineages. BMPs and Shh maintain dorsal–ventral patterning of the NT by setting borders that control the specification of neuronal fates, while Wnt-1/3a, expressed in the dorsal NT(dNT), control proliferation. We and others have shown that ectopic expression of Wnt-3a in the NT causes an increase in proliferation and an expansion of the Pax3/7 expression domain in the dNT. Conversely, our studies indicate that ectopic expression of dominant-negative TCF(dnTCF), an inhibitor of β-catenin mediated Wnt signaling, results in a loss of Pax3/7 expression. It has previously been shown that Pax3/7 transcripts are expressed in the dNT in a pattern that overlaps with Wnt1/3a expression. Consistent with the redundant roles for Pax3/7 in the dNT, we now show that Pax3/7 proteins are indeed co-expressed in the majority of cells in the dNT. Thus, we hypothesize that Pax3/7 are downstream effectors of the β-catenin pathway. To test this hypothesis, we are overexpressing Pax3/7 in the NT and determining if the resulting phenotype mimics that of ectopic Wnt-3a. Secondly, we are testing if ectopic expression of a fusion protein consisting of the Pax-3 DNA binding domain and the Engrailed repressor domain (Pax3-EnR) and Pax7-EnR mimic the phenotype of dnTCF. Finally, to directly assess whether Pax3/7 are downstream mediators of Wnt signaling, we are testing if Pax3-EnR or Pax7-EnR can block the effects of activated β-catenin.

268. Analysis of the Role of Porcupine in the Formation of the Wnt Gradient in the Chick Neural Tube. Stephanie S. Secrest, Martha J. Skalak, Lisa M. Galli, William T. O'Connor, and Laura W. Burrus. Department of Biology, San Francisco State University, 1600 Holloway Avenue, SF, CA 94132.

It has long been a goal of developmental biology to understand how morphogens establish gradients to promote proper tissue patterning. Wnts are a family of signaling molecules thought to function as morphogens. In invertebrates, the *Drosophila* homolog of Wnt has been observed in an extracellular gradient. Megason and McMahon (2002) identified a gradient of Wnt-1 and Wnt-3a induced proliferation in the neural tube of chick embryos. Porcupine (Porc) is a component of the Wnt signaling pathway that is found in the cell that synthesizes Wnt. Porc is thought to function as a membrane-bound O-acyltransferase (MBOAT), mediating the palmitoylation of Wnt. We hypothesize that Porc plays a role in the formation of this gradient of proliferation in the chick neural tube. We isolated the cDNA for chick Porc and found that porc transcripts are ubiquitously expressed in the chick embryo. We found mouse Porc and mouse Wnt-3a co-localize to the endoplasmic reticulum in COS cells. We constructed a variant of mouse PorcD that is mutated in the active site and is designed to act as a dominant negative mutation. To determine the role of Porc in vivo, we electroporated constructs encoding wild-type or mutant Porc into the neural tube of the chick embryo. Comparison of proliferation in the electroporated side of the neural tube to the contralateral side of the neural tube shows that proliferation is increased upon addition of wild-type Porc and decreased with mutant Porc. We are now specifically investigating the effect of Porc on the Wnt gradient in the neural tube.

269. Directly Visualizing Shh in the Developing CNS of the Mouse. Chester E. Chamberlain,[1] Juhee Jeong,[2] Ben L. Allen,[1] and Chaoshe Guo[1]. [1]Harvard University; [2]Univ. of California-San Francisco.

Sonic hedgehog (Shh) is a member of the Hedgehog (HH) family of secreted proteins. It is critical for the proper patterning and growth of vertebrate organisms. Shh mutants in the mouse show extreme midline defects, severe cranial-facial deformation and reduced limb development. In the developing central nervous system (CNS), it has been postulated that Shh directly specifies distinct ventral cell identities by assuming a graded distribution. In principle, a Shh gradient could allow for threshold responses along the dorsal–ventral axis to confer positional identity to neuronal precursors based on local concentration. The mechanism by which Shh gets distributed into the ventral CNS is an interesting trafficking problem that can be broken into at least four parts: (1) Shh trafficking to the cell surface; (2) Shh release from the cell surface; (3) Shh trafficking within the target field; (4) and the establishment of an appropriate stable gradient of Shh. To study the mechanism of Shh trafficking, we targeted GFP into the Shh locus to create a Shh::GFP chimeric protein. Shh::GFP is sufficient to induce all Shh-dependent ventral cell identities and can be directly visualized in the ventral CNS. However, analysis of the neural tube and other Shh-dependent tissues suggest that Shh::GFP is most likely a hypomorphic protein. I will discuss progress in using this allele to understand Shh's action in patterning the mammalian neural tube.

270. PTEN and AKT are Critical Regulators of Cell Fate and Position in the Developing Murine Brain. Anthony D. Hill[1] and Christopher A. Walsh[2]. [1]Beth Israel Deaconess Medical Center; [2]Beth Israel Deaconess Medical Center/HMS/HHMI.

Phosphatase and tensin homolog (PTEN) and AKT kinases play antagonistic roles in a signaling pathway that has been shown to control organ size, cell size, and cell proliferation in multiple systems. In order to better understand the function of this pathway during brain development, we have inactivated PTEN or AKT in the developing murine cerebral cortex in utero. Inhibition of AKT kinases results in premature cell cycle exit. Conversely, PTEN inactivation leads to increased progenitor cell proliferation, inappropriate positioning of multiple cell types, and increased brain size. These findings suggest that AKT and PTEN are critical regulators of multiple aspects of cerebral cortical development.

271. The Rx Gene and Pituitary Gland Development. Elena A. Kozhemyakina,[1] Brian Harfe,[2] and Peter Mathers[1]. [1]Sensory Neuroscience Research Center, West Virginia University, Morgantown, WV; [2]Dept. of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL.

Targeted deletion of the mouse Rx gene causes defects in eye and forebrain development, leading us to investigate its role in patterning these tissues. Mouse pituitary development starts with evagination of oral ectoderm (Rathke's pouch), which makes contact with the evaginating ventral forebrain (infundibulum) at E10.0, leading to the formation of the anterior and posterior pituitary, respectively. We found that the infundibulum is not formed in Rx-null embryos resulting in the lack of the posterior pituitary. Surprisingly, analysis of Rx-null animals with anterior pituitary markers showed a rostral and lateral expansion of the anterior pituitary, with the oral ectoderm adopting an anterior pituitary fate. We also demonstrated that transformation of oral ectoderm into Rathke's pouch in Rx-null animals is a result of the rostral and lateral expansion of BMP4 and Fgf10 expression domains in the hypothalamus, suggesting that these factors are sufficient to induce anterior pituitary cell fate. This growth factor expansion, along with reduction of Shh, in the hypothalamic region also prevents palate and cranial bone formation. As a result, the anterior pituitary forms along the roof of the oral cavity. We performed conditional inactivation of the Rx gene in the pituitary using a Shh-driven Cre, thus preserving its activity in the developing eyes. The defects observed in the conditional mutants are similar to those observed in Rx-null animals, demonstrating that Rx has a primary role in patterning the ventral forebrain and pituitary. Support: EY12152, Ziegler Found.

272. Studies on the Mechanism of Neuronal Differentiation by the Homeodomain Transcription Factor Phox2a. Maryline Paris and Ourania Andrisani. Purdue University, West Lafayette, IN 47907.

In primary neural crest cultures, the cAMP pathway is a necessary instructive signal in sympathoadrenal cell specification. The CNS-derived

murine, catecholaminergic CAD cell line provides an invaluable cellular model for further defining the molecular affects of cAMP on neuronal differentiation. CAD cell differentiation is initiated by serum deprivation, or by co-treatment with BMP2 + IBMX (a cAMP elevating agent) in the presence of serum (differentiation medium). We defined 3 growth phases of CAD cells: a proliferative, a differentiation and an apoptotic phase. Activation of Phox2a in differentiation medium is correlated with CAD cell differentiation and accumulation of the cells at the G1 phase of the cell cycle. By contrast CAD cells grown in differentiation medium in presence of 1 nM of okadaic acid (OA), an inhibitor of PP2A, exhibit an active proliferative phase, absence of differentiation, and accumulation in the G2/M phase, followed by apoptosis. To investigate how Phox2a activity mediates cell cycle exit and differentiation, we performed time course experiments and monitored the expression of cyclins and the expression of the G1/S Cyclin dependent kinase inhibitors $p27^{Kip1}$. An increase of $p27^{Kip1}$ expression is observed in differentiation medium concurrent with its exclusive localization in the cytoplasm, as opposed to the nucleus. In contrast, upon treatment with OA, $p27^{Kip1}$ is localized in the nucleus, the cell cycle is prolonged, and the cells undergo apoptosis. Together, these results emphasize the cAMP pathway as a major player in CAD cell neuronal differentiation, acting on cell cycle progression via regulation of Phox2a activity.

273. Regulation of Neuronal Differentiation by a Novel Neuron-Specific EGF-Like Repeat Domain-Containing Protein, NELL2. Nan Sung Choi, Jungil Choi, and Byung Ju Lee. Dept. of Biological Sciences, Univ. of Ulsan, Ulsan 680-749, South Korea.

We have recently identified a novel gene named as NELL2 in the developmental procedure of the rat brain using differential display PCR. NELL2 contains six EGF-like repeat domains and is exclusively expressed in the neural tissues of developing and adult brains. NELL2 mRNA was found in the differentiating neuronal cell layers, such as preplate of developing cerebral cortex. In this study, we found that NELL2 is expressed only in the cells expressing a neuron-specific protein, NeuN in the adult rat brain. Moreover, NELL2 mRNA was colocalized only with glutamatergic neuronal markers, VGLUT-1 and -2. Using immunoprecipitation and immunoblot assays, we also found that NELL2 physically interacts with protein kinase C beta. Protein kinase C-induced phosphorylation of ERK and transcription of cfos were significantly inhibited by NELL2. When HiB5 neuroprogenitor cells were transfected with NELL2 expression vectors, the cell numbers were significantly decreased and contents of some proteins involved in the cell cycle regulation such as cyclin D2 and p53 were dramatically changed. Moreover, survival cells after transfection with NELL2 began to express neurofilament-M, a neuronal marker as well as VGLUT-1. The present results suggest that NELL2 plays a role in the cell cycle regulation of the neuroprogenitor cells and in the neuronal differentiation of glutamatergic neurons via regulating protein kinase C-ERK signaling pathway.

274. Intersectional Fate Mapping Reveals Embryonic Origins of Hindbrain Cochlear Neurons. Anna F. Farago, Rajeshwar B. Awatramani, and Susan M. Dymecki. Harvard Medical School, Boston, MA 02115.

The hindbrain cochlear nuclei are essential to the auditory circuit, relaying sensory information from the cochlea of the inner ear to higher order central processing centers. While studies using S-phase tracers suggest that cochlear neurons are derived from dorsal hindbrain neuroepithelium, the specific molecular origins of these structures remain elusive. To precisely map the origins of the cochlear and other brainstem structures, we developed a novel dual recombinase-dependent reporter line designed to activate reporter gene expression in cells that have undergone both Cre- and Flpe-mediated recombination events. Utilizing dorsally restricted Flpe-expressing and rhombomere specific Cre-expressing lines, we show that the dorsal neuroepithelium indeed generates cochlear neurons. Furthermore, our data indicate that, among dorsal progenitors, cells from distinct rhombomeric levels give rise to distinct sub-populations of cochlear neurons. These studies illustrate the specificity achievable by an intersectional fate mapping approach and uncover how molecular organizations within the rhombencephalic neuroepithelium predict mature neuronal fate.

275. The FGF8 Signaling Pathway Regulates Differentiation During the Development of the Cochlear Sensory Epithelium. Bonnie E. Jacques,[1] Erynn M. Layman,[1] Mark Lewandoski,[2] and Matthew W. Kelley[1]. [1]Developmental Neuroscience NIDCD-NIH, Bethesda, MD 20892; [2]Cancer and Developmental Biology NCI-NIH, Frederick, MD 21702.

FGF8 regulates the development of many organ systems by signaling through FGFR3. FGFR3 has been shown to be crucial for the development of the organ of Corti (OC), the mammalian cochlear sensory epithelium. The OC is characterized by many highly specialized cell types arranged into rows. FGFR3 is initially expressed in a pool of precursor cells that abuts a row of mechanosensory hair cells, the inner hair cells (IHCs). At E15.5, FGFR3-positive cells directly adjacent to the IHCs develop as two rows of pillar cells (PCs). Given that *Fgf8* begins to be expressed exclusively in IHCs at E15.5, we predict that secreted FGF8 sets up a gradient leading to differential activation of FGFR3 within the OC and subsequent cellular differentiation. We used a cre-lox strategy to generate an ear-specific *Fgf8* knock-out mouse. PCs of *Fgf8*-deficient cochleae are smaller and lack a characteristic pillar head process, suggesting a defect in differentiation. Similarly, complete inhibition of PC development was observed in whole-organ cochlear explant cultures treated with an antibody that specifically inhibits FGF8. In contrast, treatment with exogenous FGF8 had no effect on PCs. Semi-quantitative PCR analysis indicates, however, that exogenous FGF8 induces strong expression of *Sprouty2* and *Sprouty4*, known antagonists of FGF8 signaling, suggesting that treatment with FGF8 initiates a strong negative feedback loop. These results suggest that differentiation, position and number of PCs are regulated through inductive interactions with IHCs.

276. A Role for Hedgehog Signaling in Development of the Mammalian Cochlear Sensory Epithelium. E.C. Driver,[1] S.P. Pryor,[2] P. Hill,[3] J. Turner,[4] U. Rüther,[3] L.G. Biesecker,[4] A.J. Griffith,[2] and M.W. Kelley[1]. [1]NIDCD, NIH, Bethesda, MD; [2]NIDCD, NIH, Rockville, MD; [3]Heinrich-Heine University, Düsseldorf, Germany; [4]NHGRI, NIH, Bethesda, MD.

Mutations in components of the hedgehog signaling pathway cause a number of human diseases, from sporadic cancers to severe congenital defects. Mutations in the transcription factor *GLI3*, which acts both as an activator and repressor of hedgehog signaling, result in at least five clinical syndromes including Pallister–Hall syndrome (PHS). PHS is associated with imperforate anus, hypothalamic hamartoma, central polydactyly, and other defects. Recently, we have found that some PHS patients also exhibit mild to moderate hearing loss, with more severe loss at lower frequencies. To determine possible causes of the auditory defect, we examined the inner ears of a mouse model of PHS ($Gli3^{\Delta}$), which produces only a truncated Gli3 protein. We found that the cochleae of $Gli3^{\Delta/\Delta}$ mice have a variable phenotype, from nearly normal to much shorter and broader than wild-type. There are also defects on a cellular level, most notably large ectopic patches of hair cells with vestibular rather than cochlear characteristics. As truncated Gli3 represses hedgehog signaling, we also treated cochlear explants with Sonic hedgehog (Shh) to

determine the effect of pathway activation on patterning. Consistent with an inhibitory role for Shh in hair cell development, we find that Shh treatment represses development of hair cells in vitro. These findings suggest both a role for hedgehog signaling in development of the cochlear sensory epithelium, and that hearing loss is an important component of PHS.

277. Hh Signaling Regulates Formation of Purkinje Neurons in the Zebrafish Cerebellum. Karen McFarland,[1] Jolanta Topczewska,[2] and Bruce Appel[1]. [1]Department of Biological Sciences, Vanderbilt University, Nashville, TN; [2]Children's Memorial Institute for Education and Research, Northwestern University Feinberg School of Medicine, Chicago, IL.

Hedgehog (Hh) signaling is a conserved mechanism that is well known for its role in patterning the dorsoventral axis of the spinal cord. In the cerebellum, Hh signaling has a different role wherein Sonic Hedgehog (Shh), secreted by Purkinje neurons, promotes proliferation of granule neuron precursors in the external granule layer. Whether Hh signaling patterns the cerebellum as it does the spinal cord has not been thoroughly examined. We are using zebrafish as a model system to investigate consequences of abnormal Hh signaling in cerebellum development. Our data show that embryos that lack Hh signaling have excess Purkinje neurons and enlarged cerebellums. This suggests that, in contrast to its role as a mitogen for granule neurons, Hh signaling promotes the timely exit of Purkinje neuron precursors from the cell cycle.

278. Zebrafish *isl1* Promotes Motoneuron Formation and Inhibits Interneuron Formation. Sarah A. Hutchinson, and Judith S. Eisen. University of Oregon, Eugene, OR 97403.

Zebrafish primary motoneurons (pmns) provide the opportunity to study vertebrate motoneuron formation at the single cell level. We are investigating regulation of motoneuron formation by the LIM-homeodomain protein Islet1 (Isl1). In mouse, Isl1 is required but insufficient for motoneuron formation; its absence results in widespread cell death in the ventral neural tube. Zebrafish have three pmns in every spinal hemisegment; each pmn has a unique subtype identity recognizable by morphology and gene expression. As in mouse, all pmns are lost when Isl1 function is knocked down with morpholino antisense oligonucleotides (MOs). However, in contrast to mouse, MO knockdown of zebrafish Isl1 does not cause increased cell death in the ventral neural tube, but instead induces ectopic interneuron formation. Thus, zebrafish Isl1 is necessary both to promote pmn formation and to inhibit interneuron formation. Another contrast to mouse is that overexpression of zebrafish Isl1 is sufficient to induce ectopic pmns in the ventral neural tube. We are currently examining the role of Isl1 in establishing pmn subtype identity.

279. Identification of Domains in Xath5 Required for Inhibition by Notch Signaling. Constance M. Dooley, Kathryn B. Moore, and Monica L. Vetter. University of Utah, Salt Lake City, UT 84132.

Proneural basic-helix–loop–helix (bHLH) transcription factors regulate multiple aspects of vertebrate neurogenesis. Proneural bHLH factors are in turn regulated transcriptionally as well as posttranslationally. Notch signaling is one regulatory pathway responsible for controlling the expression and activity of many proneural bHLH factors, however, the precise mechanisms of posttranslational regulation by Notch remain largely undefined. In order to explore how Notch inhibits the activity of proneural bHLH factors, we have analyzed the domains within the *Xenopus* atonal homologue, Xath5, that are responsible for mediating sensitivity to Notch. The related atonal homologue, XNeuroD, is relatively insensitive to Notch signaling, which allowed us to replace domains in XNeuroD with the corresponding domains from Xath5 and assess the ability of Notch to inhibit proneural activity during primary neurogenesis. A chimeric XNeuroD with the Xath5 bHLH domain was effectively inhibited by Notch signaling, and we demonstrate that this is due to the HLH dimerization motif and not the basic DNA binding domain. However, we found that the basic DNA binding domain may play a role in reducing the sensitivity of Xath5 to Notch since XNeuroD with the Xath5 HLH domain alone is more sensitive to Notch than XNeuroD with the entire Xath5 bHLH domain. Our results suggest that different regions within the bHLH domain positively and negatively regulate the sensitivity of Xath5 to Notch inhibition.

280. The Roles of Xebf3 During *Xenopus* Neurogenesis. Yangsook S. Green and Monica L. Vetter. University of Utah, Salt Lake City, Utah 84132.

Collier/Olf/EBF (COE) proteins have important roles in many aspects of animal development, such as head and muscle development, neurogenesis, and B-cell and adipocyte differentiation. Xebf3 is a member of the COE protein family expressed in the developing *Xenopus* nervous system. Xebf3 is expressed in the neural plate during early neurogenesis and in many other neuronal populations later. The overexpression of Xebf3 has been shown to cause ectopic expression of n-tubulin, a differentiated neuronal marker. The purpose of this study is to understand how Xebf3 affects neural differentiation in the developing embryo in general, and the developing retina in particular. After overexpressing Xebf3 in the early *Xenopus* embryo, we found a decrease in the expression of markers of neural progenitors (Sox2 and Slug). This suggests that Xebf3 may act to encourage progenitor cells to differentiate, consistent with its previously demonstrated role in promoting neuronal differentiation. In the retina, we found by in situ hybridization that Xebf3 is expressed specifically in the ganglion cell layer and in some inner nuclear layer cells. Overexpression of Xebf3 in cells destined to become retina cells lead to a modest increase in ganglion cell production. Finally, we have generated a DNA construct in which a human glucocorticoid receptor region is fused to Xebf3. This construct will allow us to control the activity of Xebf3 in *Xenopus* animal caps with exogenous application of hormone. We plan to use this technique to identify transcriptional targets of Xebf3 by performing a microarray comparison of genes expressed in tissue with and without active Xebf3.

281. Role of N-Cadherin-Mediated Cell Adhesion in Regulating Neurogenesis. Kavita Chalasani,[1] Keisha John,[2] Sang-Yeob Yeo,[3] Ajay Chitnis,[3] and Rachel Brewster[1]. [1]UMBC, Baltimore, MD; [2]Watson school of Biological Sciences, CSHL, NY; [3]NIH/NICHD, Bethesda, MD.

During neurogenesis, precursor cells differentiate to form different neuronal cell types in response to various molecular signals. Regulation of neurogenesis is therefore a critical factor for normal development. Following neural tube formation, neural precursor cells are arranged as an epithelial layer in the ventricular zone, while neurons are loosely associated mesenchymal cells found in more basal regions of the neural tube. As precursor cells undergo differentiation, they loose contact with their neighbors, in a process characterized by loss of adherens junctions. Down-regulation of cell adhesion is thus an important feature of neurogenesis. However, it is not understood whether loss of adhesion is a prerequisite or a mere consequence of differentiation. Previous studies have demonstrated that dissociated neuroectodermal progenitor cells generate neurons at a higher frequency than cells maintained in close proximity. Moreover, in the zebrafish, loss of N-Cadherin (N-Cad) results in enhanced neurogenesis. Using zebrafish as a model system,

we have now begun to investigate the hypothesis that loss of adhesion might be a pre-requisite for neuronal differentiation. Here, we further analyze the neurogenic defect in N-Cad mutants and investigate the potential interaction between N-Cad and the Notch and Wnt signaling pathways that are both implicated in neurogenesis.

282. Asymmetry of a Cholinergic System in the Zebrafish Brain. Kirankumar Santhakumar,[1] Christine Thisse,[2] Bernard Thisse,[2] and Marnie E. Halpern[1]. [1]Carnegie Institution of Washington, Baltimore, MD 21210, USA; [2]IGBMC, CU de Strasbourg, 67404 Illkirch cedex, France.

Connectivity between the diencephalic habenular (Ha) nuclei and midbrain interpeduncular nucleus (IPN) is highly conserved from fish to man. In lower vertebrates, left and right habenulae can differ in subnuclear divisions, degree of dense neuropil and gene expression. The habenulo-interpeduncular (Ha-IPN) system contains cholinergic neurons and is enriched for specific nicotinic acetylcholine receptor subtypes. We examined expression of markers of cholinergic neurons [choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAChT), high affinity choline transporter (HACT) and acetylcholinesterase (AChE)] in the zebrafish Ha-IPN system. A newly identified gene with high similarity to VAChT (VAChT-r) shows distinct asymmetric expression in the habenulae, with transcripts localized to the right medial habenula but not the left. To identify ENU-induced mutations that affect cholinergic neurons and left–right asymmetry of the Ha-IPN system, we assayed VAChT-r expression. In mutant c210 larvae, VAChT-r expression is variably disrupted in the habenulae at 4 days; however, expression of other habenula-specific genes appears normal. VAChT-r expression is unaffected in other brain nuclei or in spinal cord motoneurons. Interestingly, c210 mutants also display ectopic VAChT-r expression in the superficial retina, and lack AChE expression in a deeper cell layer of the retina. We are determining the identity of the retinal cells that are affected in the mutant and confirming that the c210 mutation is selectively disrupting cholinergic habenular neurons.

283. Corneal Stromal Cells Can De-Differentiate and Contribute to Multiple Neural Crest Derivatives. Peter Y. Lwigale, Paola A. Cressy, and Marianne Bronner-Fraser. California Institute of Technology.

Cranial neural crest cells migrate into the periocular region of the eye to contribute to the cornea. Upon reaching the presumptive corneal stroma, neural crest cells differentiate, synthesize extracellular matrix and become entrapped. We investigated whether corneal stromal cells were terminally differentiated or could de-differentiate and contribute to neural crest derivatives when challenged with a new environment. Stromal cells were enzymatically isolated from E10 to 16 quail corneas, as a single cell suspension and then either cultured in vitro or injected alongside the dorsal neural tube of stage 9 chick embryos, a normal neural crest migratory pathway. Analysis of embryos at various stages of development revealed that the injected stromal cells migrated ventrally adjacent to the host neural crest cells. Forty hours after injection, majority of the stromal cells had lost their state of differentiation and expressed markers characteristic of neural crest cells in their new environment. Contrary, stromal cells maintained their state of differentiation in vitro. Immunohistochemistry with tissue-specific markers indicated that the injected stromal cells could contribute to the corneal endothelial and stromal layers, mandibular process, blood vessels and cardiac cushion tissue. However, they were never found within cranial ganglia. Our data indicate that stromal cells can migrate, de-differentiate and contribute to the non-neuronal cranial neural crest derivatives. These results suggest that corneal stromal cells even at late embryonic stages are not terminally differentiated, but rather maintain plasticity and multipotentiality.

284. Cellular and Genetic Characterization of the Zebrafish ENS Mutant Enema. Meaghann Guyote,[1] Jean-Marie Delalande,[1] William S. Talbot,[2] and David W. Raible[3]. [1]Dept. of Biology, Emory University, RRC 1510 Clifton Road, Atlanta, GA 30322; [2]Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305; [3]Department of Biological Structure, University of Washington, Box 357420, Seattle, WA 98195.

The enteric nervous system (ENS) is the largest most complicated subdivision of the peripheral nervous system and is completely derived from the neural crest. Enema (enm) was identified in a genetic screen that looked for zebrafish ENS development mutants. enm mutants have a significant reduction in the number of enteric neurons at 96 hpf. In addition to its ENS phenotype, this mutation is also characterized by other abnormalities affecting development of the eyes, the thymus, the jaw cartilages and the heart. With the exception of the eyes, all of these structures are entirely derived, or receive cellular contributions, from the post-optic NCCs. This suggests that the enm mutation is in a gene that is essential for the normal development of this NCC population. To determine the earliest stage at which we could identify a defect in ENS development in enm mutants, we examined the expression of phox2b. Preliminary results suggest that the enm mutation affects the ability of ENS precursors to colonize the gut. Experiments are currently being undertaken to assess if the decrease in enteric precursors is due to reduced proliferation or increased apoptosis in the enm ENS precursors. To determine the genetic basis of the enm mutation we have generated a mapping hybrid. We have fine mapped the mutation and find that it is tightly linked to the pax9 locus. We will report our progress in determining the genetic basis for the enm mutation.

285. Zebrafish prdm1 is Required for Neural Crest and Sensory Neuron Specification. Kristin B. Artinger, Laura Hernandez-Lagunas, Irene F. Choi, Christy Cortez, Takao Kaji, and Peter Simpson. University of Colorado Health Sciences Center, Aurora, CO.

prdm1 is the only known gene that is expressed in a putative progenitor population of cells at the neural plate border: Neural crest cells and Rohon–Beard (RB) sensory neurons. We hypothesize that both these cell types arise from either a multipotent precursor or several unipotent precursors within the border domain. We have designed several experiments to determine whether prdm1 functions as a cell fate switch in the segregation of this lineage. The zebrafish narrowminded (prdm1) mutation has defects that are observed in both cell populations: It leads to a complete absence of RB neurons and a reduction of neural crest cells and their derivatives. Additionally, over-expression of prdm1 mRNA promotes the development of both cell types and mosaic analysis suggests that prdm1 acts cell autonomously for both neural crest and RB sensory neurons. Cell lineage analysis is underway to determine if neural crest and RB sensory neurons can arise from a common progenitor or from two separate progenitors. These data suggest that prdm1 is not a simple molecular switch promoting neural crest fate verses RB sensory neurons. In order to define the molecular pathway for neural crest and RB sensory neuron development, we analyzed expression of prdm1 in BMP mutant embryos. prdm1 expression is eliminated at the neural plate border in swr (BMP2b), expanded in sbn (Smad5) and reduced in snh (BMP7) mutant embryos. These data in combination indicate that prdm1 acts cell autonomously to regulate cell fate specification at the neural plate border, acting downstream of the initial BMP-inducing signal.

286. Role of Egfr/Ras Pathway in the Specification of Sensory Organs of the Wing Margin in *Drosophila melanogaster*. Pallavi Kshetrapal and Shashidhara L.S. Centre for Cellular and Molecular Biology, Hyderabad 500 007, India.

The wings of the fruitfly *Drosophila melanogaster* are specialized structures comprising of the wing lamina, veins and sensory bristles on

the margin, all of which are made up of different cell types. Interplay of different signaling pathways brings about this diversity in cell type. Sensory organs of the wing margin are composed of the mechanosensory and chemosensory types of bristles. The accumulation of proneural proteins (of the Achaete–Scute complex) in the sensory-mother cells is critical for the determination and differentiation of these bristle types. The expression of proneural genes is dependent on the function of Wingless (Wnt/Wg) at the dorso-ventral boundary. We have observed that down-regulation of Egfr/Ras pathway cell-autonomously suppresses sensory bristle development, while ectopic activation of the pathway causes ectopic bristle development. However, ectopic bristle development was conditional to the presence of high levels of Wnt/Wg signals. Thus, our observations suggest that the Wnt/Wg pathway specifies the pro-neural field and the Egfr/Ras pathway sets up the developmental program for the sensory bristle formation along the wing margin. Detailed results of loss- and gain-of-function genetic experiments would be presented, which also suggests a mechanism for Engrailed-mediated repression of sensory bristle development in the posterior compartment.

287. Late Specification of the Larval Peripheral Nervous System in the Ascidian *Ciona intestinalis*. Robert W. Zeller, Mark A. Pellatiro, Anthony Okamura, and Kevin LaFontaine. San Diego State University.

The ascidian larval peripheral nervous system (PNS) consists of a series of ciliated cells, called epidermal sensory neurons (ESNs), which are located along the dorsal and ventral midlines of the larval tail and on the dorsal and anterior trunk. Experiments from other laboratories have identified several genes that are expressed in these cells and have suggested that Notch-Delta lateral inhibition may play a role in the formation of the ESNs. We have now identified and characterized a number of regulatory genes from *Ciona intestinalis* that are involved in later aspects of PNS specification. We propose a model in which one or more Atonal-related bHLH genes are expressed in broad stripes along the midline of the dorsal and ventral tail epidermis. Notch-Delta lateral inhibition then selects certain midline cell for the PNS fate while the remaining cells become epidermis. Next, genes encoding two transcription factors, a different Atonal-related bHLH gene and a Pou-IV class gene, are expressed in the presumptive PNS cells. Ectopic expression of a dominant-negative Suppressor of Hairless gene throughout the embryonic epidermis disrupts Notch-Delta lateral inhibition resulting in the appearance of supernumerary ESNs, but only along the dorsal and ventral midlines. Ectopic expression of several of the Atonal-related genes in the epidermis also produces supernumerary ESNs along the dorsal and ventral midlines and additionally in the lateral epidermis where ESNs are not normally produced. This suggests that the domain of Notch-Delta lateral inhibition has expanded beyond the midline epidermis into epidermal territories that never give rise to ESNs.

288. The Role of Nutrition in Creation of the Eye Imaginal Disc and Initiation of Metamorphosis in *Manduca sexta*. David T. Champlin,[1] J. Paul Allee,[1] Steven G. MacWhinnie,[1] Charles A. Nelson,[2] Lynn M. Riddiford,[2] and James W. Truman[2]. [1]University of Southern Maine, Portland, ME 04103; [2]University of Washington, Seattle, WA 98195.

With the exception of the wing imaginal discs, the imaginal discs of *Manduca sexta* are not formed until early in the final larval instar. An early step in the development of these late-forming imaginal discs from the imaginal primordia appears to be an irreversible commitment to form pupal cuticle at the next molt. Similar to pupal commitment in other tissues at later stages, activation of broad expression is correlated with pupal commitment in the adult eye primordia. Feeding is required during the final larval instar for activation of broad expression in the eye primordia and dietary sugar is the specific nutritional cue required. Dietary protein is also necessary during this time to initiate the proliferative program and growth of the eye imaginal disc. Although the hemolymph titer of juvenile hormone normally decreases to low levels early in the final larval instar, eye disc development begins even if the juvenile hormone titer is artificially maintained at high levels. Instead, creation of the late-forming imaginal discs in Manduca appears to be controlled by unidentified endocrine factors whose activation is regulated by the nutritional state of the animal.

289. The Functional Role of Pax-3 in Ophthalmic Trigeminal Placode Development. James R. Bradshaw and Michael R. Stark. Brigham Young University, Provo, UT.

In vertebrate embryos, the ophthalmic trigeminal (opV) placode contributes sensory neurons to the trigeminal ganglion during development of the peripheral nervous system. Pax-3 is one of the earliest genes expressed in opV placode cells, and while it has been used extensively as a marker of the opV placode, its functional role in the development of these cells has not been investigated. Here, we present data showing that Morpholino knockdown of Pax-3 in cranial ectoderm results in a loss of Pax-3 protein as well as all other known opV placode markers, including FGFR4, Ngn2, Brn-3a, and other neuronal markers. From this, we conclude that Pax-3 is necessary for normal opV placode development. We also present data showing upregulation of FGFR4 in response to ectodermal Pax-3 misexpression. Other opV placode markers are only minimally upregulated, or show no response to Pax-3 misexpression, indicating Pax-3 is not sufficient for complete opV placode cell differentiation.

290. Olfactory and Lens Placode Precursors are Independently Specified Despite a Shared Spatial Origin. Sujata Bhattacharyya,[1] Andrew Bailey,[2] Andrea Streit,[2] and Marianne Bronner-Fraser[1]. [1]California Institute of Technology, Pasadena, CA, USA; [2]King's College, London, UK.

Sensory placodes arise transiently as discrete thickenings of the embryonic ectoderm and contribute significantly to the cranial peripheral nervous system. Of these, the olfactory placode gives rise to the olfactory epithelium, whereas the lens placode forms the lens of the eye. How and when are olfactory and lens placode precursor cells specified? Using focal dye injections, we have determined that precursors for both placodes coexist at the border between the anterior neural plate and the adjacent non-neural ectoderm at early neurula stages in chick (HH6,7). In fact at this time, a small population of labeled cells often gives rise to progeny in both the olfactory and lens placodes. Is the heterogeneity of this common lens-olfactory territory reflected at the single cell level? To address this, we injected single cells in this territory and determined the fate of their progeny. Surprisingly, we find no evidence for a shared olfactory/lens placode lineage from single precursors even at early neurula stages suggesting that these precursors are specified towards their respective fate prior to any overt signs of placode formation. Furthermore, in isolation, the common lens-olfactory region is capable of generating Pax6+, delta-crystallin+ lentoid bodies in addition to Dlx3+ cells. Neuronal specification only occurs when the anterior neural ridge is included. We are currently testing the hypothesis that FGF signaling may play a role in specifying cells along an olfactory pathway while placode precursor cells unresponsive to this signal adopt a lens fate.

291. Induction and Cell Fate Specification in Early Eye Development. Peter Mathers,[1] Vera Voronina,[2] Elena Kozhemyakina,[1] Vicki Bennett,[1] Brian Zamora,[1] and Mark Lewandoski[2]. [1]Sensory Neuroscience Research Center, West Virginia U., Morgantown, WV; [2]Genetics of Vertebrate Development Section, NCI, Frederick, MD.

Bmp and Fgf families are implicated in inductive signaling between the optic vesicle and lens ectoderm. We have studied the role of the Rx

homeobox gene in regulating inductive signaling and cell fate determination at the optic vesicle/cup stages of eye development. As Rx-nulls fail to form optic vesicles, we developed conditional deletion and hypomorphic Rx alleles to monitor its function at later stages of ocular formation. Deletion of Rx activity by Foxg1-Cre during optic vesicle formation blocks lens placode induction, in part through the lack of Bmp4, Fgf8, and Fgf15 in the optic vesicle. The optic vesicle in these mutants fails to develop a neural retina, with the distal vesicle adopting a retinal pigment epithelial (RPE) fate. Using an Fgf9 transgene that converts RPE to neural retina, the Rx conditional mutant phenotype is not rescued by Fgf9, suggesting that Rx acts cell-autonomously in neural retina downstream of Fgf activation. Embryos with a single hypomorphic Rx allele also fail to develop neural retina and again RPE forms. Homozygous Rx hypomorphs exhibit microphthalmia, cataracts, and ectopic RPE within the neural retina. Deletion of Rx during optic cup stages leads to reduced proliferation of retinal stem cells and altered neural retinal cell fates, with a bias toward early born cells. Our current hypotheses are that Rx promotes neural retinal identity and retinal stem cell proliferation, while deletion of Rx causes premature cell cycle exit and altered cell fate determination. Support-EY12152, Ziegler Found.

292. Role of Dkk2 in the Maintenance of Ocular Surface Morphology. Mahua Mukhopadhyay,[1] Maria Morasso,[2] Chang Ma,[1] and Heiner Westphal[1]. [1]Laboratory of Mammalian Genes and Development, NICHD, National Institutes of Health, Bethesda, MD; [2]Developmental Skin Biology Unit, NIAMS, National Institutes of Health, Bethesda, MD.

Dickkopf (Dkk) genes enclode a family of secreted cysteine-rich proteins. Here, we report on the consequences of a Dkk2 knockout in the mouse. Our analysis provides genetic evidence for an inhibitory role of the Dkk2 gene in Wnt signaling. The cornea of Dkk2−/− mutants shows a dramatic transformation from a stratified epithelium to a cornified epithelium. We observe features normally associated with skin and conjunctiva, respectively, including hair follicles, sebaceous glands and goblet cells. The eyes of wild-type mice open 12−14 days after birth. Thereafter, there is a turnover of the corneal epithelium every 3−4 weeks as a result of normal loss of apical cells by desquamation and apoptosis. Corneal epithelial stem cells located in the basal layer of the limbus are responsible for the maintenance of the epithelial turnover and ocular surface integrity. We propose that this process is governed by Dkk2 mediated inhibition of Wnt pathway.

293. Function of Bhlhb5 (Beta3) Transcription Factor in Retinal Development. Lin Gan and Liang Feng. University of Rochester, Rochester, NY 14642.

Targeted deletion of Math5 in mice leads to the absence of retinal ganglion cell (RGC) and concurrently, the increase in the number of cone and displaced amacrine cells, suggesting Math5′s dual roles in promoting RGC and inhibiting non-RGC differentiation pathways. It remains unclear what factors mediate the suppressing effects of Math5 on non-RGC differentiation. We show here that Bhlhb5 is expressed in developing retina starting at E12. In adult, its expression is limited to a subset of cone bipolar and non-type II, GABAnergic amacrine cells. Intriguingly, Bhlhb5 expression is up-regulated in math5-null retina throughout embryogenesis. In adult math5-null mice, a significant increase in Bhlhb5-positive displaced amacrine cells were seen in the ganglion cell layer. To investigate its role in retinal neurogenesis, we used retrovirus-mediated gene transfer method to ectopically express Bhlhb5 in developing chicken retinas and demonstrated that the gain-of-function of Bhlhb5 resulted in the increase of amacrine cell differentiation. These results imply that Bhlhb5 plays a positive role in the amacrine cell development and that the suppression of amacrine cell differentiation by Math5 is likely mediated by Bhlhb5.

294. Pax6 Function in the Vertebrate Retina. Yi-Wen Hsieh, Xiang-Mei Zhang, Joseph Horwitz, and Xianjie Yang. Molecular Biology Institute and Jules Stein Eye Institute, David Geffen School of Medicine, University of California, Los Angeles.

Pax6 is expressed in all retinal progenitor cells (RPC). As retinal development progresses, Pax6 expression is maintained in post mitotic retinal ganglion cells (RGC), amacrine cells, and horizontal cells; while Pax6 in photoreceptor cells and bipolar cells becomes undetectable. Previous studies using mutant and transgenic mice indicate that the dosage of Pax6 gene expression is critical for eye/retinal development. Furthermore, retinal specific Pax6 knockout in RPC leads to the production of only amacrine cells and the loss of other retinal cell types including photoreceptors, suggesting that Pax6 is required for the maintenance of RPC to be multipotent. Overexpression of Pax6 in embryonic chicken retinal cultures causes increased numbers of RGC and decreased the number of photoreceptor cells. Therefore, we have used a temporal RNAi knockdown strategy to investigate Pax6 function in the developing retina. Embryonic chicken retinas were electroporated with DNA constructs containing a nuclear GFP cassette and U6 promotor with or without Pax6 targeting short hairpin RNA. The transfected retinas were cultured as explants and labeled with BrdU towards the end of the culture period. Immunostaining analyses of RPC and photoreceptor markers indicated that RNAi-mediated knockdown of Pax6 did not affect RPC proliferation during the period of BrdU labeling and decrease in the level of Pax6 drives progenitor cells towards a photoreceptor cell fate. These results suggest that temporal regulation of Pax6 is critical for retinal cell type differentiation.

295. Math5 is Required for Early Retinal Neuron Differentiation and Cell Cycle Progression. Nadean L. Brown,[1] Emily Wroblewski,[2] Sima Patel,[2] and Tien T. Le[1]. [1]Children's Hospital Research Foundation, Cincinnati, OH; [2]Children's Memorial Institute for Education and Research, Chicago, IL.

In the mammalian retina, removal of the bHLH factor Math5 causes the loss of retinal ganglion cells (RGCs) and appearance of excess cone photoreceptor and amacrine neurons. We have found that Math5 mutant cells are unable to adopt the earliest fates, particularly that of an RGC, at E11.5. Along with the loss of early retinal neurons, surrounding progenitors undergo abnormal mitotic cell cycles in the absence of Math5. Math5 suppression of cone and amacrine determination correlates with the upregulation of NeuroD1 and Ngn2, which induce these fates. However, Math5 regulates each process differently as NeuroD1 and amacrine genesis increase cell-autonomously while Ngn2 and cone genesis are upregulated in cells surrounding those that express Math5. Thus, bHLH factors regulate neurogenesis via both intrinsic and extrinsic mechanisms.

296. Frizzled 5 Signaling Governs the Neural Potential of Progenitors in the Developing *Xenopus* Retina. Kathryn B. Moore,[1] Terence J. Van Raay,[2] Ilina Iordanova,[3] Michael Steele,[1] Milan Jamrich,[4] William Harris,[3] and Monica L. Vetter[1]. [1]Univ. of Utah, Salt Lake City, UT 84054; [2]Vanderbilt Univ., Nashville, TN 37235; [3]Univ. of Cambridge, Cambridge, UK; [4]Baylor College of Medicine, Houston, TX 77030.

An important step in neural development is the process by which neural precursors acquire and then maintain neural potential, allowing them to undergo neuronal differentiation. SoxB1 transcription factors function as intrinsic regulators of this process, affecting the formation and maintenance of neural-competent precursors in multiple regions of the CNS. There is evidence that their expression is regionally regulated suggesting that specific signals regulate neural potential in subdomains of

the developing CNS; however, these extrinsic signals have not been defined. Using the developing *Xenopus* retina as a model system for CNS development, we show that the frizzled (Fz) transmembrane receptor Xfz5 selectively governs neural potential in the retinal subdomain by regulating the expression of Xsox2. Inhibiting either Xfz5 or canonical wnt signaling within the developing retina does not alter the expression of multiple progenitor markers, but blocks Xsox2 expression, reduces cell proliferation, inhibits the onset of proneural and neurogenic gene expression, and biases individual progenitors towards a non-neural fate. Rescue experiments indicate that Xsox2 is downstream of Xfz5 while blocking Xsox2 function mimics the effects of Fz5 inhibition. Thus, the regulation of neural potential in a subset of CNS progenitors is dependent upon Fz signaling.

297. Analysis of Pax6 Alpha Transcripts Using BAC Technology Reveals Restricted Expression in Amacrine Cells. Jorn Lakowski and James D. Lauderdale. University of Georgia, Athens, GA 30602.

The layered architecture of the vertebrate retina depends on the correct generation and assembly of seven distinct cell types, which are derived from a common, multipotent population of progenitors. In these progenitors, cell fate decisions, which result in the appearance of different cell types in a defined histogenetic order, are controlled by both extrinsic and intrinsic factors. One of the intrinsic factors controlling the retinogenic potential of the precursor cells is the evolutionarily conserved transcription factor Pax6. In the quail neuroretina, Pax6 is expressed both as paired-less and paired-containing isoforms. However, the function of the paired-less form is not known. In *C. elegans*, it was found that a similarly truncated Pax6 protein was crucial to development of the male specific sensory organ by controlling cell fate. To test our hypothesis that the paired-less isoform plays a role in cell fate decisions during retinogenesis, we used bacterial artificial chromosome (BAC) technology. Using two different reporter genes, a BAC containing the zebrafish Pax6a gene, was modified in such a way, that one could distinguish between transcripts giving rise to either of the two Pax6 isoforms. We show that transcripts which correspond to the truncated form of Pax6 are exclusively detected in amacrine cells of the developing zebrafish retina. The spatial and temporal onset of expression of these transcripts suggests that the short isoform is involved in the cell fate decision leading to the generation of amacrine cells.

298. *Math5* Establishes Ganglion Cell Competence in Postmitotic Retinal Cells. Tom Glaser and Joseph A. Brzezinski. University of Michigan, Ann Arbor, MI 48109.

The *atonal* homolog *Math5* (also *Atoh7*) is transiently expressed during early retinal histogenesis. This bHLH factor is absolutely required for retinal ganglion cell (RGC) fate specification and optic nerve development. We have traced the lineage of *Math5* expressing cells. *Math5-Cre* BAC transgenic mice were created and crossed to Z/AP and R26-GFP reporter strains. We also co-stained *Math5-lacZ* knock-in retinas for *lacZ* and phase-specific cell cycle markers. *Math5*+ cells contribute to every major cell type in the adult retina, but are heavily skewed toward fates with early birthdates, consistent with the timing of *Math5* expression. In addition to RGCs, the Math5-Cre transgenes label 30% of cones and horizontal cells, 10% of amacrines, 1.5% of rods, and $< 0.1\%$ of bipolar and Müller cells. When Z/AP and GFP reporters were tested simultaneously, the concordance of labeling was $> 90\%$ in every cell type. Activated Z/AP reporter and perdurant *Math5-lacZ* are excluded from M, S and G2 phases of the cell cycle. *Math5* is therefore expressed by postmitotic cells. Approximately 5% of retinal neurons descend from *Math5*+ cells, but $\leq 10\%$ of these develop as RGCs. In *Math5* mutants, these cells adopt a variety of non-RGC fates. Math5 is thus a permissive factor, creating an RGC competence state within a cohort of progenitors after they exit the cell cycle. Only some of these cells are selected to form RGCs. Our findings demonstrate remarkable plasticity in postmitotic retinal cells and suggest a general mechanism for fate specification. We are currently testing the conditions and potential cofactors that ultimately direct *Math5*+ cells to become RGCs. Supported by NIH grant EY14259.

299. DLX1 and DLX2 Regulation of the Neurotrophin Receptor Trk-B and Survival of Late-Born Retinal Ganglion Cells in the Vertebrate Retina. J. de Melo, Q.P. Zhou, S. Cheng, and D.D. Eisenstat. Depts. of Anatomy and Pediatrics, University of Manitoba, Winnipeg, Canada.

Dlx homeobox genes are expressed in retinal ganglion cells (RGC), amacrine and horizontal cells of the developing and postnatal retina. Phenotypic analysis of the Dlx1/Dlx2 null retina demonstrates a reduced ganglion cell layer (GCL), with loss of late-born differentiated RGCs due to increased apoptosis. Signaling through the neurotrophin (BDNF, NT-4/NT-5) receptor TrkB is proposed to regulate the dynamics of RGC apoptosis throughout development. Onset of TrkB expression is E12.5 and extends postnatally through to the adult. Expression becomes limited to the GCL by E18.5. TrkB is co-expressed with Dlx1 and Dlx2 and with RGC markers such as Brn3b and Isl-1. In the Dlx1/Dlx2 null retina, TrkB expression is reduced in the GCL as early as E13.5. We have explored TrkB as a candidate transcriptional target of Dlx genes. Using a modified chromatin immunoprecipitation (ChIP) assay of embryonic retina, DLX2 binds to specific regions of the TrkB promoter in situ. In vitro confirmation of binding by gel shift assays and the functional consequences of DLX binding to regions of the TrkB promoter by reporter gene assays signify the potential importance of TrkB regulation by Dlx homeoproteins. The generation of RGC from retinal progenitors and their subsequent terminal differentiation and survival require the coordinated expression of transcription factors, such as Math5, Brn3b, Dlx1 and Dlx2. Signaling mediated by the neurotrophin receptor TrkB may contribute to survival of late-born RGCs whose terminal-differentiation is regulated by Dlx function.

300. Wnt-3a Regulation of Somite Myogenesis. Michelle Baranski and Stephen Hauschka. University of Washington, Seattle, WA 98109.

Skeletal muscle development in somites is mediated by signals from the neural tube, notochord, ectoderm and the somites themselves. Nine different Wnts are expressed in the somites or in surrounding tissues. To investigate the role of Wnts in somite myogenesis, undifferentiated somites were explanted from stage 12 chick embryos and incubated in the presence of conditioned medium (CM) from L cells stably transfected with Wnt-3a or CM from control L cells. Additionally, some explants were treated with commercially available semi-purified Wnt-3a to examine the affects of Wnt-3a in the absence signaling factors present in CM. Treated explants were assayed for myogenic induction via immunostaining and quantitative RT-PCR. Treatment of explants with control CM or unconcentrated Wnt-3a CM results in little or no myogenesis. However, concentrated Wnt-3a CM is sufficient to induce myogenesis, and $30\times$ Wnt 3a CM induces at least 4 times more cells to become myosin heavy chain positive as $3\times$ Wnt-3a CM. Semi-purified Wnt-3a protein is also sufficient to induce myogenesis in explanted immature somites. Quantitative RT-PCR of explants indicates concentrated Wnt-3a CM or semi-purified Wnt-3a protein is sufficient for upregulation of the myogenic regulatory factor myf-5. To determine if a subset of somite cells is myogenically responsive to Wnt-3a, portions of immature somites were incubated in concentrated Wnt-3a CM. Results indicate that cells from different parts of the immature somite differ in their Wnt responses. Overall, these ongoing studies suggest that undifferentiated cells in certain regions of the somite are myogenically responsive to Wnt-3a, and that this response can be assayed through measuring myf-5 expression.

301. Potency of Skeletal Muscle Stem Cells in the Early Chick Embryo. Mindy George Weinstein,[1] Jacquelyn Gerhart,[1] Christine Neely,[1] Luis Narciso,[1] Justin Elder,[1] Tage Kvist,[1] and Karen Knudsen[2]. [1]Philadelphia College of Osteopatic Medicine, Philadelphia, PA 19131; [2]Lankenau Institute for Medical Research, Wynnewood, PA 19096.

The differentiation of skeletal muscle begins in the somites. Although myogenesis is regulated in part by tissues surrounding the somites, the skeletal muscle specific transcription factor MyoD is expressed in the embryo prior to somite formation. MyoD is present in a subpopulation of cells within the epiblast, the epithelium that gives rise to all tissues of the embryo. These cells appear to be committed to the skeletal muscle lineage since they undergo skeletal myogenesis when implanted into the heart-forming fields. MyoD positive, but not MyoD negative epiblast cells, downregulate E-cadherin, upregulate N-cadherin, and differentiate into skeletal muscle in vitro. MyoD positive cells stimulate skeletal myogenesis and N-cadherin synthesis in MyoD negative cultures by releasing an inhibitor of the bone morphogenetic protein signaling pathway. Furthermore, ablation of MyoD positive cells in the pregastrulating epiblast results in herniation of the organs through the ventral body wall and a reduction in differentiated muscle in the myotomes and limbs. These findings demonstrate that MyoD positive epiblast cells recruit pluripotent cells to the skeletal muscle lineage in vitro by blocking the BMP signaling pathway and promoting N-cadherin mediated cell–cell adhesions. They also suggest that skeletal muscle stem cells that emerge prior to the onset of gastrulation are crucial for skeletal myogenesis during later stages of development.

302. MyoD Positive Cells of the Pregastrulating Embryo are Critical for Morphogenesis and Skeletal Muscle Differentiation. Justin Elder,[1] Jacquelyn V. Gerhart,[1] Christine Neely,[1] Howard Eisenbrock,[1] Tage Kvist,[1] Karen Knudsen,[2] and Mindy George-Weinstein[1]. [1]Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131; [2]Lankenau Institute for Medical Research, Wynnewood, PA 19096.

Messenger RNA for the skeletal muscle specific transcription factor MyoD is expressed in a subpopulation of chick epiblast cells. MyoD positive cells recruit pluripotent epiblast cells to the skeletal muscle lineage in vitro by producing an inhibitor of the bone morphogenetic protein signaling pathway. A technique was developed to study the role of MyoD positive cells during myogenesis in vivo. Embryos were removed from the shell on the yolk and labeled with the G8 monoclonal antibody that binds specifically to epiblast cells that express MyoD. G8/MyoD positive cells were lysed by incubating embryos in complement. After treatment, embryos on the intact yolk were returned to the shell, covered with plastic wrap, and incubated at 37°. Control embryos treated with G8 or complement alone developed normally for at least 10 days. Embryos treated with G8 and complement exhibited herniation of the heart and abdominal organs through the ventral body wall. Immunofluorescence localization of sarcomeric myosin revealed a decrease in differentiated skeletal muscle in the dorsal and ventral myotomes and limbs. These results provide strong evidence that skeletal muscle stem cells of the epiblast play a role in regulating skeletal myogenesis at later stages of development.

303. MyoD Positive Cells of the Pregastrulating Embryo are Committed to the Skeletal Muscle Lineage. Luis Narciso,[1] Jacquelyn Gerhart,[1] Jordanna Perlman,[1] Christine Neely,[1] Kersti Linask,[2] and Mindy George-Weinstein[1]. [1]Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131; [2]University of South Florida, St. Petersburg, FL 33701.

Although skeletal muscle differentiation begins in the somites, the presomitic mesoderm of the chick embryo contains cells that express mRNA for the skeletal muscle specific transcription factor MyoD. A population of MyoD expressing cells also is present in the epiblast, the tissue that gives rise to mesoderm during gastrulation. When MyoD positive cells are isolated from the epiblast cultured, they differentiate into skeletal muscle. To determine whether epiblast cells with MyoD are stably committed to the skeletal muscle lineage, we tested their potential to differentiate into cardiac muscle when implanted into the heart-forming fields. Stage 1 embryos were labeled with the G8 MAb antibody that binds MyoD positive epiblast cells. Cells labeled with G8 were isolated by magnetic cell sorting, stained with Hoechst dye, and microinjected into the precardiac mesoderm of stages 4–5 embryos. After culturing the embryos on nucleopore filters for 48 h, the hearts were removed and dissociated with trypsin. Cells were centrifuged onto slides and stained with antibodies to sarcomeric myosin heavy chain, the skeletal muscle specific 12101 antigen, and cardiac muscle specific troponin T. Most MyoD/G8 positive epiblast cells implanted into the heart differentiated into skeletal muscle. By contrast, MyoD negative epiblast cells formed cardiac muscle. Since cells that express MyoD mRNA undergo skeletal myogenesis in the developing heart, they appear to be stably committed to the skeletal muscle lineage.

304. Muscle Specification in *Xenopus laevis* Gastrula-Stage Embryos. Syed A. Rizvi,[1] Kathleen Wunderlich,[2] Jean K. Gustin,[3] and Carmen R. Domingo[1]. [1]San Francisco State University, San Francisco, CA 94132; [2]Biomolecular Resource Center, UCSF, San Francisco, CA 94143; [3]Oregon Health Sciences University, Portland, OR 97201.

Recent fate maps of *Xenopus laevis* gastrulae show that mesodermal tissue surrounding the blastopore gives rise to muscle (Keller, 1991; Lane and Smith, 1999). In contrast to earlier data, the new fate maps reveal that cells from the ventral blastopore region are precursors to a significant portion of trunk somites. However, these posterior somites are not formed until tadpole stages (stages 38–44). We therefore set out to determine the timing of muscle specification within the ventral half of the marginal zone. Our approach was to generate a series of tissue explants from gastrula-stage embryos (stages 10–12) and then culture them to either stage 28 (tailbud) or stage 44 (tadpole). At each end point, the presence of muscle in these explants was assessed with a muscle-specific antibody (12/101). We found that while muscle specification occurs by the onset of gastrulation, muscle tissue is not detected in ventral explants until they have reached tadpole stages. This is not unexpected, as this is the point at which this tissue normally gives rise to muscle. We further show that muscle specification occurs throughout the marginal zone and does not change over the course of gastrulation. Lastly, we found that the mesoderm derived from the ventral half of the blastopore gives rise to muscle in the absence of notochord tissue. Together, these results suggest that dorsalizing signals emanating from the midline are not necessary for muscle specification of the ventral half of the involuting marginal zone.

305. Hedgehog Signaling Negatively Regulates pax3/7 Expression in Presumptive Myogenic Precursors in Zebrafish. Xuesong Feng, Eric G. Adiarte, and Stephen H. Devoto. Biology Department, Wesleyan University, Middletown, CT 06459.

Vertebrate skeletal muscle growth is regulated by signals from tissues around the somite. In zebrafish, the first fibers to differentiate derive from a layer of presomitic cells adjacent to the notochord, called adaxial cells. They receive Hedgehog signal from the midline and undergo differentiation, then migrate to the lateral aspect of the zebrafish somite. At the end of the segmentation stage (24 h), they form a layer of mononucleated embryonic slow muscle fibers on the surface of the somite. The myogenic precursors medial to the surface embryonic slow fibers express myoD and develop into embryonic fast muscle fibers independently of Hedgehog signaling. At this time, there is a group of cells expressing pax3 and pax7, known as early myogenic markers within the somite. We found a dynamic expression pattern of pax3/pax7 in these cells. Hedgehog signaling is not

required for the induction of pax3/pax7 expression. However, Hedgehog signaling negatively regulates pax3/pax7 expression after 16–18 h stage. We are currently testing which components of the Hedgehog signaling pathway are required for this regulation.

306. Identification of a Myogenic Precursor Population Underlying Growth in Zebrafish. Frank Stellabotte, Xuesong Feng, Sara E. Patterson, Daniel A. Fernandez, and Stephen H. Devoto. Wesleyan University, Department of Biology, Middletown, Connecticut 06459.

Myogenic precursor cells in amniotes originate in the dermomyotome epithelium of embryonic somites. In zebrafish, the cells responsible for early embryonic myogenesis have been identified prior to segmentation; however, the myogenic precursors for subsequent growth are yet to be identified. We wish to identify a source of myogenic precursor cells that underlie this growth. Cells at the anterior margin of newly formed somites differentiate later than cells at the posterior margin, as indicated by the expression of myogenic regulatory factors such as myf5 and myoD. Using vital dyes, we labeled these anterior cells and compared their behavior to cells that were more posterior. Twenty-four hours after segmentation about one third of the labeled anterior cells remained undifferentiated, whereas all of the labeled posterior cells had elongated into muscle fibers. Seventy-two hours after labeling some of the anterior cells remained undifferentiated. We found that anterior cells came to lie lateral to the zebrafish myotome on their way to becoming muscle. Their position and morphology is consistent with the position of cells expressing pax3 and pax7. These pax genes are markers for amniote dermomyotome and are required for the development of amniote muscle fibers. Altogether, our data suggest that anterior cells may represent a myogenic precursor population responsible for the expansion of the zebrafish myotome.

307. Zebrafish Mib^{ta52b} Antagonizes Miblike in Neurogenesis and Compromises DeltaD in Somitogenesis. Chengjin Zhang, Qing Li, Chiaw Hwee Lim, and Yun-Jin Jiang. Institute of Molecular and Cell Biology, Singapore 138673.

Zebrafish *mind bomb* (mib) encodes a novel RING E3 ligase, which activates Notch via ubiquitylation-dependent Delta endocytosis. Detailed morphological and molecular examinations revealed that the genetic severity among different mib alleles is mib^{ta52b} (M1013R) $\gg$ mib^{m132} (C785stop) $\geq$ mib^{tfi91} (Y60stop). The antimorphic phenotypes seen in mibta52b and mibm132 mutants, compared to those of the null allele, $mibt^{fi91}$, are due to Mib^{ta52b} and Mib^{m132} proteins, respectively. We have identified a new *mib* homolog, *miblike* (*mibL*), whose protein has similar structure domains and biochemical properties as those of Mib. Both proteins are unstable and possess an E3 autoligase activity, which requires their C-terminal-most RING finger. Mib and MibL can form homo- and hetero-oligomers and ubiquitylate themselves and each other. Biochemical and genetic analyses indicate that Mib^{ta52b} and Mib^{m132} have a dominant-negative effect on MibL and injection of their corresponding mRNAs leads to a similar phenotypic severity. DeltaC is a common substrate of Mib and MibL, whose mutant forms interfere with its internalization. While Mib and MibL target DeltaC for internalization with similar efficacy, MibL is much less efficient in facilitating DeltaD internalization compared to Mib. Moreover, the mib^{ta52b} somite abnormality is mainly through DeltaD but not DeltaC. Finally, we showed that Notch signaling negatively regulates *mib* expression in a Su(H)-dependent manner, forming a negative feedback loop in modulating Notch activation. This work was supported by the Agency of Science, Technology and Research (A*STAR), Singapore.

308. *fbf-1* and *fbf-2*: Nearly Identical Genes with Unique Roles in *C. elegans* Germline Cell Fate Determination. Liana B. Lamont[1] and Judith Kimble[2]. [1]University of Wisconsin-Madison; [2]University of Wisconsin-Madison and HHMI, Madison, WI 53706.

The *fbf-1* and *fbf-2* genes are required for continued mitotic divisions of stem cells in the *C. elegans* germ line, as well as for germline sex determination. A *fbf-1 fbf-2* double mutant possesses no germline stem cells in adults, and fails to make the hermaphrodite switch from spermatogenesis to oogenesis. The FBF-1 and FBF-2 proteins are >90% identical throughout their entire amino acid sequence (Zhang et al., 1997). FBF-1 and FBF-2 are largely redundant: both *fbf-1* and *fbf-2* single mutants make germline stem cells and also make the hermaphrodite switch from spermatogenesis to oogenesis (Crittenden et al., 2002; Lamont et al., 2004). However, the two single mutants do have distinct effects. Whereas *fbf-1* single mutants have fewer stem cells and more spermatocytes than normal, *fbf-2* single mutants have more stem cells and fewer sperm than normal. Therefore, *fbf-1* and *fbf-2* do indeed substitute for each other, but each has minor and opposing defects in germline development (Lamont et al., 2004).

What causes these unique, but subtle phenotypes? We previously reported that *fbf-1* and *fbf-2* have unique patterns of mRNA and protein expression in the adult germ line. Indeed, the *fbf-2* gene, but not *fbf-1*, appears to be under the direct control of GLP-1/Notch signaling (Lamont et al., 2004). We are now examining FBF-1 and FBF-2 protein expression during development, both in wild-type and in mutants that alter the timing of the mitosis/meiosis and sperm/oocyte decisions. This work will extend our understanding of how these two nearly identical proteins pattern a field of cells during development.

309. Fgf9 Signaling is Required for Sertoli Precursor Cells to Establish the Male Pathway. Yuna Kim,[1] Akio Kobayashi,[2] Ryo Sekido,[3] Blanche Capel,[1] Richard R. Behringer,[2] and Robin Lovell-Badge[3]. [1]Dept. of Cell Biology, Duke Univ. Medical Center, Durham, NC; [2]Dept. of Molecular Genetics, Univ. of Texas, MD Anderson Cancer Center, Houston, TX 77030; [3]Division of Developmental Genetics, MRC National Institute for Medical Research, London NW7 1AA, UK.

The gonad primodium is remarkable in that all of its cells are believed to be bipotential, and can differentiate into ovarian or testicular lineages. Consistent with the idea that cells in this primodium are poised between two developmental pathways, either male or female pathway. *Fgf9* is essential for normal testis development. To understand the function of *Fgf9* in sex determination, we analyzed the genetic hierarchy of *Fgf9* among other testis determining genes using transgenic reporter mice and knockout mutant mice. We show that *Fgf9* is expressed in Sertoli cells and functions downstream of *Sry* to maintain *Sox9* expression in Sertoli precursor cells by a positive feedback loop regulation. Using knockout mutant mice and an embryonic gonad explant culture system, we show that *Fgf9* and *Wnt4* act in an antagonistic relationship. Based on these findings, we will describe a model in which the opposing activity of *Fgf9* and *Wnt4* in bipotential gonads controls switching the fate of supporting precursor cells from the female pathway to the male pathway.

310. Competence Windows for Endocrine Differentiation in the Developing Mouse Pancreas. Kerstin A. Johansson,[1] Umut Dursun,[1] Nathalie Jordan,[1] Gérard Gradwohl,[2] and Anne Grapin-Botton[1]. [1]ISREC, 1066 Epalinges, Switzerland; [2]INSERM unit 381, 67200 Strasbourg, France.

The pancreas, a highly branched mixed endocrine and exocrine gland, starts developing around E8.5 from a foregut domain expressing Pdx1. Starting at E9.5 and continuing until birth, cytodifferentiation of the four pancreatic endocrine lineages, producing glucagon, insulin, somatostatin or PP, occurs in a sequential yet overlapping fashion in scattered epithelial cells that transiently express the bHLH transcription factor ngn3. All endocrine lineages strictly depend on ngn3, but it is not understood how cell fate choices are made in NGN3+ progenitors.

Early expression of ngn3 in Pdx1-ngn3 transgenic mice increases the number of glucagon-positive cells, with no reported increase of insulin-positive cells. To study the possible temporal fate restriction of NGN3+ progenitors, we generated transgenic mice expressing a tamoxifen (TAM)-inducible NGN3 fusion protein under the control of the Pdx1 promoter. The Pdx-ngn3ERTAM-ires-nGFP transgene was further back-crossed into the ngn3−/− background, devoid of endogenous endocrine cells. Embryos of pregnant females injected with TAM at different time points were analyzed at E14.5. As expected, injection of TAM at E8.5 not only induced a marked increase in glucagon cells, but also pancreatic hypoplasia, reflecting an early depletion of pancreas progenitors. In contrast, activation of NGN3 at E12.5 triggered the differentiation of insulin-producing cells. These analyses should now be extended to the other endocrine lineages as well as later developmental time points.

311. Regulation of Pancreatic Islet Cell Differentiation. Lori Sussel, Catherine Prado, and Christina Chao. University of Colorado Health Sciences Center, Aurora, CO 80045.

Pancreatic islet cell development and differentiation is coordinately regulated by many well-characterized transcription factors. We previously determined that the homeodomain protein Nkx2.2 is essential for the differentiation of all insulin-producing beta cells and a subset of glucagon-producing alpha cells. Mice lacking Nkx2.2 have relatively normal sized islets, but the number of glucagon cells is reduced and insulin is undetectable. Strikingly, a large number of cells within the mutant islet appear to be converted into an alternative cell fate. These new cells produce ghrelin, an appetite-promoting peptide predominantly found in the stomach. We have determined that a small population of ghrelin-producing cells normally resides in the pancreas and defines a new endocrine cell population within the islet. We propose that insulin and ghrelin cells share a common progenitor and Nkx2.2 is required to specify differentiation into the beta cell fate; in the absence of Nkx2.2, progenitor cells differentiate into ghrelin cells by default. Additional studies have demonstrated that Nkx2.2 is also required for the appropriate differentiation of many enteroendocrine lineages in the intestine. In the absence of Nkx2.2, many of the peptide hormones are absent or reduced, including serotonin, gastrin and GIP. Interestingly, we observe a concomitant upregulation of ghrelin-producing cells suggesting that in the intestine, as in the islet, Ghrelin cells may be the default endocrine cell in the absence of Nkx2.2. We are now performing lineage tracing experiments to determine the lineage relationship between ghrelin cells and the endocrine cells of the islet and intestine.

312. Wnt/β-Catenin Signaling Induces Synovial Joint Formation and Specifies Osteoblast and Chondrocyte Cell Fates. Yingzi Yang, Timothy F. Day, Xizhi Guo, and Lisa Garret-Beal. Genetic Disease Research Branch, NHGRI, NIH.

We have found that the β-catenin-mediated canonical Wnt signaling is required for two fundamental developmental processes in mammalian skeletal system: synovial joint induction and chondrocyte versus osteoblast cell fate determination from common mesenchymal progenitors, which is believed to be controlled by two distinct processes, intramembranous and endochondral ossification. We show that several Wnts including Wnt14 were expressed in overlapping patterns during early skeletal development, correlating with upregulated β-catenin protein levels and transcription activity. Ectopic expression of an activated β-catenin or Wnt14 in mouse embryos inhibited chondrocyte differentiation, induced ectopic joint formation and enhanced osteoblast differentiation. In contrast, genetic inactivation of β-catenin promoted chondrocyte formation, caused joint fusion and inhibited osteoblast differentiation in both intramembranous and endochondral ossification. Moreover, β-catenin inactivation in mesenchymal progenitors in vitro causes chondrocyte differentiation under conditions that only allows osteoblasts to form. These studies demonstrate that Wnt/β-catenin signaling is necessary and sufficient for synovial joint induction and determining whether mesenchymal progenitors will form osteoblasts or chondrocytes regardless of the regional location or the mechanism of ossification. Our work reveals that controlling β-catenin protein level is the common molecular mechanism linking chondrocyte and osteoblast cell fate determination with specification of intramembranous and endochondral ossification.

313. Transcription Factors Coordinately Expressed During Adult, Murine T-Lymphocyte Specification. E.-S. David,[1] C.C. Tydell,[1] M.K. Anderson,[2] G. Buzi,[1] T. Taghon,[1] L. Rowen,[3] and E.V. Rothenberg[1]. [1]California Institute of Technology, Pasadena, CA 91125; [2]University of Toronto, Canada M4N 3M5; [3]Institute for Systems Biology, Seattle, WA.

Insight into possible gene regulatory networks of early T-cell development has been acquired. As hematopoietic stem cells are specified to T-cell fates, the transcription factor network state of thymic pro-T cells changes. To determine identities of these cell-intrinsic regulatory players, and circumvent the limitations of micro-arrays, we developed a series of structural and expression screens for a pro-T cDNA library. We used cell-lines, and mutant and wild type thymocytes; followed by two subtractive screens between select hematopoietic cell types. The results identified > 120 transcription factors that were not previously known to be up-regulated in thymocytes. While the bulk of the novel and new factors are zinc fingers, a large number of them are repressive in function. Using quantitative PCR, we interrogated the expression of over 90 transcription factors across hematopoietic cells, focusing on expression in subsets of wild type and mutant pro-T cells, for high-resolution expression analysis. Kinetics of factor expression in T-cell lineage specification in response to essential, initiating Notch signals were assayed by using a stromal co-culture system, the OP9 cells. Patterns of expression were sorted based on correlation values and resulting clusters of genes give insight into possible networks and functions of the regulators and some of their families. With these results, we aim to construct webs of changing factors essential to the transcriptional state of developing pro-T cells in thymíí.

314. Cellular Assessment of Calcium Aluminate Materials for Bone Tissue Engineering. John S. Doctor,[1] Jade T. Leung,[1] and Kenneth A. McGowan[2]. [1]Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282; [2]Westmoreland Advanced Materials, Arnold, PA 15068.

As a potential biomaterial for bone regeneration, we are evaluating calcium aluminate (CA)-based materials in which phase composition, micro-porosity, surface texture and dissolution rate are controlled, thus creating a three dimensional structure with defined macro-porosity and volume stability. As a prelude to animal studies, we are assessing the in vitro biocompatibility of the CA materials using human MG-63 osteoblast-like cells and human bone marrow-derived adult mesenchymal stem cells (hAMSC). Attachment and viability of hAMSC and MG-63 cells on CA has been assessed through Live/Dead viability staining, scanning electron microscopy, and CyQUANT assays. The viability of MG-63 cells and hAMSC is greater than 90% through 6 days of culture. MG-63 cells proliferate on CA as indicated by an increase in cell numbers between day 2 and day 6 of culture. hAMSC attach and differentiate on CA. We are currently assessing a variety of CA chemistries for in vitro biocompatibility in preparation for animal studies. Preliminary studies using the chorioallantoic membrane assay in developing chick embryos shows no CA toxicity in this in vivo assay. Based on our studies, calcium aluminate-based materials should prove to be effective for use in bone tissue engineering.

315. Expression Profiles of Genetically Identical Mesenchymal Stem Cells Indicate Diversity of Multipotent Cell Populations. Kenro Kusumi,[1] Vladimir Markov,[2] Dilusha A. William,[1] Vitali Lounev,[2] Dorian M. Hall,[1] Eric F. Rappaport,[1] Rick I. Cohen,[2] and Biagio Saitta[2]. [1]The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA 19104; [2]The Coriell Institute for Medical Research, Camden, NJ 08103.

Expression profiling has uncovered unanticipated differences among phenotypically similar stem cell lines. This diversity has been attributed to genetic heterogeneity between donors or to different developmental microenvironments. To minimize both variables, we identified and characterized two mesenchymal stem cell (MSC) populations isolated from the umbilical cord blood of a single human donor. These cell populations were isolated as individual colonies of adherent spindle-shaped cells with differences in cell morphology, colony formation assay efficiency, and growth kinetics. Both types displayed the cell surface markers of MSCs, and the ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages as expected for MSCs. Microarray analysis of both cell types was compared with bone marrow MSCs from an unrelated donor, and showed distinct expression profiles. We identified groups of genes with expression specific to each cell type. The presence of unique molecular signatures for genetically identical cells suggests diversity within MSCs, which may impact on their therapeutic use. (Supported by NIH grant AR050687 and grants from the Burroughs Wellcome Fund and the Coriell Institute for Medical Research.)

316. Generation of Peripheral Sensory and Sympathetic Neurons and Neural Crest Cells from Human Embryonic Stem Cells. Ronald S. Goldstein,[1] Oz Pomp,[1] Reubinoff E. B,[2] and Irina Brukhman. [1]Bar-Ilan Univ, Ramat-Gan, 52900, Israel; [2]Human Embryonic Stem Cell Research Center, Jerusalem, 91120 Israel.

Human embryonic stem cells (hESC) have been directed to differentiate into neuronal cells using many cell culture techniques. Central nervous system cells with clinical importance have been generated from hESC. However, there have not been any definitive reports of generation of peripheral neurons from hESC. We used a modification of the method of Sasai et al. for mouse and primate ESC to elicit neuronal differentiation from hESC. When hESC cells are co-cultured with the cells of line PA6 for 3 weeks, neurons are induced that co-express: (1) peripherin and Brn3a, and (2) peripherin and tyrosine hydroxylase, combinations characteristic of sympathetic and peripheral sensory neurons, respectively. In vivo, peripheral neurons develop from the neural crest (NC). Analysis of expression of mRNAs used in other species as NC markers revealed that the PA6 cells induce NC-like cells from hESC. Several NC markers including *SNAIL* are increased at 1 week of co-culture relative to naive hESC. Furthermore, the expression of several marker genes known to be down-regulated upon in-vivo differentiation of NC derivatives, is lower at 3 weeks of PA6-hESC co-culture than at 1 week. Our report is the first of the expression of molecular markers of NC-like cells in primates in general, and in humans, specifically. Our results suggest that this system can be used for study of molecular and cellular events in the almost inaccessible human NC, as well as producing normal human peripheral neurons for developing therapies for diseases such as Familial Dysautonomia.

317. Epigenetic Reprogramming of Somatic Nuclei with Human Embryonic Stem Cells. Kevin C. Eggan, Chad A. Cowan, and Brian Haas. Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138.

Nuclear transplantation experiments in animals indicate that both oocytes and embryonic stem (ES) cells have the capacity to reprogram the epigenetic state of somatic nuclei. We have tested whether human ES cells can also reprogram somatic nuclei, leading to the restoration of developmental potency. Our results indicate that following fusion of human somatic cells with human ES cells, stable cell-hybrids can be produced. These cell-hybrids express markers consistent with an hES cell identity and when differentiated give rise to cell-types from the three embryonic germ-layers. Expression profiling has demonstrated that within these cell-hybrids transcription of somatic specific genes was extinguished while embryonic genes were upregulated. Our experiments suggest that human embryonic stem cells provide a model system for both investigating and executing the reprogramming of human somatic nuclei.

318. Primitive Endoderm-Like Cells Derived from Human Embryonic Stem Cells Support Maintenance of Pluripotency. Rodolfo Gonzalez,[1] Xuejun Parsons,[1] Tim McDaniel,[2] Shawn Baker,[2] Steffen Oeser,[2] David Barker,[2] Evan Y. Snyder,[1] and Jeanne F. Loring[1]. [1]Burnham Institute, La Jolla, CA 92037; [2]Ilumina Inc., San Diego, CA 92021.

Feeder-layer free culture systems allow the expansion of undifferentiated human embryonic stem cells (hESC) without the need for mouse or human feeder cell layers. Our data show that feeder layer-free culture conditions allow the spontaneous differentiation from hESC of a population of cells that morphologically resemble fibroblasts. We produced a morphologically homogeneous population of these cells through repeated passaging, and used immunohistochemical and gene expression analysis (RT-PCR and Illumina's BeadArray) to discover that these cells express many of the markers associated with the primitive endoderm cell lineage. We tested the ability of this cell type as a feeder layer to support undifferentiated growth of hESC, and showed that HESCs cultured on mitotically inactivated cells (which we tentatively term hES-primitive endoderm-like, or hES-PEL) expressed the undifferentiated stem markers OCT-4, Nanog, SSEA-4, and Tra-1-60, and develop into teratomas when injected into SCID mice. We conclude that, in feeder layer-free culture systems, hESCs generate primitive endoderm-like (PEL) cells that promote hESC proliferation and maintain their pluripotent state. This would be consistent with the early events in embryonic development, in which the first differentiated cell type generated by the inner cell mass is the primitive endoderm. Future studies will focus on identifying the factors produced by the hES-PEL cells that regulate hESC proliferation and differentiation.

319. Appositional Bone Growth and Digit Regeneration in Mice and Humans. M. Han,[1] X. Yang,[1] J. Lee,[1] C. Brownyard,[1] S. Escudero,[1] K. Johnson,[1] C. Allan,[2] and K. Muneoka[1]. [1]Tulane University, New Orleans, LA 70118; [2]University of Washington, School of Medicine, Seattle, WA 98104.

Regeneration of the distal phalanx of the perinatal mouse digit is associated with the nail and involves direct ossification, whereas during development this bone forms by endochondral ossification. We characterized the development and growth of the distal phalanx to provide insight into this unique regenerative response. The distal phalanx forms an endochondral growth plate at its proximal end, which is gone by 3 weeks of age, and at its distal end, bone forms by direct ossification. By using the vital dye calcein and BrdU incorporation, we show much of its distal elongation can be attributed to appositional growth at the distal tip. We carried out level-specific amputations of the distal phalanx to investigate if a regeneration response is associated with normal phalangeal elongation versus an association with the nail. We found that distal amputations regenerated while proximal amputations failed to regenerate even though both amputations included nail tissue. We also present a case study of a 2-year-old child who was subjected to proximal amputation injury of the distal phalanx that failed to regenerate. Thus, our evidence suggests both

mice and humans display a level-specific regeneration response within the distal phalanx. These data suggest postnatal appositional ossification is responsible for the phenomenon of digit tip regrowth following amputation injury in neonatal mice and children.

320. Withdrawn.

321. Wnt Signaling and Lineage Segregation in Mouse Embryonic Stem Cells. Nicole Slawny, Theresa Gratsch, and Sue O'Shea. Department of Cell and Developmental Biology, University of Michigan.

The processes of neural induction and differentiation require precise control of multiple redundant signaling pathways in time and space. Mouse embryonic organizing centers secrete molecules that block or stimulate key pathways such as TGFβ, Wnt, and Nodal. Embryos null for select members of the canonical Wnt signaling pathway are characterized by an expansion of neural tissue at the expense of mesoderm, suggesting that repression of Wnt signaling is critically involved in lineage choice at gastrulation. Embryonic stem cells (ES cells) are derived from the inner cells mass of the mouse blastocyst and form all embryonic tissues. Because they express genes typical of the inner cell mass and epiblast, ES cells are an excellent model system to study signaling cascades in lineage segregation. Previous studies in our lab have suggested that repression of Wnt signaling is involved in lineage choices of ES cells. We have developed ES cell lines to allow for episomal replication of plasmids, resulting in superior transfection efficiency. We have used these cell lines to over express a dominant-negative Tcf-4 protein (blocking canonical Wnt signaling) and the Cerberus-like protein (blocking nodal and BMP signaling) in addition to challenging the transfected cells with growth factors. Preliminary observations suggest that down regulation of canonical Wnt signaling is required for the initial steps in differentiation. Investigations are in progress to both determine which specific Wnt proteins are involved using RNAi to knock down expression, and to compare Wnt gene expression in the early embryo itself. Supported by NIH grant NS-39438.

322. Ciliated Cells Serve as Progenitor During Regeneration of the Respiratory Epithelium. Kwon-Sik Park,[1] James M. Wells,[2] Aaron M. Zorn,[2] Susan E. Wert,[1] Victor E. Laubach,[3] Lucas G. Fernandez,[3] and Jeffrey A. Whitsett[1]. [1]Division of Pulmonary Biology; [2]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, OH 45229; [3]Department of Surgery, University of Virginia Health System, Charlottesville, VA 22908.

Since the lung is repeatedly subjected to injury by pathogens and toxicants, maintenance of pulmonary homeostasis requires rapid repair of epithelial surfaces. We show that ciliated bronchiolar epithelial cells, previously considered as terminally differentiated, rapidly undergo squamous cell metaplasia, proliferate, and re-differentiate to restore ciliated and non-ciliated cell types lining the bronchioles after injury. Sox17 expression, normally restricted to ciliated cells in the adult lung, was enhanced during regeneration of the bronchiolar epithelium following naphthalene injury and during compensatory lung growth following unilateral pneumonectomy. Dynamic changes in immunostaining of transcription factors, which play important roles in lung morphogenesis, accompanied the regeneration process. In transgenic mice, Sox17 was sufficient to induce ectopic differentiation of multiple cell types, as well as hyperplasia of both ciliated and non-ciliated cells in the peripheral lung, and to induce ciliated cell differentiation in the fetal lung. Taken together, these findings demonstrate that ciliated epithelial cells serve as a source of multipotent progenitor cells. Sox17 regulates ciliated cell differentiation and influences progenitor cell behavior in the bronchiolar epithelium.

323. Fibroblast Growth Factor 8: A Crucial Signaling Molecule for Olfactory Epithelium Neurogenesis. Rosaysela Santos, Shimako Kawauchi, and Anne L. Calof. Univ. of California, Irvine.

Signaling molecules of the fibroblast growth factor (FGF) family have been shown to promote neurogenesis in vitro. Among FGFs, Fgf8 has been shown to be important for development in neural regions, but its mechanism of action is unclear. To understand this, we are investigating the role of Fgf8 in the olfactory epithelium (OE). The OE is a useful model system to study neurogenesis because neural development proceeds in a linear fashion in which each stage of the lineage can be identified. FGFs promote proliferation of olfactory stem cells in vivo, suggesting that FGFs have a regulatory role in OE neurogenesis, however, the identity of FGFs crucial roles for this process in vivo have not been investigated. To study the role of Fgf8 in the OE, we used a genetic approach in which the Fgf8 gene becomes inactivated at the earliest stage of OE development. These Fgf8flox/D23;Foxg1+/cre (mutant) mice showed strong defects in several neural regions including the OE. Using in situ hybridization, we found that cells of the OE neuronal lineage are initially produced in mutant mice during development (e10.5), but then fail to develop, indicating that Fgf8 is required for OE neurogenesis. To test the hypothesis that OE neuronal cells require Fgf8 for survival, we performed TUNEL assays to identify apoptotic cells. A large increase in the number of TUNEL+ cells was found in the OE of mutant mice when compared to their wild-type littermates at e10.5, and this increase in apoptotic cells is maintained in the mutant mice until approximately e14.5.These results suggest that Fgf8 is a crucial signaling molecule for OE neurogenesis.[Δ]

324. Recapitulation of in Vivo Gene Expression During Hepatic Differentiation from Mouse Embryonic Stem Cells. Yusuke Yamamoto,[1] Takumi Teratani,[1] Gary Quinn,[1] Takashi Kato,[2] and Takahiro Ochiya[1]. [1]Sec. Meta., National Cancer Center Research Institute, Tokyo, Japan; [2]Waseda University, Tokyo, Japan.

Embryonic stem cells were isolated from the inner cell mass of mouse blastocyst and can be propagated indefinitely in an undifferentiated state but have the capacity, when provided with the appropriate signals, to differentiate, presumably via the formation of precursor cells, to almost any mature cell phenotype. Hepatic differentiation at the molecular level still remains poorly understood, mainly due to the lack of a suitable model. Recently, using an adherent monoculture condition, we demonstrated the direct differentiation of functioned hepatocytes from ES cells. In this study, we have exploited the direct differentiation model to compare the gene expression profiles of ES cell-derived hepatocytes with adult mouse liver using microarray technology. The result showed that the gene expression pattern of ES cell-derived hepatocyte is quite similar to mouse liver. By further analysis of gene ontology categories for the 232 most radically altered genes we demonstrated that the significant categories related to hepatic function. Further, by the use of small interfering RNA (siRNA) technology and RT-PCR analysis of liver-enriched transcription factors in vitro, HNF3beta/FoxA2 was identified as having an essential role in hepatic differentiation, and ES cells differentiated into hepatocytes via endoderm differentiation. These results demonstrate that ES cell-derived hepatocytes recapitulate the gene profile of adult mouse liver to a significant degree, and indicate that our direct induction system progresses via endoderm differentiation.

325. Evolutionary Filtering for Genes in the Oct4 (Pou5F1) Pathway. Shawn M. Burgess, Zengfeng Wang, Lisa Garrett-Beals, Abdel Elkahloun, and Yidong Chen. National Human Genome Research Institute/National Institutes of Health.

The transcription factor Oct4 (POU5f1) plays a crucial role in early embryonic lineage specification. In mouse, the level of Oct4 expression

determines the fate of different cell types in the pre-implantation embryo. Its expression is elevated in the inner cell mass (ICM) and suppressed upon differentiation into the trophoblast lineage. In zebrafish, the gene Pou2 has been demonstrated to be the ortholog of mammalian Oct4. Inhibition of Pou2 by injection of antisense morpholino oligos causes an early arrest phenotype similar to the mouse knockout of Oct4. We studied the regulatory network of Oct4 in mouse ES cells and zebrafish embryos in parallel by over expressing or reducing the levels of Oct4 (and Pou2) followed by microarray analysis of the transcriptional changes. The major emphasis of this project is the meta-analysis of transcriptional profiles across species to identify to define a "core" regulatory pathway for stem cell identity. In comparing the mouse and zebrafish profiles we have established 350 genes in common that have detectable changes in transcript level. Multidimensional scaling and clustering of these genes reveal a group of approximately 18 genes that closely mirror Oct4 expression in both mice and zebrafish. Remarkably, all of the genes in this cluster are signaling molecules or transcription factors directly responsive to signaling molecules. Direct testing of several genes in zebrafish embryos confirms the early roles for these genes in maintaining pluripotency. This suggests that evolutionary filtering is an effective method for identifying core regulatory pathways.

326. Expression of Immune-Related Genes During Limb Regeneration in *Xenopus laevis*. M.W. King,[1] L. Abu-Niaaj,[1] A.L. Mescher,[2] E.A. Moseman,[2] and A.W. Neff[2]. [1]IU School of Medicine, THC, Terre Haute, IN 47809; [2]IU School of Medicine, MSP, Bloomington, IN 47405.

Limb regeneration in amphibians serves as the best-studied model for the analysis of the cellular and molecular aspects of vertebrate appendage regeneration. Regenerative capacity in *Xenopus* hindlimbs is near perfect when amputation occurs at premetamorphic stages but declines during prometamorphic stages. We have hypothesized that developmental changes in the immune system during this pre/post-metamorphic phase is responsible, in part, for limb regeneration decline. To assess this hypothesis we have analyzed, using RT-PCR, the expression of several immune system genes following limb amputation at regeneration-competent and -incompetent stages. Our initial focus has been on the genes for IL-1β, MyD88, MHC class II, CRP, and complement proteins: C3, C4, and factor B. None were found to be expressed exclusively in regeneration-competent or -incompetent tissues, but we have observed dynamic differences in the timing of expression. Most notably, IL-1β expression is rapidly and dramatically initiated within 1 day after amputation at the regeneration-competent stage followed by down-regulation at 3 days. At the regeneration-incompetent stage the transient rise of IL-1β occurs 2 days later. Expression of C3 and C4 was not seen in intact limbs but induced following amputation. Induced C3 expression was steady from days 1 to 5 in regenerating limbs, but only transient in nonregenerating limbs. Examining the temporal and spatial expression of these genes and their proteins will add significantly to our understanding of the function of these factors in limb regeneration.

327. Characterization of tgf-β1 Expression During Wound Healing and Limb Regeneration in the Axolotl. Mathieu Lévesque,[1] Mireille Pilote,[1] and Stéphane Roy[2]. [1]Department of Biochemistry; [2]Department of Stomatology, Université de Montréal, Montréal, QC H3T 1J4, Canada.

Urodele amphibians (e.g., axolotls) have the unique ability, among vertebrates, to perfectly regenerate many parts of their body including limbs, brain, spinal cord, skin and tail. Limb regeneration is a complex process, divided into two main phases that require multiple cellular and molecular mechanisms in order to take place. The preparation phase represents the first part of the regeneration process which includes wound healing and cellular dedifferentiation. The redevelopment phase represents the second part when dedifferentiated cells proliferate and redifferentiate to give rise to all missing structures. In the axolotl, when a limb is amputated or an incisional wound is made, the amputated or wounded tissue is regenerated/healed perfectly with no scarring. Therefore, we were interested in characterizing the expression of the TGF-β1 gene, which is implicated in wound healing and scar tissue formation in many organisms, during regeneration. With the use of molecular biology and in situ hybridization techniques, we were able to clone the full-length sequence of the axolotl gene and characterize the temporal and spatio-temporal expression patterns of TGF-β1 in regenerating tissues. Our data demonstrate that TGF-β1 is highly conserved in axolotls compared to other vertebrates. We also show that it is upregulated during limb regeneration specifically during the preparation phase. This upregulation corresponds to the formation of the wound epithelium and the apical ectodermal cap as well as the dedifferentiation of mesenchymal cells.

328. A Proximodistal Gradient of Fgf Signaling Defines Position-Dependent Rates of Zebrafish Fin Regeneration. Yoonsung Lee, Sara Grill, Maureen Murphy-Ryan, and Kenneth D. Poss. Duke University Medical Center, Durham, NC 27710.

During appendage regeneration in lower vertebrate species, regeneration is precisely regulated such that only the appropriate structures are replaced. One of the major unexplained issues of regeneration is how adult cells in the injured area recognize the positional information required for this. In many regenerative lower vertebrates, positional information is encoded in part by a gradient in the rate of regenerative growth along the proximodistal (PD) axis of the appendage. Remarkably, the regenerative rate is faster at proximally amputated appendages than distally amputated ones. The differences in underlying regulation are currently unexplained at the cellular and molecular levels. Here, we identified a PD gradient in the rate of regenerative growth of the amputated zebrafish caudal fin. This gradient was associated with additional PD gradients in blastemal length, mitotic index, and expression of Fgf target genes. To address the function of Fgf signaling in regulating these gradients, we used transgenic fish that facilitate heat-inducible expression of a dominant-negative Fgfr1. We observed a dose-dependent effect of Fgfr inhibition on target gene expression, cellular proliferation, and regenerative rate. Thus, our experiments indicate that PD-regulated amounts of Fgf signaling define the rate of fin regeneration. Our current research is focused on understanding mechanisms of this regulation.

329. Inhibition of bmp Signaling During Zebrafish Fin Regeneration Disrupts Fin Growth and Bone Matrix Deposition. A. Smith, F. Avaron, B.K. Padhi, and M.A. Akimenko. Ottawa Health Research Institute, University of Ottawa, Canada.

The zebrafish caudal fin provides a simple model to study the molecular mechanisms of bone regeneration. Following fin amputation the lost structures are replaced by proliferation and differentiation of blastemal cells. We have previously shown that ectopic expression of both sonic hedgehog (shh) and bone morphogenetic protein (bmp2b) within the regenerate results in ectopic bone deposition and that a bmp factor acts as a downstream target of shh signaling in dermal bone regeneration. To further investigate the role of bmp signaling we transfected blastema cells with chordin, a BMP inhibitor. Chordin misexpression results in inhibition of both regenerate outgrowth and deposition of new bone matrix. Growth arrest, accompanied by decreased cell proliferation and a down-regulation of *msxC* expression, is likely a result of inhibition of bmp4 signaling in the distal blastema. Bmp inhibition did not affect expression of *shh* or *Indian hedgehog* (*ihh*), a newly characterized *zebrafish hedgehog* (*Hh*) gene, further suggesting that a bmp factor acts downstream or independently of Hh signaling in this system. The reduced bone deposition is likely the result

of a defect in the differentiation and maturation of bone-secreting cells as further suggested by downregulation of the expression of *runx2a*, *runx2b* and *collagen type 10a1* (*col10a1*). Finally, this analysis unraveled the surprising finding of *col10a1* and *ihh* expression in scleroblasts, since these genes are uniquely expressed in cartilage cells of endochondral bone, whereas fin rays are described as dermal bone forming through direct ossification of bone matrix.

330. Pax7 in Adult and Regenerated Tails of the Teleost *S. macrurus*. Christopher Weber and Graciela A. Unguez. NMSU, Las Cruces, NM 88003.

The electric fish *S. macrurus* is unique among vertebrates in that it can regenerate its tail including spinal cord, skin, skeleton, muscle, and the muscle-derived electric organ (EO). This regeneration capability is parallel to that of urodeles which can replace all tissue types after limb amputation. The origin of the blastema in *S. macrurus* is thought to derive from myogenic stem cells (Patterson and Zakon, 1993), whereas in urodeles the blastema forms by the dedifferentiation of mature cells (Nye et al., 2003). To further test whether myogenic stem cells contribute to the regeneration blastema in *S. macrurus*, we have examined the distribution of the myogenic stem cell marker Pax7. In mature tails, Pax7 immunolabel is detected in satellite cells in muscle and EO, with a higher predominance in EO. One week after tail amputation, the number of Pax7-positive cells appeared to increase in muscle and EO proximal to the cut site. In 1- and 2-week regeneration blastemas, Pax-7 label was found in developing muscle and EO. These preliminary data suggest that myogenic stem cells contribute to the regeneration blastema. We will combine Pax7 and BrdU labeling to identify the source of proliferating cells and their distribution at different states of regeneration. We have cloned a 444bp Pax7 cDNA from 1-week blastema using RT-PCR and heterologous protein sequences from various vertebrates. This fragment contained 148 amino acids spanning the paired domain with a 95% and 97% similarity to zebrafish and human Pax7, respectively. Characterization of Pax7 transcript expression in different tissues will compliment experiments that determine the identification of proliferative cells during regeneration.

331. Spherule Cells and their Role in the Intestinal Regeneration of *Holothuria glaberrima*. José E. García-Arrarás, Christian Schenk, Roxanna Rodríguez-Ramírez, Irma I. Torres, Arelys Cabrera-Serrano, Ann G. Candelaria, Sharon File, and Griselle Valentín-Tirado. University of Puerto Rico, Rio Piedras, PR 00931.

Regeneration processes involve dramatic changes in the extracellular matrix (ECM) component. We have shown that ECM remodeling occurs during the regeneration of the intestine in the sea cucumber, *Holothuria glaberrima*. In this process the ECM loses some of its components and a new ECM is eventually formed. We have now identified the cells associated with this event. These are large, round cells, full of vesicles or spherules found within the connective tissue of various tissues. During the regeneration process, the number of spherule cells increases, peaking at 2-weeks following evisceration. The increase in cell numbers suggests that the cells are migrating into the regenerating structure at a time when the blastema-like structure is growing in size and the intestinal lumen is forming. As regeneration progresses, their morphology changes and they appear to release their vesicular content within the internal connective tissue. In experiments where regenerating animals were treated with (0.8–10 mg/ml) RGDS (Arg-Gly-Asp-Ser), a peptide that competes with the RGD sequence–integrin interaction, there is a reduction in the number of spherule cells and a concomitant delay in intestinal regeneration. Thus, the data suggest that the spherule cells migrate into the regenerating structure via the mesentery and release their vesicular content, contributing in this way to the formation of the new extracellular matrix in the regenerating intestine. Funded by IBN-NSF, MBRS-NIGMS, NIH-RCMI, and the UPR.

332. The Totipotent Stem Cell Systems of the Flatworm *Macrostomum lignano* and the Acoel *Convoluta pulchra*. Peter Ladurner, Daniela Pfister, Robert Gschwentner, Katharina Nimeth, Bernhard Egger, and Reinhard Rieger. University of Innsbruck, Austria.

Flatworms possess a totipotent stem cell system giving rise to all cell types including germ cells. These stem cells are responsible for the extraordinary regenerative power and plasticity of the Platyhelminthes. We are characterizing the stem cell system of *Macrostomum lignano* and the acoel *Convoluta pulchra* using a molecular, ultrastructural and histological approach. Sequencing of 8000 *Macrostomum* and 100 *Convoluta* ESTs revealed candidate genes as markers for stem cells and the germ line. We use neoblast-specific genes (piwi, MCM, PCNA) and germ line-specific genes (vasa, pumilio, nanos) to study expression and function. BrdU and phosphorylated Histone H3 staining was used to show distribution, migration, and differentiation of neoblasts. We generated monoclonal antibodies specific for certain cell types of *Macrostomum* to follow neoblast differentiation. Immunogold labeling corroborated the existence of morphologically distinguishable neoblasts. We want to understand if (1) a true germ line exists in basal Platyhelminthes and in Acoels, (2) germ line cells are derived from the totipotent neoblasts during postembryonic development and regeneration, and (3) a totipotent stem cell system is ancestral for the Bilateria. Taking advantage of the unique stem cell system in Platyhelminthes and in Acoels, our study may reveal the mechanisms of stem cell and germ line regulation in development, growth and regeneration in these primitive bilaterians. Supported by FWF project 15204 and APART fellowship to L.P.

333. The Totipotent Stem Cell System as a Source for Germ Line Cells During Development and Regeneration in the Flatworm *Macrostomum lignano*. Daniela Pfister and Peter Ladurner. Institute of Zoology, University of Innsbruck, Austria.

To study stem cell biology flatworms are excellent models as they feature a totipotent stem cell system that gives rise to all cell types including germ cells. To study the origin of the totipotent stem cell system and the derivation of germ line cells from neoblasts during postembryonic development and regeneration of *Macrostomum lignano*, we are isolating stem cell-specific molecular markers for neoblasts, such as MCM, piwi or PCNA, and vasa, pumilio or nanos for germ line cells. We report results of expression studies with the germ line-specific gene vasa at mRNA and protein level. Vasa was expressed in the testis, ovaries, and in eggs. During postembryonic development freshly hatched worms did not show vasa staining. Vasa-expressing cells were found in the male gonad at day 3 after hatching and more prominent staining appeared at day 4 in testis and ovaries. In order to follow reformation of gonads from totipotent stem cells, gonads were completely removed by cutting animals at the post-pharyngeal level. Regenerating heads did not show expression of vasa shortly after cutting. Vasa signal was present in the blastema up to 3 days after cutting. From day 4 on, discrete vasa expressing cells were specified. At day 7 functional gonads can be seen. We are currently developing and refining an RNAi method to result in a simple soaking protocol for easy analyses of stem cell and germ line gene function in regeneration, embryonic development, and postembryonic development of the flatworm *Macrostomum lignano*. Supported by FWF project 15204 and APART fellowship to L.P.

334. Germ Cell Lineage Behaves Differently from Somatic Stem Cells During the Regeneration of *Enchytraeus japonensis*, an Oligochaete Worm that Undergoes Both Asexual and Sexual Reproduction. Ryosuke Tadokoro,[1] Mutsumi Sugio,[2] Junko Kutsuna,[2] Shin Tochinai,[2] and Yoshiko Takahashi[3]. [1]NAIST, Nara, Japan; [2]Hokkaido Univ, Sapporo, Japan; [3]CDB, RIKEN, Kobe, Japan.

Animals that can regenerate their entire body have provided valuable information concerning pluripotency. However, both neoblasts and germ

cells are pluripotent stem cell populations in these organisms, and very little is known about the behavior of the germ cell lineage during this regeneration. We addressed this issue by studying *E. japonensis*, a tiny oligochaete worm, which propagates both asexually and sexually. We found that tissue regenerated from body segments that normally have no gonad can produce mature gonads upon sexualization. To understand how the germ cell lineage participates in the gonad regeneration, we examined the behaviors of cells that express piwi, a gene known to be involved in the germ cell lineage in other animals. *Piwi*-positive cells, localized to the 7th and 8th segments of the asexually growing animal, are indeed primordial germ cell-like cells, and they can differentiate into germ cells upon experimental sexualization. After amputation to remove the gonad-containing segments of an asexually growing worm, somatic stem cells including neoblasts make a major contribution to the early regeneration blastema, while the *piwi*-positive cells emerge in the developing blastema only at a later stage. These latter cells appear to derive from the preexisting *piwi*-positive cells, which are sparsely and widely distributed in the body. This is the first report that the germ cell lineage behaves differently from somatic stem cells during regeneration.

335. Silencing of the CREB Pathway Prevents Hydra Head-Regeneration Through Inhibition of Apoptosis in Regenerating Tips. Brigitte Galliot, Luiza Ghila, Kevin Dobretz, and Simona Chera. Dept. of Zoology and Animal Biology, Univ. of Geneva, CH 1211-Geneva 4, Switzerland.

The MEK/RSK/CREB signaling pathway was previously identified as a key regulator of early head regeneration in hydra (Kaloulis et al., PNAS 2004). In this work, we investigated the cellular function of three components of this pathway, RSK, CREB and CBP, during early regeneration. In immunohistochemistry experiments, we recorded a co-expression of RSK, CREB and CBP in all cell types including endodermal and ectodermal epithelial cells, interstitial stem cells, dividing nematoblasts and sensory neurons. When we compared the cellular modifications exhibited by CREB and RSK-expressing cells within the first hours following bisection, we noticed that interstitial cells underwent a dramatic apoptotic process in head- but not foot-regenerating tips. In addition, in head-regenerating tips, the endodermal epithelial cells were transiently transformed into "bomb" cells that phagocytosed and digested apoptotic bodies. When RSK, CREB and CBP were silenced upon feeding hydra with bacteria expressing dsRNA, this apoptotic process did not take place, the dramatic increase in the transcript abundancy of these genes normally observed in endodermal cells of the stumps was abolished and the head regeneration process was significantly delayed under non-lethal conditions. These data show that in hydra (i) a massive but spatially restricted apoptosis occurs before head organizer activity is established in head-regenerating tips, (ii) this cellular process requires a functional CREB pathway, and (iii) head and foot regeneration rely on distinct cellular and molecular mechanisms.

336. Stem Cell-Specific Gene Expression in Leech. Daniel H. Shain and Kristi A. Hohenstein. Rutgers, The State University of New Jersey, Camden, NJ 08102.

The glossiphoniid leech, *Theromyzon tessulatum*, displays particularly large and accessible embryonic stem cells during early developmental stages. Among these are the bilaterally paired mesodermal (M) and neuroectodermal (N) embryonic stem cells that give rise to the bulk of segmental tissue, and their respective progenitor cells, DM and NOPQ. By differential display – PCR analysis – we have identified ~200 differentially expressed cDNAs among the DM, NOPQ, M and N cell populations, ~20 of which appear to be turned on upon stem cell birth (i.e., M/N specific). The transient abrogation of several stem cell-specific genes with antisense (AS) oligonucleotides disrupted the normal formation of embryonic stem cells and their progeny. In particular, knockdown of clone K110 in progenitor cells prevented stem cell birth and caused dramatic proliferation of the injected cells. Microinjection of the same AS oligos into newly born stem cells, however, had little effect on subsequent development. Collectively, these results suggest that clone K110 represents a novel gene with a critical role in stem cell genesis.

337. A Planarian Etr-1 Homolog is Required for Stem Cell Maintenance. Tingxia Guo and Phillip A. Newmark. University of Illinois at Urbana-Champaign, IL 61801.

Elav-type RNA-binding proteins (Etr) belong to the Bruno-like family of transcriptional regulators, and they have been found in mammals, zebrafish, *Drosophila*, *Xenopus* and *C. elegans*. These RNA-binding proteins play roles in many diverse processes, from germ cell development and synaptic vesicle release to pre-mRNA processing. In a screen to identify stem cell markers in the freshwater planarian, *Schmidtea mediterranea*, we identified a planarian homolog of zebrafish *etr-1*. This gene is expressed in the mesenchymal region of the animal, in which the stem cells reside, as well as in the brain and ventral nerve cords. In the sexual strain of *S. mediterranea*, this gene is also expressed in the testes and ovaries. During early regeneration, elevated expression of planarian *etr-1* was detected in the blastema; while from day 3 after amputation the mRNAs were observed in the regenerating brain primordia. After selectively eliminating the stem cell population by x-irradiation, the mesenchymal expression of this gene was no longer detectible; however, the neuronal expression of planarian *etr-1* was not affected. RNA interference (RNAi) experiments were used to test planarian *etr-1* function. After RNAi knockdown of planarian *etr-1*, animals failed to regenerate properly, and both the intact and regenerating animals died in a manner similar to what is observed after x-irradiation-induced stem cell loss. Immunostainings with numerous stem cell markers confirmed the loss of stem cells in the RNAi-treated animals. These results suggest that the planarian Etr-1 homolog is required for stem cell maintenance in planarians.

338. Chromatin Architecture in the Planarian *Schmidtea mediterranea*. Sofia M. Robb and Alejandro Sanchez Alvarado. University of Utah School of Medicine, Department of Neurobiology and Anatomy.

Planarians are well known for their regenerative capacities. Understanding the biological principles guiding this remarkable attribute is likely to shed light on our understanding of how cellular pluripotentiality may be regulated. Whole genome shotgun sequencing of the planarian *Schmidtea mediterranea* is currently under way at the Washington University Genome Sequencing Center. At least 10e6 unassembled trace reads are already accessible to the public at the National Center for Biotechnology Information. Given the role chromatin architecture is likely to play in defining the genomic output of a given cell, and the multipotentiality found in the stem cells (neoblasts) of *S. mediterranea*, we have computationally scanned these trace reads to identify sequences homologous to all known histones. Canonical histones, H3, H4, H2A, H2B, and H1 and the histone variants H3.3, H2A.X, H2A.Z are present in the S. mediterranea genome. We identified conserved epitopes for commercially available anti-Histone antibodies and tested their cross-reactivity with planarian chromatin. Western blot analysis and immunocytochemistry has confirmed, for example, the existence of acetylated and methylated post-transcriptional modifications of histone H3. In addition, chromatin immunoprecipitation (ChIP) methods have been successfully adapted for experimentation in planarians. ChIP of histone H3 acetylated at lysine residues 9 and 14 and of histone H3 tri-methylated at lysine residue 9 are currently being conducted. These experiments aim to introduce robust and reliable assays to gauge chromatin states in the undifferentiated neoblasts and their division progeny.

339. Identification of Stem Cell Gene Candidates in the Planarian *Schmidtea mediterranea*. George T. Eisenhoffer and Alejandro Sánchez Alvarado. University of Utah School of Medicine.

Research into the in vivo activation and migration of multipotent progenitor cells is likely to improve our understanding of tissue homeostasis and organ regeneration in multicellular organisms. The general inaccessibility of stem cells continues to pose a challenge for in vivo studies. In an attempt to overcome this limitation, we have chosen to study the abundant and experimentally accessible stem cells (neoblasts) of the flatworm *Schmidtea mediterranea*. Neoblasts are the only mitotically active cells in planarians and, as such, are responsible for replacing tissues lost to turnover and/or injury (amputation). We have used gamma-radiation to selectively target the mitotically active neoblasts, which was confirmed by Fluorescence Activated Cell Sorting. Gene expression profiles of planarians exposed to different radiation dosages were monitored using cDNA microarrays. We identified a subset of genes that disappeared after exposure to radiation. Whole mount in situ hybridization was used to both validate the array data and to determine the spatial distribution of the candidate transcripts. Many transcripts were found in cells that are morphologically indistinguishable from neoblasts. The data suggest that the various expression patterns may represent different subpopulations of cells. We expect that the identified transcripts will provide us with markers for stem cells as well as shed light on the functional regulation of the *S. mediterranea* neoblasts during the processes of tissue homeostasis and regeneration.

340. Cell Cycle Dynamics of a Stem Cell Population in Vivo: the Neoblasts of the Planarian *Schmidtea mediterranea*. Hara Kang and Alejandro Sánchez Alvarado. University of Utah School of Medicine.

It is well known that differentiated cells oftentimes respond to environmental changes by either undergoing cell cycle arrest or activation. Whether stem cells respond to such changes using similar strategies remains largely unknown. To address this question, we are studying the stem cells of the planarian *Schmidtea mediterranea*, also known as neoblasts. Neoblasts are constantly entering the cell cycle at a relatively rapid rate, and it is possible to label these cells and follow their in vivo activities, making neoblasts one of the most experimentally accessible stem cell populations known. Prior studies have shown that neoblasts can respond to both global and local homeostatic signals. For example, it has been noted that starvation can lead to an allometric reduction in size of the entire organism, and that such changes are mediated by cell loss. This is likely to entail a reduction in cell proliferation, which may be mediated at the level of the neoblast cell cycle. Locally, amputation leads to a burst of neoblast proliferation near the amputation plane without significantly affecting the proliferative rates of neoblasts elsewhere in the animal. By taking advantage of flow cytometry, we have begun to characterize the cell cycle parameters of neoblasts in vivo under a variety of conditions, e.g., feeding, starvation and/or irradiation. Information derived from these pilot studies will help us identify aspects of the cell cycle that may be under homeostatic regulatory control, and help us identify the signals and the target molecules driving such regulation.

341. Dissecting the Molecular Basis of Neural Regeneration in Planarians. Ricardo M. Zayas, Francesc Cebrià, and Phillip A. Newmark. Department of Cell and Structural Biology, Neuroscience Program, University of Illinois at Urbana-Champaign, IL, USA.

It has been well documented that neurons are replaced in specific regions of the central nervous system (CNS) of both vertebrates and invertebrates. Yet, we still do not understand why neurogenesis is highly restricted in the CNS, how it is regulated, and how these new neurons are incorporated into a functional nervous system. Planarian flatworms are known for their ability to regenerate entire worms from small body pieces, a remarkable ability conferred by a population of pluripotent stem cells these animals maintain throughout life. Thus, planarians are able to repair and regenerate their nervous system and regain normal function, making them a useful system in which to examine how neural regeneration is achieved at the molecular and cellular levels. As a first step in this process, we have taken advantage of a collection of ~27,000 expressed sequence tags (ESTs) generated from the sexual strain of the planarian *Schmidtea mediterranea* to identify genes implicated in neural regeneration. Using bioinformatics we have identified candidate genes that are known to play important roles in the function and development of the CNS. We have screened the expression pattern of many of these ESTs by in situ hybridization of intact and regenerating planarians and have identified ~200 that are expressed in the planarian nervous system. We are currently investigating the role(s) of these genes in neural regeneration using RNA interference.

342. Withdrawn.

343. The *indeterminate gametophyte1* Gene of Maize Encodes a Member of the Lateral Organ Boundaries Domain Protein Family. Matthew M. Evans. Department of Plant Biology, Carnegie Institution, Stanford, CA 94305.

Plants have two phases to their life cycle: the diploid sporophyte and the haploid gametophyte separated by meiosis at one end and fertilization at the other. Female gametophyte, or embryo sac, development in many angiosperms is characterized by a phase of synchronous, free nuclear divisions that produce an eight nucleate syncytium. Mutations in the *indeterminate gametophyte1* (*ig1*) gene of maize cause these free nuclear divisions to be variable in number. Consequently, mature *ig1* mutant embryo sacs have a variety of structural defects, including extra egg cells, extra polar nuclei, and extra central cells. These abnormalities in turn lead to a variety of abnormal fertilization events. We have cloned the *ig1* gene using a combination of comparative genomics and transposon tagging. The *ig1* gene encodes a member of the LOB domain protein family of plant-specific transcriptional regulators. Plants homozygous for a Mutator transposon insertion allele of *ig1* have abnormal leaf morphology as well as abnormal embryo sac development. The identity of *ig1* and phenotype of *ig1* mutants in the gametophyte and sporophyte suggest that common logic is used to regulate sporophytic and gametophytic development.

344. Functional Genomic Analysis of Germ Cell Specification in Planarians. Joel M. Stary,[1] Yuying Wang,[1] Ricardo M. Zayas,[1] George T. Eisenhoffer,[2] and Alejandro Sánchez Alvarado[2]. [1]Dept. of Cell and Structural Biology, Neuroscience Program, University of Illinois at Urbana-Champaign; [2]Dept. of Neurobiology and Anatomy, Univ. of Utah School of Medicine.

The propagation of sexually reproducing organisms is dependent on the development of primordial germ cells. In many animal models, germ cells are specified early in embryogenesis by maternally supplied, cytoplasmic determinants. However, germ cell determination in other organisms proceeds epigenetically, requiring inductive interactions. Planarian flatworms are excellent models to study epigenetic germ cell specification because the germ cells are not formed embryonically; rather, they appear to derive from stem cells present in the adult. There are two strains of the planarian, *Schmidtea mediterranea*: hermaphroditic, sexual worms and fissiparous, asexual worms. To investigate the molecular basis of planarian germ cell specification, we have generated ~27,000 expressed sequence tags (ESTs) from the sexual strain (corresponding to ~10,000 unique transcripts). Microarray analysis comparing gene expression levels between the asexual and sexual strains shows that hundreds of genes are differentially expressed in the sexual strain. We are examining the cell

types expressing these genes using automated whole-mount in situ hybridization. This approach has led us to identify new markers of the reproductive organs in sexual planarians. Combined with functional analysis using RNAi, these studies will help elucidate the mechanisms by which inductive interactions specify germ cell fate and the extent to which these mechanisms have been evolutionarily conserved.

345. Early Development of Polarized Oogenic Cysts in the Polychaete Worm, *Ophryotrocha labronica*. John L. Brubacher and Erwin Huebner. Dept. of Zoology, University of Manitoba, Winnipeg, MB, Canada.

Oocytes in annelids of the genus *Ophryotrocha* develop in connection with single nurse cells as 2-cell syncytial cysts, free of an enveloping follicular epithelium. The elegant simplicity of these polarized cysts, and the phylogenetic position of annelids, make these worms ideal for comparative studies of nurse-cell/oocyte specification. Oogenesis in *Ophryotrocha* spp. comprises two phases: an initial intraovarian phase, in which cysts arise within rudimentary ovaries, and a later extraovarian phase, in which cysts are released to the coelom and mature. Pfannenstiel and Grünig (1982) proposed that in the intraovarian phase of *O. puerilis*, two germline cells in each segment undergo 4 incomplete mitotic divisions to produce 16-cell cysts, which then fragment into eight 2-cell units each. Cells originally connected to the parent cysts by a single cytoplasmic bridge are fated to become oocytes (*Zoomorphology* 100: 203–214). We have studied the early stages of oogenesis in a related species, *O. labronica*, by a variety of light and electron microscopy techniques. Our observations show there is an early, intraovarian distinction between nurse cells and oocytes. However, in *O. labronica*, 2-cell cysts are not produced by fragmentation of a 16-cell cyst, but instead, arise as independent units. Thus, cystocyte fate is not determined by the hierarchy of cells within a parent cyst. To more fully understand the process of cystocyte differentiation, we are currently examining the temporal and spatial dynamics of intraovarian germ cells using 3D reconstruction of serial TEM sections, and bromodeoxyuridine labeling. We gratefully acknowledge NSERC funding.

346. *C. elegans* Germline Mitotic Region Possesses Germline Stem Cells. Sarah L. Crittenden, Kim Leonhard, Dana Byrd, Myon Hee Lee, and Judith Kimble. University of Wisconsin-Madison and HHMI, Madison, WI 53706.

The adult *C. elegans* germ line includes mitotically dividing cells in the distal gonad and meiotic cells more proximally. A single somatic cell, the distal tip cell (DTC), provides a "stem cell niche" that promotes germline mitoses by GLP-1/Notch signaling. We will present experiments that address the self-renewal and division pattern of germline stem cells and that further define the DTC niche. To assay self-renewal, we have done BrdU pulse-chase experiments in germline cells in the mitotic region. Multiple regimens all gave the same result. A long pulse of BrdU (~24 hours) effectively labeled all germline cells in the mitotic region, but after a chase of 14 hours, virtually no label was detected in the mitotic region. Instead, the labeled cells were observed in the meiotic region of the gonad. We conclude that the labeled mitotically dividing cells were able to generate a new population of unlabelled mitotically dividing cells as well as differentiating gametes—the two hallmarks of stem cells. We observed no asymmetric or oriented cell divisions. Our current model is that distal cells within the mitotic region reside in the DTC niche and remain undifferentiated, while more proximal cells begin the transition into differentiation.

347. Dangerous Liaisons: The *Caenorhabditis* Kinase KGB-1 Targets GLH-1; Both Associate with the COP9 Signalosome Subunit CSN-5. K. Bennett, W. Li, and A. Orsborn. MMI Department, University of Missouri.

How the germline is determined, regulated and maintained are fundamental questions in developmental biology. To understand these processes we study proteins in *Caenorhabditis elegans* with germline functions. One such protein is KGB-1, a novel MAP kinase that binds the germline RNA helicases (GLHs). The GLHs are constitutive components of P granules, non-membranous aggregates of protein and RNA that pattern the *C. elegans* embryo and segregate with the germline throughout development. In examining KGB-1 function, we find that GLH-1 levels are greatly increased in the *kgb-1*(*um3*) deletion strain, which exhibits temperature sensitive sterility at 26°C and endomitotic replication of oocytes (EMO). Northern analyses show no change in *glh-1* levels with temperature change or KGB-1 loss. KGB-1 does not bind GLH-1 in pull-down analyses when a region with a potential MAP kinase docking site and a putative phosphodegron is deleted. Kinase assays show KGB-1 phosphorylates GLH-1; this modification may be used to target GLH-1 for degradation. CSN-5 (COP9 signalosome subunit 5), part of a conserved multi-protein complex important for protein stability, also associates with the GLHs. In *C. elegans* the loss of CSN-5 mimics the loss of GLH-1 and GLH-4, resulting in small, under-proliferated gonads and sterile worms. Based on evidence of CSN-5/KGB-1 interactions, we predict CSN-5 blocks KGB-1-mediated GLH-1 degradation. Thus, KGB-1 regulates GLH-1, with CSN-5 likely involved; these liaisons appear crucial for proper germline function.

348. Transcription Regulation of Endogenous Genes by *C. elegans* RNA Helicase A. Katherine M. Walstrom, Chris Bauer, and Deborah Schmidt. New College of Florida, Division of Natural Sciences, Sarasota, FL 34243.

Transcriptional silencing is an important process required for early germline development in some organisms such as *C. elegans* and *Drosophila* (Leatherman and Jongens (2003) BioEssays 25, 326–335). RNA helicase A (RHA) is a conserved protein with roles in transcription regulation and histone modification in numerous organisms such as flies and humans. We are studying the role of *C. elegans* RHA (RHA-1) in transcription regulation and germline development. A null mutant, *rha-1*(tm329), has a temperature-dependent defect in germline transcriptional silencing of transgene arrays (Walstrom, Schmidt, Bean, and Kelly (2005) Mech. Dev. In press). In addition, histone modifications are mis-localized on the transgene arrays, on autosomes, and on the single male X chromosome. These defects in gene regulation lead to a sterile phenotype in hermaphrodite and male worms. We are using real-time RT-PCR to determine if endogenous genes have altered expression in the mutants, and we are focusing on the X chromosome in the mutant males. The *rha-1* mutant also has a temperature-dependent sterile phenotype, and we hope to identify genes with altered expression that could lead to the sterile phenotype. Recent results will be presented at the meeting. Research Funding Source: NIH.

349. Identifying GLD-2 Target mRNAs in *C. elegans*. Nayoung Suh[1] and Judith Kimble[2]. [1]Department of Biochemistry; [2]Departments of Biochemistry, Genetics, and Molecular Biology, University of Wisconsin-Madison, and Howard Hughes Medical Institute, 433 Babcock Drive, Madison, Wisconsin 53706, USA.

Translational regulation plays a key role in animal development. In the *C. elegans* germ line, GLD-2 is the catalytic subunit of a cytoplasmic poly(A) polymerase critical for the decision between continued mitotic divisions and entry into meiosis. GLD-2 acts as a heterodimer with an RNA binding partner called GLD-3. No substrate mRNAs for the GLD-2/GLD-3 poly(A) polymerase have been identified to date. We suggest that GLD-2/GLD-3 polyadenylates and thereby activates meiosis-promoting mRNAs. Here, we provide three lines of evidence that *gld-1*, another key regulator of germline development, is a direct target mRNA of GLD-2. First, the *gld-1* poly(A) tail is longer in wild-type than in *gld-2* null mutants. Second, GLD-1 protein

accumulates to a higher level in wild-type than in *gld-2* null mutants. Third, *gld-1* mRNA co-immunoprecipitates with GLD-2 protein. The identification of *gld-1* mRNA as a target of GLD-2 activation is of particular interest because this same mRNA is also a target of FBF repression. FBF is a Pumilio homolog and key regulator of germline stem cells. We are in the process of identifying other GLD-2 substrates, both to learn more about the circuitry controlling the mitosis/meiosis decision and to compare the regulation of various mRNAs by this atypical poly(A) polymerase.

350. Loss of Pan-1 Causes a Peter Pan-Like Phenotype in *C. elegans*. Ge Gao and Karen Bennett. University of Missouri-Columbia.

P granules are complexes of proteins and RNA found surrounding the nuclei of *Caenorhabditis elegans* germ cells and germ cell precursors. GLH (germline RNA helicase) proteins are components of the germline-specific P granules, which are necessary for fertility in *C. elegans*. PAN-1, a P granule associated novel protein, was identified as a GLH-binding partner in yeast two hybrid assays. PAN-1 contains some N-terminal conserved amino acids of F-box motifs, as well as 16 leucine-rich repeats and a weak FOG-2 homology (FTH) motif; these two additional motifs are also found in some of the many (> 400) F-box proteins in *C. elegans*. F-boxes, which are part of the SCF (SKP-1, Cullin, F-box) complex, utilize ubiquitin-mediated degradation after binding to their specific substrates. When *pan-1* is eliminated by RNA interference (RNAi), the F1s of treated worms are arrested, staying at a size similar to the L2 stage and surviving up to 8 days at 20°C. (In 8 days, wild-type worms go through at least two generations!) A *pan-1*(*gk142*) deletion strain exhibits the same "forever-young" phenotype as seen by RNAi. mRNA analysis and protein expression studies show that PAN-1 is not germline specific but is germline enhanced, with PAN-1 surrounding germ cell nuclei in the adult gonad. Experiments are ongoing to separate potential germline and somatic functions of PAN-1. To begin to address the PAN-1/GLH relationship, we have looked at germ cell numbers in *pan-1(gk142)* arrested larvae where we see increased numbers of germ cell nuclei compared to wild-type L2 larvae. Thus, if PAN-1 is a GLH-specific F-box protein, it may regulate GLH levels, along with two other GLH binding proteins, CSN-5 and KGB-1.

351. A Genetic Screen to Identify the Targets of puf-8, a Puf Family Gene Involved in the Meiotic Progression of *C. elegans* Spermatocytes. Kuppuswamy Subramaniam and Mohd Ariz. Dept. of Biological Science and Bioengineering, Indian Institute of Technology, Kanpur 208016, India.

PUF-8, a member of the Puf family of RNA-binding proteins, is essential for the proper progression of spermatocytes through meiosis. The germ cells of the puf-8(−) worms enter normally into both meiosis and spermatogenesis, but do not complete either of the processes. Instead, the primary spermatocytes exit meiosis after reaching diakinesis and dedifferentiate back into mitotically cycling germ cells that form rapidly growing tumors. Since two members of the Puf family, namely the *C. elegans* protein FBF and the *Drosophila* protein Pumilio, have been shown to control the translation of the target mRNAs, we reasoned that the PUF-8 protein may also have mRNA targets. To identify these target mRNAs, we conducted a genetic screen to isolate mutants that suppress the puf-8(−) phenotype. By this approach, we have so far identified two mutants that completely suppress the tumor formation in the puf-8(−) worms. We will present our results on the characterization of these two mutations. This work was supported by a Wellcome Trust Senior Research Fellowship grant to K. Subramaniam.

352. Identification and Analysis of New Germline Sex Specific Genes. Abbie L. Casper and Mark Van Doren. Department of Biology, Johns Hopkins University, Baltimore, MD 21218.

Gamete development is necessary for fertility and for the survival of a species. In *Drosophila melanogaster*, previous research has shown that the sex of both germ cells and somatic gonadal precursor cells (SGPs) must match for proper gamete development. In the *Drosophila* embryo, we are studying the communication between germ cells and SGPs and how it contributes to germ cell sex determination through controlling gene expression in a sex-specific manner. *Drosophila* germline sex determination has been studied mostly in the adult stages; however, embryonic gonads are already sexually dimorphic. To focus on early germline sex determination, we first conducted a molecular screen to identified genes expressed in the germline during embryogenesis. Some germline genes identified are expressed in male germ cells and not in female germ cells following gonad coalescence. We found using sexual mosaics that the sex of the soma control expression of some of the identified genes and the germline sex regulates the expression of other genes. In addition, our preliminary work indicates that altering the known germline sex determination does not effect the expression of the male germline markers suggesting that there are genes upstream of the understood pathway. By studying the expression of the newly identified genes in the germ cells, we are hoping to clarify the contributions of the soma and the germ cell autonomous cues to germline sex determination.

353. Characterization of Genes Required for Germ Cell Development in *Drosophila melanogaster*. Juli D. Bodensteiner and Clark R. Coffman. Iowa State University, Ames, IA 50014.

Germ cell migration and programmed cell death are important aspects of the development and maintenance of many organisms. Defects in these processes are causative in diseases such as congenital heart disease and cancer in higher organisms. We utilize *Drosophila* germ cell development as a model to elucidate the mechanisms of cell migration and programmed cell death. In *Drosophila*, germ cells initially form outside the embryo, migrate into the embryo, and pathfind to the gonads during embryogenesis. Less than 50% of the germ cells initially formed are incorporated into the gonads. The germ cells that fail to reach the gonad are disposed of by programmed cell death. We will present the initial characterization of previously unreported X-linked mutations that disrupt germ cell migration and/or programmed cell death.

354. Germ Cell Migration and Death: Characterization of Scattershot and Ousiders Expression in *Drosophila melanogaster*. Keri D. Andersen and Clark R. Coffman. Iowa State University, Ames, IA 50011.

Cell migration and programmed cell death are essential components of animal development and homeostasis. They are critical for numerous processes including the migration of lymphocytes, eradication of extranumerary or misplaced cells, and nervous system development. Two genes, *scattershot* (*sctt*) and *outsiders* (*out*), are required during *Drosophila melanogaster* embryogenesis for germ cell migration and programmed cell death. The *sctt* mutation is a partial loss of function allele of *tre1*, a G protein-coupled receptor. Both germ cell migration and programmed cell death are disrupted by the *sctt* mutation. Another gene, *out*, is required for the programmed cell death of germ cells. We will report our analyses of the RNA and protein expression patterns of the *out* and *sctt* gene products, providing data informative to the functions of these genes during germ cell development.

355. Disruption of Germ Cell Migration and Programmed Cell Death in *Drosophila* Embryogenesis: the *scattershot* mutant. Angela R. Kamps and Clark R. Coffman. Iowa State University, Ames, IA 50011.

G protein-coupled receptors (GPCRs) have critical functions in many processes including development, immune response, and wound healing.

They are integral to a vast array of networks constituting the intracellular signaling pathways that regulate cell proliferation, survival, growth, and migration. Tre1, a GPCR expressed in *Drosophila melanogaster* germ cells, has roles in the migration and programmed cell death decisions of these cells during embryogenesis. In wild-type *Drosophila*, the germ cells originate at the posterior pole and become incorporated into the posterior midgut primordium. From their location within the midgut primordium, the germ cells migrate across the surrounding epithelial layer, transit through the mesoderm, and reach the somatic gonad tissue to be incorporated into the gonads of the embryo. During migration, over half of the germ cells that were present in the midgut primordium are destroyed via programmed cell death. The *scattershot* (*sctt*) mutation is a partial loss of function allele of *tre1*. In *sctt* mutants, the germ cells initiate migration, but few reach the gonads. Rather, they scatter throughout the posterior half of the embryo. In addition, these errant germ cells are not eradicated as in wild-type embryos. Interestingly, the cell migration and cell death phenotypes can be genetically uncoupled. This poster highlights our recent work studying the role of the Sctt/Tre1 GPCR in germ cell development.

356. The Outsiders Gene is Necessary for the Programmed Cell Death of Primordial Germ Cells During *Drosophila* Embryonic Development. Yukiko Yamada and Clark R. Coffman. Iowa State University, Ames, IA 50010.

Successful germ cell development is essential for the propagation of life in sexually reproducing organisms. We are exploring the molecular mechanisms regulating programmed cell death (PCD) of primordial germ cells in *Drosophila melanogaster*. In the early *Drosophila* embryo, approximately 50% of the germ cells that form at the posterior pole successfully migrate to and become incorporated into the developing gonads. The rest are eliminated via PCD. To investigate the regulation of germ cell PCD, we are studying the *outsiders* (*out*) gene. In *out* mutants, germ cells ectopic to the gonads fail to undergo PCD but continue to express germ cell markers. Normally these cells are eliminated. Interestingly, germ cell migration in *out* mutant embryos is wild type. Currently, seven recessive alleles of *out* exist. Genetic studies reveal that *out* function is required zygotically. We are using genetic approaches to gain insights into the roles of *out* and its possible interactions with other components of PCD networks during germ cell development. These efforts will allow construction of a more complete and integrated model for germ cell PCD.

357. *Vreteno*, a New Gene Required for Germ Line Stem Cell Differentiation in *Drosophila*. Marie Y. Davis, Eric Staeva-Vieira, and Ruth Lehmann. Skirball Institute/HHMI, New York University School of Medicine, New York, NY 10016.

Drosophila oogenesis results from many interactions between the germline tissue and adjacent somatic tissues. We have isolated a gene named *vreteno* from a forward EMS mutagenesis screen for maternally contributed genes on the 3R chromosome that display defects in both the soma and germline during oogenesis. In weak mutants, ovaries display a spindle eggshell phenotype and abnormal karyosome, as well as weak Gurken localization, indicating defects in egg patterning. In strong *vret* mutants, ovaries display severe defects in the germline stem cells, later cysts and egg chambers, as well as abnormal somatic follicle cells, indicating that *gurken* is not the only target of *vret*. In strong *vret* alleles the number of germline stem cells is increased and *dpp* signaling is upregulated in the germline. Genetic experiments suggest that vret acts upstream or in parallel to the germ line differentiation gene *bam*.

Clonal analysis suggests that the dorsal–ventral eggshell patterning defect is germ line autonomous, while the early oogenesis defects are not due to an autonomous role for *vret* in the germline or the somatic follicle cells. Further analysis using positively marked clones and pole cell transplantation will be used to determine where *vret* function is required during early development.

Not only females but also males homozygous for strong alleles are sterile, suggesting that *vret* is a gonad-specific gene. Further characterization of the *vret* phenotype in ovaries and testes and molecular identification of *vret* should elucidate the role of this new gene in soma–germline interaction.

358. Direct Control of Germline Stem Cell Division and Cyst Growth by Neural Insulin in *Drosophila*. Daniela Drummond-Barbosa and Leesa LaFever. Vanderbilt University Medical Center, Nashville, TN 37232-8240.

Stem cells reside in niches that provide signals required for their maintenance and division. Tissue-extrinsic signals can also modify the activity of stem cells, although this is a poorly understood process. We have previously shown that germline and somatic stem cells, as well as their dividing progeny, adjust their rates of division and development in response to nutrition in the *Drosophila* ovary, and that the insulin pathway is required for this response. Here, we report that brain-derived insulin-like peptides (DILPs) directly regulate the rate of germline stem cell division, demonstrating that signals mediating the ovarian response to nutrition can modify the activity of stem cells in a niche-independent manner. We also reveal a crucial direct role of DILPs in controlling the rate of germline cyst growth and progression through vitellogenesis. Our data also suggest that somatic cells in the ovary receive a relay signal from the germline that maintains the two tissue types coordinated with each other.

359. Zebrafish Staufen in Germ Cell Specification. Srinivas Ramasamy, Wang Hui, and Karuna Sampath. Laboratory of Vertebrate Development, 1, Research Link, Temasek Life Sciences Laboratory, National University of Singapore, Singapore-117604.

Maternally localized factors influence embryonic polarity and the specification of the germ line. In Zebrafish, the role of maternally localized factors and RNA binding proteins in RNA localization and specification of embryonic axis and germ line is poorly understood. *Drosophila staufen* encodes an RNA-binding protein that is essential for the correct localization of several transcripts in oocytes, embryos and germ plasm assembly. We have isolated and characterized two zebrafish *staufen homologs*. Zebrafish *stau2* is expressed maternally and throughout embryogenesis. stau2 transcripts are localized in oocytes. During embryogenesis, stau2 transcripts are ubiquitously expressed in cleavage and blastula stages and also expressed in peri-ventricular neurons at 24 hpf. To determine the function of stau2 in early embryos, GST-STAU2 peptides were expressed in *E. coli*. Purified peptides were injected into 1 cell stage embryos. A dominant-negative STAU2 peptide disrupts the localization of GFP-nanos-3′UTR fusion RNA to germ cells. In situ hybridization with various marker genes showed that expression of *vasa*, a germ cell specific gene, is also abolished in STAU2 peptide-injected embryos. In addition, STAU2 peptide-injected embryos show cell death in the anterior and midline regions. These phenotypes were also observed in embryos injected with an antisense morpholino oligonucleotide targeting stau2. Injection of *Drosophila melanogaster* staufen RNA rescues the germ cell and neural cell death phenotypes. These results suggest that STAUFEN protein has an evolutionary conserved role in germ line specification.

360. The RNA Binding Protein Hermes, May Have Several Important Functions During Early Development of *Xenopus laevis*. Hye-Won Song and Laurence D. Etkin. University of Texas-M.D. Anderson Cancer Center, Graduate school of Biomedical Sciences Program in Genes and Development, Houston, TX.

Hermes protein is 197 amino acids long and contains a single RRM (RNA Recognition Motif) domain. Several lines of evidence indicate that Hermes protein is a bona fide RNA binding protein (Gerber et al., 2002 and unpublished results). Hermes mRNA and protein are both localized within the germ plasm at the vegetal cortex of the oocytes. EM immunogold labeling analysis demonstrated that the Hermes protein is detected within the germinal granules in a pattern very similar to that of the Xcat2 mRNA. Recent evidence suggests that Hermes is part of an RNP complex with Xcat2 (M.L.K, unpublished results). Hermes protein level decreases during maturation and is not detected in embryos during early embryogenesis. Injection of antisense morpholino oligonucleotides (HE-MO) caused a precocious depletion of Hermes protein during maturation, which resulted in cleavage defects in vegetal blastomeres (Zearfoss et al., 2004). This result suggests a distinct function of Hermes related to the regulation of cleavage. Recently, we observed that the precocious depletion of Hermes by HE-MO injection also caused the acceleration of maturation. We are carrying out studies to understand the fundamental role of Hermes RNA binding protein in regulating both oocyte maturation and early cleavage of the vegetal blastomeres. Our preliminary data suggest that Hermes may be involved in regulating translation of a group of maternal RNAs involved in these processes; perhaps through a cytoplasmic polyadenylation (CPE)-related mechanism.

361. FGF9 is Required for Male Germ Cell Survival. Leo DiNapoli and Blanche Capel. Duke University, Dept. of Cell Biology, Durham, NC 27710.

The integration of germ and somatic cell development is crucial for the reproductive fitness of organisms. Improper gonad development can result in an array of defects including gonad dysgenesis, sexual ambiguity, and sterility. Fibroblast growth factor 9 (*Fgf9*) has been shown to play an important role in male gonad development, as loss of *Fgf9* results a reduction in the number of Sertoli cells, lower rates of proliferation, and improper testis development. Our data have also revealed a separate role for FGF9 in male germ cell survival. In XY *Fgf9*−/− mice, germ cells decline in numbers after embryonic day 11.5, while germ cells in XX gonads are unaffected. We present evidence that FGF9 directly affects germ cell survival in male gonads and can rescue germ cell survival in culture. Interestingly, exogenous FGF16, which is in the same subfamily of FGFs as FGF9, can partially rescue germ cells in culture, while exogenous FGF7, from a different subfamily, cannot. Also, we find that the XY *Fgf9* null gonad undergoes true male to female sex reversal as it expresses the female markers *Wnt4*, *BMP2*, and *Follistatin*. This research was funded by NSF.

362. The Role of the Forkhead Gene Foxc1 in Gonad Development. Deidre M. Mattiske and Brigid Hogan. Duke University Medical Center, NC 27710.

Foxc1 encodes a forkhead/winged helix transcription factor expressed in many embryonic tissues, including mesonephric and metanephric mesenchyme. Previous studies have shown that Foxc1 plays an important role in the specification of the intermediate mesoderm, in which the mesonephros, gonad, and metanephros arise. Homozygous null Foxc1LacZ mice die at birth with multiple developmental defects, including duplex kidneys and hydroureters. This study investigates the role of Foxc1 in the development of the mesonephros and gonad. Foxc1 homozygotes have ectopic mesonephric tubules, along with posterior expansion of expression of markers of nephric tissue such as Pax2. At 11.5 dpc, the gonads of homozygous mutants are significantly smaller than wild type with a reduction and disorganization of tissue in the posterior region of the gonad. Although germ cells are specified correctly in Foxc1 homozygotes, germ cells do not migrate correctly to the gonadal ridge, remaining trapped in the hindgut mesentery. By 11.5 dpc, when germ cells have populated the gonads, the number of germ cells in homozygous mutants is less that 25% of the number in wild-type gonads. At birth, germ cell number is significantly less than wild type with fewer developing type I follicles in the ovary. In both male and female gonads, the structure of the gonad is disorganized, the gonadal volume is smaller and the gonads are located in a more anterior position within the embryo. This study provides evidence that Foxc1 has essential functions in gonad development alongside its previously known roles in kidney, cardiovascular and eye development.

363. Oocyte-Specific MATER Affects Microtubule Formation and May Suppress Protein Secretion. Mami Ohsugi, Sonja Hess, and Jurrien Dean. NIDDK, NIH, Bethesda, MD 20892.

Mater is a single copy, maternal effect gene expressed in growing oocytes that encodes a ~125 kDa member of the NALPS family of cytoplasmic proteins. Initially present uniformly in the cytoplasm of growing oocytes, MATER migrates to the periphery of fully grown oocytes. Following ovulation, MATER transcripts disappear, but the protein persists until the early blastocyst. Embryos derived from *Mater*−/− females die early in development and the phenotype is not rescued by normal males. Although spindle formation, midbodies and cleavage appear normal in the absence of MATER, microtubules are irregular with decreased levels of α-tubulin, and atypical mitochondria localization observed late in oocyte growth. The absence of MATER affects the abundance of other proteins within ovulated eggs, some of which have been identified by mass spectrometry. One, a novel protein designated FILIA, co-localizes and forms a complex with MATER. Another, CALRETICULIN, accumulates in abnormal large envelopes in the periphery of eggs that stain positively with *trans*-Golgi- but not endoplasmic reticulum-marker proteins. These data suggest that MATER interacts with other oocyte-specific proteins and may affect protein secretion.

364. Understanding the Functional Evolution of Trunk *Hox* Genes in Arthropods. Cheryl C. Hsia and William McGinnis. University of California, San Diego.

Hox genes encode transcription factors that specify segmental identity along the *a*–*p* axis. Changes in *Hox* gene number, expression or in target gene regulation are correlated with morphological transitions within and between phyla (Gellon and McGinnis, 1998). Elucidating the mechanistic changes affected by changes in *Hox* gene function can lead to a better understanding of the evolution of diverse animal body plans. Recent studies provide evidence that modifications within Hox protein sequences may have played a large role in their functional evolution. Based on studies of Ubx orthologs from *Drosophila melanogaster* and the crustacean *Artemia franciscana*, Ronshaugen et al. (2002) proposed that the insect lineage lost phosphorylation sites within the C-terminus. This loss allowed a domain found outside the C-terminus to repress limbs in the abdominal trunk segments, contributing to the evolution of a hexapod body plan from a multi-limbed crustacean body plan. Using ectopic expression assays in *Drosophila*, we set out to map the key phosphorylation sites in *Artemia* Ubx and to determine if this is a mechanism shared by the *Artemia* AbdA protein. Our studies indicate that a single serine within a CK2 phosphorylation site is required and sufficient to inhibit *Artemia* Ubx from repressing limbs. The mechanism through which *Artemia* AbdA is inhibited from repressing limbs, however, is quite different. The C-terminus of the AbdA protein appears to inhibit the protein from repressing limbs by preventing protein accumulation during early development. This finding is the first example of a stage-specific function of a Hox protein controlled via post-transcriptional regulation of protein accumulation.

365. The Role of *Hox* Genes in Crustacean Appendage Specialization. Danielle M. Liubicich, Julia M. Serano, and Nipam H. Patel. University of California, Berkeley/HHMI CA 94720.

Changes in gene expression patterns during development may play a critical role in the evolution of morphological diversity among metazoans. For example, homeotic mutants provide extreme illustrations of how altered expression of *Hox* genes results in transformation of segmental identity as well as modification of structures that develop on those segments. Previous studies revealed a correlation between *Hox* gene expression patterns and appendage specification in crustaceans. Here, we focus on understanding the role of three *Hox* genes, *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*), and *Ultrabithorax* (*Ubx*), during the development of modified trunk appendages, called maxillipeds, in the amphipod *Parhyale hawaiensis*. Maxillipeds are thoracic appendages that function in feeding rather than locomotion, and *Parhyale* develop one pair of maxillipeds that can be morphologically distinguished from other thoracic appendages during early limb bud elongation (at about 40% of development, 96 h after egg laying). Analysis of *Parhyale Scr*, *Antp*, and *Ubx* mRNA and protein expression suggests each of these *Hox* genes may play a role in establishing maxilliped identity, and this event may occur quite early in development, as *Hox* gene expression begins when individual parasegment precursors are just single-cell wide rows of ectodermal cells. To test this correlation between *Hox* gene expression and appendage morphology, we are currently attempting gene knockdown experiments using RNAi and morpholinos.

366. *Hox* Gene Expression Initiates in Single-Cell Wide Parasegment Precursors in the Amphipod Crustacean *Parhyale hawaiensis*. Julia Serano,[1] Danielle M. Liubicich,[1] William E. Browne,[2] and Nipam H. Patel[1]. [1]University of California, Berkeley/HHMI CA 94720; [2]University of Hawaii.

Hox genes have been extensively studied in *Drosophila* and other insects, where they are expressed in overlapping domains along the anterior–posterior axis and establish segmental identity. Recent studies of *Hox* gene expression in crustaceans, sister group to the insects, have reported similar correlations between segment morphology and Hox expression domains. Most previous studies, however, have focused on relatively late stages of development, but our analyses have revealed that *Hox* genes in the amphipod crustacean *Parhyale hawaiensis* are first expressed remarkably early in development. *Hox* gene expression occurs prior to *engrailed* expression and is coincident with the first visible signs of parasegmental organization when unpatterned ectodermal precursor cells begin to align themselves into highly ordered single-cell wide rows, called Roman Numeral Rows, each of which is the precursor to precisely one parasegment. *Parhyale Hox* genes demonstrate both temporal and spatial colinearity in their expression—the only exception thus far being *proboscipedia*, which is not expressed until the time of appendage formation. We are currently examining the potential function of these early expression patterns through a variety of experimental manipulations.

367. Evolution of *Hox* Gene Expression and Function in Mammals. Chih-Hsin Chen,[1] Chris J. Cretekos,[1] John J. Rasweiler IV,[2] George B. Adebayo,[1] and Richard R. Behringer[1]. [1]University of Texas MD Anderson Cancer Center, Houston; [2]SUNY Downstate Medical Center, Brooklyn.

Body plan divergence exists in mammals. Most of this diversity is found in limb and vertebral morphologies. This morphological diversity could be caused by divergent gene expression profiles and/or functions. Unlike other mammals, the 2nd–5th digits of bat forelimb are relatively elongated to form the major part of the wing. In vertebrates, *Hoxd13* is one of the important factors for digit growth and patterning, and thus its divergent expression between species may lead to divergent growth and patterning of the digits. We have cloned *Hoxd13* from *Carollia perspicillata*, the short-tailed fruit bat. The expression patterns of bat *Hoxd13* during early limb development were compared to those of mouse. In bat and mouse hindlimbs, their expression patterns of *Hoxd13* are similar. However, both mammals have divergent *Hoxd13* patterns in forelimb from late limb bud stages to limb plate stages; the anterior expression boundary of bat *Hoxd13* is posterior-shifted relative to the mouse. Compared with the *Hoxd13* expression profiles of other vertebrates, the above results suggest that divergent *Hoxd13* expression profiles may contribute to different digital morphologies in vertebrates. To functionally examine the consequences of divergent expression profiles of developmental control genes among mammals, we have used human *HOXB1-9* containing DNA to rescue mice homozygous for a *HoxB1-9* deletion to determine human and mouse *HoxB1-9* function in vertebral anterior–posterior patterning. Preliminary data suggest that human *HOXB1-9* could partially rescue the mouse homeotic vertebral phenotypes.

368. Evolution of Gastrulation Gene Networks in the Honeybee *Apis mellifera*. Jessica D. Cande and Michael Levine. University of California Berkeley.

In *Drosophila melanogaster*, the early regulatory events leading to the patterning of the dorsal/ventral axis are well understood. The transcription factor dorsal is present as a nuclear gradient. High levels of nuclear dorsal activate transcription of mesoderm specific factors such as *twist*, the *snail* repressor and the FGF receptor *heartless*, which is required for mesoderm spreading. Lower levels of dorsal pattern the mesectoderm, neurogenic ectoderm and dorsal ectoderm in a threshold readout response. Intermediate levels of dorsal lead to the activation of neurogenic ectoderm-specific transcription factors such as *sim*, *vnd* and *ind*, which, together with *msh*, are crucial for the early specification of neuroblast identity. Dorsal acts as a repressor to limit expression of genes such as *zen*, a transcription factor crucial for the formation of the amnioserosa, to the dorsal ectoderm. Ultimately, this leads to the patterning of the various germ layer precursors, and the initiation of programs of cell movement (gastrulation) and of differentiation. By comparing protein sequences, expression patterns and *cis*-regulatory sequences from the honeybee (*Apis mellifera*) dorsoventral patterning genes with their fly orthologs we are beginning to understand how the regulatory mechanisms governing gastrulation have evolved and how changes in gene expression relate to the phenotypic differences seen in gastrulation in these two insects.

369. Nodal Signaling and the Evolution of Deuterostome Gastrulation. Helen K. Chea and Billie J. Swalla. Friday Harbor Laboratories, University of Washington, Seattle, WA 98195.

Deuterostomes are animals in which the blastopore becomes the anus and the mouth forms secondarily in another location during embryonic development. The evolution of gastrulation within the deuterostomes has allowed for the formation of the various developmental and morphological differences seen in larval and adult deuterostomes today. Although gastrulation is an amazingly complicated developmental process, involving wide-scale cell movements and signaling mechanisms, there are some common mechanisms that are beginning to emerge. An important signaling pathway involved in regulating gastrulation is that of Nodal. Nodal is a member of the transforming growth factor-β (TGF-β) superfamily of signaling molecules, and it plays a regulatory role in cell interactions that are responsible for patterning the early embryo. *Nodal* was initially discovered in vertebrates, but has also been found in invertebrate chordates and, most recently, in non-chordate deuterostomes. We examine *Nodal* expression in each of the vertebrate model systems, then compare them to *Nodal* expression in invertebrate deuterostomes. When *Nodal* expression within the deuterostomes is examined, several conserved features emerge. We have also conducted experiments to determine when and where *Nodal* is expressed throughout tunicate development, in order to understand how Nodal signaling has evolved in the chordates. *Nodal* is expressed early during gastrulation and later to specify left–right asymmetry in chordates,

but the ancestral role of *Nodal* may have been in positioning the mouth during larval and/or adult development.

370. Embryonic TGF-Beta Signaling in Cnidaria and Higher Animals: Similar Genes-Similar Functions? G.H. Thomsen,[1] D. Matus,[2] K. Pang,[2] T. Kalkan,[1] and M.Q. Martindale[2]. [1]Stony Brook University, Stony Brook, NY; [2]University of Hawaii, Kewalo Marine Lab, Honolulu, HI.

We are interested in the evolutionary origin and molecular mechanisms of embryonic polarity, bilateral symmetry and embryonic germ layer formation (particularly the mesoderm). The cnidarian *Nematostella vectensis*, the starlet sea anemone, is a new model system with which to tackle questions about the evolution of animal developmental mechanisms. Our recent efforts have focused on identifying and testing the embryonic function of the TGFβ signaling system in Nematostella. We have found a full TGFβ signaling toolbox, complete with ligands, ligand antagonists, receptors, Smad signal transducers and transcriptional cofactors. We have identified Nematostella activin, BMP-6/7 and a new DPP/BMP-related gene, in addition to previously reported orthologs of DPP/BMP-4 and GDF-5 that are expressed asymmetrically along an axis that defines a plane of bilateral symmetry and may be an ancient precursor of the dorso-ventral axis of higher animals (Finnerty et al., 2004; 304:1435). We have also identified orthologs of the DPP/BMP antagonists, chordin and noggin in Nematostella that function in triploblastic animals to specify dorso-ventral polarity and pattern the germ layers. We will discuss the patterns of expression and potential function of TGFβ ligands and their inhibitors in development of the sea anemone, which lacks a defined mesodermal germ layer, and discuss how these patterns inform us about the evolution of dorso-ventral axial patterning.

371. Melanocyte Development of the Invertebrate Chordate *Ciona intestinalis*. Angela C. Cone and Robert W. Zeller. San Diego State University.

In vertebrate embryos, neural crest cell (NCC) derivatives produce much of the structural complexity that distinguishes craniates from invertebrates. NCCs, a vertebrate synapomorphy, are defined as a multipotent population of embryonic cells that arise at the border of the neural and non-neural ectoderm, migrate at the end of neurulation, and express characteristic genes. Many transcription factors are expressed in overlapping domains in developing NCCs, but it is unknown if each NCC expresses the same suite of factors. Analysis of single cell gene expression has remained technically difficult in vertebrate embryos, but profiling of the transcriptome of individual developing NCCs is required. The evolutionary origin of neural crest is being examined by studying the development of neural crest-like cell types in the urochordate, *Ciona intestinalis*. A careful analysis of gene expression patterns in a single neural plate border cell lineage in *Ciona* indicates that many NCC regulatory genes are expressed at this border region as well as in melanocytes. Preliminary evidence suggests that the melanocyte lineage in ascidians is derived from cells at the neural plate border, and gives rise to multiple cell types, some of which may migrate. Further characterization of the melanocyte lineage with regards to cell fate, migration, gene expression, and the functional linkages of these genes in a regulatory network is helping to determine what aspects of neural crest development were present in the common chordate ancestor. This will add to our current understanding of neural crest development in vertebrate embryos where analysis at this level of cellular resolution is difficult or impossible.

372. Evidence for Complex Evolutionary Origins of a Sea Urchin Polyketide Synthase Gene. Cristina Calestani, Todd A. Castoe, Tricia Stephens, and Christopher L. Parkinson. University of Central Florida, Orlando, FL 32816.

Polyketide synthases (PKSs) are a large family of multifunctional proteins identified mainly in bacteria, fungi and plants. A polyketide synthase gene (*SpPks*) was previously isolated from sea urchin embryos and proven to be required for the biosynthesis of the larval pigment echinochrome. To address the evolutionary origin of *SpPks*, we exhaustively surveyed for PKS homologs/orthologs/paralogs across all available NCBI accessioned genomic data. Phylogenetic trees based on this amino acid alignment were estimated using maximum parsimony (MP) and Bayesian Markov-chain Monte Carlo (MCMC) phylogenetic methods. We conducted phylogenetic analyses on three partitions of the data set: (1) the entire alignment, (2) the PKS domain only, and (3) the acyltransferase (AT) domain only. Phylogenetic results support a complex evolutionary history having lead to *SpPks*. The PKS domain appears most closely related to PKSs found in planktonic bacteria and fungi. Similarly, the AT domain is closely related to fungal AT domains, although also related to AT domains of the fatty acid synthase gene family found ubiquitously in animal taxa. Collectively, our results suggest the following: (1) the evolutionary history of *SpPks* likely involves some domain-shuffling or intragenic recombination to account for the unique evolutionary history inferred for the PKS and AT domains, and (2) the phylogenetic relationships of *SpPks* conserved domains are most consistent with the historical occurrence of a horizontal transfer event, possibly from bacteria or fungi.

373. Gene Expression Analysis of Germ Layer Evolution in the Indirectly Developing Polychaete Annelid *Hydroides elegans*. Cesar Arenas-Mena. San Diego State University.

The embryo of this marine worm has broad evolutionary-developmental relevance derived from its indirect developing life cycle. The early embryonic generation of ectodermal, endodermal, and mesodermal precursors together with the relatively simple morphogenetic processes leading to the minute trochophore larva are isolated by days of feeding from the far more complicated processes involved in the formation of the macroscopic adult (i.e., terminal growth, segmentation, anteroposterior patterning, etc.). The expressions of several developmental regulatory genes with orthologs having germ layer specification functions in other metazoans (Sox, Gata, Snail, Fox, etc.) suggest derived and ancestral functions in this lineage. In addition, an EST/WMISH project is further identifying major gene expression subdivisions, which provide a first draft map for upstream embryonic regulatory events and the particular distribution of metazoan cell-type functions in the feeding trochophore larva. These gene expression patterns illuminate major questions related to metazoan germ layer specifications as well as the establishment of primary and secondary embryonic asymmetries. In particular, given the extensive ectomesodermal contributions of this embryo toward larval structures, some regulatory and differentiation gene expression patterns seem key to understand the origin and evolution of metazoan mesoderm. Furthermore, in the context of an equal cleaving embryo with no dorso-ventral polarity, they also shed light on secondary axis specification evolution.

374. The GATA Family of Receptors Within the Polychaete *Platynereis dumerilii*. William J. Gillis, Bruce A. Bowerman, and Stephan Q. Schneider. Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229.

GATA transcription factors play key roles in the specification of cell lineages and germ layers throughout bilaterians. In vertebrates two discrete classes GATA 1/2/3 and GATA 4/5/6 have been found which are playing an ectodermal and an endomesodermal role, respectively. However, the role and functions of GATA-factors in *Drosophila* and *C. elegans* are less clear due to an expansion of the GATA gene families by gene duplications and subsequent divergence. To investigate the ancestral functions of GATA factors we have recently identified two GATA factors within the polychaete

Platynereis dumerilii. Initial phylogenetic and gene expression analysis supports the placement of these two factors into a GATA1/2/3 class and a GATA 4/5/6 class, respectively. This suggests that the bilaterian ancestor had already two GATA factors, with separate endomesodermal and ectodermal functions.

375. Molecular Composition of Epithelial Cell Junctions in the Polychaete *Platynereis dumerilii*. Stephan Q. Schneider, Bruce A. Bowerman, and Chris Q. Doe. University of Oregon, Eugene, OR 97403.

Epithelial tissues are present in most metazoans and rely for their crucial functions, e.g., in homeostasis on cell junctions. However, remarkable differences exist between the molecular and structural organization and composition of cell junctions among metazoan epithelia especially between vertebrates and insects. It is not known which epithelial type reflects the prototypic epithelium from which both the vertebrate and insect types originated. To gain insight into this intriguing question we investigated epithelial cell junctions (ECJ) in the marine polychaete Platynereis. We screened for cross-reacting antibodies against key components of junctional complexes and used immunohistochemistry combined with 3D confocal microscopy to elucidate the molecular composition and organization of polychaete ECJs. Surprisingly, we found evidence that polychaete ECJs form one single unit with juxtaposed but clearly separate sub-domains that correspond in its molecular composition to tight junctions, adherens junctions, and septate junctions all combined in a structure of one. A similar organization has been observed in the nematode *C. elegans* suggesting that this single type of ECJ reflects the prototypical invertebrate condition, and that vertebrate and insect epithelia have evolved novel and separate junctional features.

376. Cocoon Production and Function in Leech Development. Daniel H. Shain, Charlene W. Sayers, Jon'elle Coleman, Corneliu Dimitriu, and Tarin A. Mason. Rutgers, The State University of New Jersey, Camden, NJ 08102.

The aquatic leech, *Theromyzon tessulatum*, secretes a proteinaceous cocoon that facilitates embryogenesis and a required parent–juvenile association in the field. The process of cocoon production is characterized by thickening of the clitellar wall due to the synthesis of cocoon components. Specifically, several hundred km of protein fiber, cocoon fluid comprising multiple proteins, and an underwater sealant are required for the construction of each cocoon. Histological comparisons of clitellar segments at different stages of cocoon synthesis reveal that small granules are channeled to the surface via an elaborate network of ducts more prominent on the dorsal surface. Globules of fibrous material appear to fuse in succession to the inner surface of the thickening cocoon membrane, generating a thin, transparent wall ~70 layers thick. Opercula (i.e., plugs that seal each end of the cocoon) become positioned asymmetrically on the upper aspect of the cocoon and appear to provide an escape route to the parent leech as juveniles hatch. In the field, parents often shuttle juveniles to their first blood meal, a duck nose.

377. Diverse Functions of Wnt Signaling During Nematode Vulva Formation: A Comparison Between *Pristionchus pacificus* and *C. elegans*. Min Zheng, Hua Xiao, and Ralf J. Sommer. Max-Planck Institute for Developmental Biology, Tuebingen 72076, Germany.

The nematode *Pristionchus pacificus* has been developed as a satellite organism in evolutionary developmental biology and its genome is currently sequenced by NIH. Detailed comparison of vulva development between *P. pacificus* and *C. elegans* indicates multiple mechanistic differences: In *C. elegans*, Wnt signaling is involved in vulva induction, whereas a Wnt pathway is involved in a negative signal counteracting vulva induction in *P. pacificus* (Zheng et al., *Nature Genetics*, 37, 300–304, 2005). Mutations in *Ppa-lin-17/Fz* and *Ppa-groucho* result in gonad-independent vulva differentiation and a multivulva phenotype. In addition, *Ppa-lin-17/Fz* is crucial for the polarization of the vulva, similar to *Cel-lin-17/Fz*; whereas *Ppa-groucho* regulates the apoptosis of a subset of vulval precursor cells. Morpholino studies in sensitized *Ppa-lin-17/Fz* and *Ppa-groucho* mutant backgrounds suggest that *Ppa*-LIN-44/Wnt, *Ppa*-EGL-20/Wnt, *Ppa*-MIG-5/Dsh, *Ppa*-APR-1/APC, *Ppa*-BAR-1/β-catenin, and *Ppa*-POP-1/TCF are involved in negative signaling. Thus, the *P. pacificus* Wnt pathway plays a crucial role in negative signaling during vulva development. How vulva induction by the somatic gonad overrides this negative signaling function remained unknown. Here, we describe the cloning of the first *P. pacificus* gene involved in vulva induction. Mutations in *Ppa-vul-2* result in a vulvaless phenotype and genetic experiments indicate that *Ppa-vul-2* acts genetically downstream of *Ppa-groucho* and *Ppa-lin-17/Fz*. Testing *Ppa*-VUL-2 physical interactions with downstream components of the Wnt pathway is ongoing.

378. Dorsoventral Patterning in a Short Germ Insect. Maurijn van der Zee and Siegfried Roth. University of Cologne, 50931 Cologne, Germany.

In the long germ insect *Drosophila* a gradient of the BMP homologue *decapentaplegic* (*dpp*) specifies cell fates along the dorsoventral (DV) axis. Dorsal peak levels activate *zerknüllt* (*zen*), which specifies the amnioserosa. Ventral inhibition of dpp signaling by the chordin homologue *short gastrulation* (*sog*) leads to the specification of the neurogenic ectoderm and mesoderm. We used RNAi to investigate the dynamics of this regulatory network in the beetle *Tribolium castaneum*, which exhibits more primitive development. Whereas absence of *sog* in *Drosophila* has minor dorsalizing effects, *sog* RNAi in *Tribolium* has a strong effect on the DV axis, revealing a similarity to vertebrates. Furthermore, again similar to chordin in vertebrates, absence of *sog* leads to the loss of anterior structures. Although the *dpp* gradient has an input in the dorsal localization of the *Tribolium zen* transcripts, the initial *zen* expression is solely dependent on the anteroposterior patterning system. Functional analysis of the two *zen* homologues shows that *Tc-zen1* acts early and specifies the serosa. After *Tc-zen1* RNAi the serosa is absent and the whole blastoderm consists of an enlarged germ rudiment, with an amnion at the dorsal side. Thus, after loss of *Tc-zen1*, *Tribolium* acquires features of long germ development with a single extraembryonic membrane. The other orthologue, *Tc-zen2* acts later and initiates the fusion of amnion and serosa required for dorsal closure. After *Tc-zen2* RNAi, the embryo closes ventrally, assuming a completely everted (inside–out) topology. In summary, RNAi studies in the short germ beetle *Tribolium* reveal similarities to vertebrates and greatly contribute to our understanding of insect evolution.

379. Waves of Runt Expression Segment the Short Germ Insect *Tribolium castaneum*. Sherry C. Rhoades and Susan J. Brown. Kansas State University, Manhattan, KS 66506.

Considerable debate continues regarding the extent to which the molecular segmentation machinery is conserved between vertebrates and arthropods. Oscillating expression patterns of Notch and Delta in the growth zone of spider embryos indicate that some aspects of the vertebrate segmentation machinery are conserved in chelicerates. However, the dynamics of the striped expression patterns of *Drosophila* pair-rule gene homologs in evolutionarily diverse arthropod species suggest little beyond superficial parallels between segmentation in insects and vertebrates. We examined the expression pattern of Tribolium runt, a pair-rule gene required for proper segmentation in *Drosophila*. Tcrunt is initially expressed in a broad domain in the posterior end of the Tribolium blastoderm, from 0% to 30% egg length. Expression at the posterior end fades as cells closer to 50%

begin to express runt. The first runt stripe stabilizes at ~ 50% egg length narrows to a stripe coexpressed with the second Engrailed stripe and eventually fades. During the resolution of the first stripe, cells at the posterior tip of the embryo express runt, their expression fades as cells more anterior cells express runt. The second stripe eventually stabilizes overlapping the fourth Engrailed stripe and then fades. Each successive stripe arises at the posterior tip of the embryo in a similar manner, until eight runt stripes have formed, resolved and faded. This is the first description of oscillating pair-rule gene expression in an insect to provide a striking parallel to the waves of Notch, Delta and hairy expression during somitogenesis in vertebrates.

380. Netrins: Creating Divergent Morphologies. Molly Duman-Scheel, Stephanie Clark, Eric Grunow, Wendy Simanton, Andrew Hasley, and Brandon Hill. Albion College, Albion, MI 49224.

In recent years, the discovery of numerous axon guidance molecules has allowed us to make great strides in understanding how axons find their targets during development. Many of these molecules are conserved among divergent organisms. For example, Netrin proteins regulate growth of axons toward the midline in a wide variety of creatures, from fruit flies to mice. To date, research has mainly concentrated on the similarities in axon guidance in different species. Such discoveries, while fascinating, have failed to explain how different neural network patterns have arisen in divergent organisms. Despite the presence of homologous neuroblasts and neurons in a variety of arthropods, we have observed divergent axon morphologies. This investigation aims to examine the molecular mechanisms underlying the creation of such novel axonal morphologies. The specific aims are to examine *netrin* gene expression in *Artemia franciscana*, a crustacean with unique midline axonal morphologies, and compare it to netrin expression patterns in *Drosophila*, an organism in which the function of netrin genes at the midline is well documented. To this end, we have used a degenerate PCR approach to clone two *Artemia franciscana netrin* genes, *afnet-1* and *afnet-2*. A polyclonal antibody generated against the AfNet-1 protein is allowing us to elucidate a novel early embryonic expression pattern for this protein. Moreover, this antibody recognizes Netrin proteins in a variety of insects and crustaceans and is serving as a useful cross-reactive tool for comparing Netrin expression patterns among distantly related arthropods.

381. Direct Regulation of *knot* by Ultrabithorax and the Evolution of *cis*-Regulatory Elements in *Drosophila*. Bradley M. Hersh and Sean Carroll. University of Wisconsin-Madison, HHMI.

The regulation of development by Hox proteins is important in the evolution of animal morphology, but the evolution of regulatory sequences of Hox target genes remains unclear. To understand the regulatory organization and evolution of these targets, we identified a wing-specific *cis*-regulatory element controlling the *knot* gene, which is expressed in the developing *Drosophila* wing but not the haltere. This element contains a single binding site critical for activation by the transcription factor cubitus interruptus (Ci), and a cluster of binding sites for repression by the Hox protein, Ultrabithorax (UBX). The negative and positive control regions are physically separable, suggesting that UBX does not repress by competing for occupancy of Ci binding sites. Although knot expression is conserved among *Drosophila* species, this cluster of UBX binding sites is not. We isolated the knot wing *cis*-regulatory element of *D. pseudoobscura*, which contains a cluster of UBX binding sites that is not homologous to the functionally defined *D. melanogaster* cluster. It is, however, homologous to a second *D. melanogaster* region containing a cluster of UBX sites that can also function as a repressor element. Thus, the *knot* regulatory region in *D. melanogaster* has two apparently functionally redundant blocks of sequences for repression by UBX. This redundancy suggests that the complete evolutionary unit of regulatory control is larger than the minimal experimentally defined control element. The span of regulatory sequences upon which selection acts may, in general, be more expansive than functional studies of regulatory elements have previously indicated.

382. Anterior Patterning in the Lower Cyclorrhaphan Flies *Megaselia* and *Episyrphus*. Steffen J. Lemke, A. Matteen Rafiqi, Sean Ferguson, and Urs Schmidt-Ott. Department of Organismal Biology and Anatomy, The University of Chicago, Chicago, IL 60637.

We use the natural diversity of developmental genetic circuitries in the insect order Diptera ('true' flies) to explore how novel regulatory factors and specific interactions evolve. We are interested in the evolution of anterior embryonic patterning, and our current aim is to reconstruct the ancestral state of this developmental network for Cyclorrhapha, a major dipteran suborder. Cyclorrhapha comprises about one-third of Diptera, including *Drosophila*. While the embryonic regulatory circuitries of non-cyclorrhaphan dipterans differ significantly from *Drosophila* (e.g., PNAS 99: 274; Development 131: 4251), Cyclorrhapha have been thought to develop in a very uniform manner. However, our recent findings question this assumption. We have established in situ staining and RNA interference protocols for two species which belong to independent basal branches of Cyclorrhapha (Phoridae: *Megaselia abdita*; *Syrphidae*: *Episyrphus balteatus*) and found divergent expression of *hunchback*, *tailless*, and *zerknüllt* homologs. Our ongoing investigations suggest that anterior regulatory interactions changed substantially during the radiation of Cyclorrhapha.

383. The Role of the Pupal Specifier, Br in the Direct-Developing Milkweed Bug, *Oncopeltus fasciatus*. Deniz F. Erezyilmaz, Lynn M. Riddiford, and James W. Truman. University of Washington, Seattle, WA 98195-1800.

Within the insects, the Holometabola, or metamorphosing insects diverged from hemimetabolous, or direct-developing ancestors about 300 million years ago. To better understand the genetic basis of this divergence, we have examined the role of the pupal specifier, Broad (Br), in the hemimetabolous milkweed bug. In Holometabola, epidermal expression of Br is restricted to the larval–pupal transition by juvenile hormone (JH); during larval life, JH inhibits Br expression, but a drop in JH titers at the last larval instar allows the expression of Br and the onset of metamorphosis. In *Drosophila*, Br null mutants develop normally until the third instar but fail to form a puparium at the end of larval life. In contrast to its expression in the Holometabola, we find that OfBr is expressed during the immature stages of the milkweed bug, where it appears at transitions between the nymphal stages, but is absent during adult development. To test the role of OfBr during the nymphal stages, we used dsRNAi-mediated knock down (dsRNA). We found that knock down of OfBr prevents nymphal heteromorphosis, or changes in cuticle pattern between nymphal instars. Injected nymphs still molt, but to a larger version of the previous instar. Moreover, by following the growth of the wing pads, legs, and antennae during the nymphal stages, we show that loss of OfBr prevents differential growth of the wings so that their growth trajectory resembles the isometric pattern of legs and antennae. Finally, we show that loss of OfBr and addition of JH during the final nymphal instar produces a supernumerary 6th stage nymph with genitalia.

384. Notch Expression in *Schistocerca americana*. Beata J. Blachuta, Kevin M. Curry, and Lisa M. Nagy. University of Arizona, Tucson, AZ 85716.

Adult appendages in *Drosophila melanogaster* develop from imaginal discs. In contrast, the appendages of most arthropods develop directly out of the body wall. Although many of the molecules that regulate limb patterning have been conserved in diverse arthropods, whether their function in the overall limb patterning network differs in arthropods that

develop their appendages directly remains unknown. We examined the expression of the transmembrane receptor Notch, which is involved in the patterning of joints in *Drosophila* legs, in the directly developing *Schistocerca americana* leg. Notch has two temporally distinct expression patterns in the developing grasshopper leg. Early in the development of the leg, Notch expression is limited to the distal portion of the leg. We are currently assaying whether early Notch expression correlates with a proliferation zone in the distal leg. Notch is subsequently turned on in stripes along the proximal–distal axis of the grasshopper leg, in a temporally and spatially distinct fashion. The stripes are not all expressed simultaneously. Instead, they are added over time as the leg continues to grow out of the body wall. Since Notch has been shown to be involved in cell proliferation in the developing *Drosophila* leg, it is possible that this early Notch expression is required for cell growth. The striping pattern may correspond to pSMAD expression, which is also present in proximal–distal stripes in the grasshopper leg. This suggests a potential regulatory interaction between the NOTCH and DPP pathways in regulating joint patterning.

385. Notch Signaling During Segmentation of the Amphipod Crustacean, *Parhyale hawaiensis*. Kira E. O'Day and Nipam H. Patel. UC Berkeley and HHMI.

The Notch signaling pathway is involved in somitogenesis in vertebrates and recent work has also demonstrated its importance during spider segmentation, where loss of Notch activity during embryogenesis leads to disorganization of segments. In *Drosophila melanogaster*, Notch signaling is not required for segmentation. However, fly segmentation occurs in a syncytium, obviating the need for cell signaling. To determine if Notch involvement in segmentation is confined to chelicerates or if it is more widespread among arthropods, we are examining the expression of components of the Notch signaling pathway during segmentation in the amphipod crustacean, *Parhyale hawaiensis*. In amphipods, the germband coalesces from previously unorganized cells into a grid-like pattern of cell rows. These rows then undergo two anterior-to-posterior waves of mitosis, giving rise to parasegments. Because of the invariant organization of the germband, it is possible to following patterning at the level of individual cells, allowing for a degree of analysis not possible in chelicerates and most other arthropods. We will investigate the role of PhNotch and its ligand, PhDelta, during amphipod segmentation by using in situ hybridization to determine their expression patterns, and by attempting to disrupt Notch function in *Parhyale* embryos through the injection of RNA coding for a dominant, constitutively active form of the Notch protein.

386. Segmentation in Branchiopod Crustaceans. Annalisa M. VanHook and Nipam H. Patel. Howard Hughes Medical Institute and University of California at Berkeley.

In contrast to our comprehensive understanding of segmentation in the long-germ insect *Drosophila melanogaster*, which forms all segments simultaneously in a syncytial environment, relatively little is known about the mechanisms of segmentation in other arthropods. Malacostracan crustaceans generate segments through stereotyped mitoses of ordered cell rows; cell rows may be generated by stem cell-like teloblasts (crayfish) or by self-organization of cells in the condensing germ disc (amphipod). Brachiopods are non-Malacostracan crustaceans that generate segments by a third mechanism. Brachiopods such as *Triops longicaudatus* and *Artemia franciscana* hatch with only their anteriormost segments formed; trunk segments emerge from an apparently unorganized posterior "growth zone" as the nauplius swims and feeds. We have characterized the expression of orthologs of the *Drosophila* pair-rule genes Pax3/7 and eve in both Artemia and Triops, and have seen a dynamic pattern which suggests that expression may be cycling within the growth zone; further experiments are underway to test this possibility. Triops is also unique in that it has a different number of dorsal and ventral segments. The first eleven or so trunk segments are aligned and present in a 1:1 ratio, but subsequent ventral segments become smaller and smaller posteriorly, while dorsal segments remain a constant size. Based on our analysis of Pax 3/7 expression we believe that this dorsal/ventral asymmetry may be due to the differential regulation of cell growth and division on the two sides of the animal.

387. Characterization of Pair-Rule Orthologs from the Crustacean, *Parhyale hawaiensis*. Ronald J. Parchem,[1] William E. Browne,[2] Matthias Gerberding,[3] Courtney C. Babbitt,[1] and Nipam H. Patel[1]. [1]University of California, Berkeley/HHMI; [2]Kewalo Marine Lab, University of Hawaii; [3]Max-Planck-Institut fr Entwicklungsbiologie-Tbingen.

The parasegment, the developmental segment unit whose boundaries lie at the *wingless/engrailed* interface, is a conserved feature of early patterning in all major groups of arthropods. Studies in *Drosophila* have shown that pair-rule genes provide direct input to properly position and pattern the parasegment, and at least some aspects of pair-rule prepatterning are likely to be conserved among insects. To determine more clearly if pair-rule patterning exists outside of insects, and whether conserved pair-rule gene interactions contribute to segmentation, we have begun characterizing orthologs of *Drosophila* pair-rule genes in the amphipod crustacean *Parhyale hawaiensis*. The simple and stereotyped cellular process of segmentation in *Parhyale* provides a powerful and precise system to study the molecular basis of segmentation. The germband of *Parhyale* condenses into an organized grid of ectodermal cells in which each transverse cell row (Roman Numeral Row (RNR)) represents the precursor to precisely one parasegment. Each RNR will undergo two rounds of highly stereotyped divisions along the anterior/posterior axis before expressing Engrailed in the anterior-most progeny to form the parasegment. We have cloned orthologs of almost all of the *Drosophila* pair-rule genes, and preliminary expression data show that some orthologs are expressed in a segment-polarity fashion, some during RNR divisions indicating a possible role in their coordination, and yet others in remarkable dynamic patterns posterior to the first RNR division.

388. Expression of Appendage Patterning Genes in the Crustacean *Parhyale hawaiensis*. Khoa D. Tran and Nipam H. Patel. University of California, Berkeley/HHMI CA. 94720.

The role of *extradenticles* (*exd*), *homothorax* (*hth*), *dacshund* (*dac*), and *Distalless* (*Dll*) in the development of *Drosophila* appendages has been studied in great detail. However, limb formation in *Drosophila* occurs via imaginal disc development during the larval stages as opposed to directly through embryogenesis as in most arthropods. Here we examine the expression of the abovementioned genes in the crustacean *Parhyale hawaiensis*, which develops limb directly from the ectoderm during embryogenesis. As in *Drosophila*, *exd* is expressed ubiquitously, *hth* is localized to the most proximal regions of the limbs, and dac and *Dll* are restricted to the "elbow" and distal region of the appendages, respectively. Preliminary results suggest that there are multiple *Dll* paralogs with potentially different patterns of expression. In *Parhyale*, the limb fields form and elaborate through a highly organized pattern of cell divisions, and we are interested in determining the relationship between the ectodermal cell lineages and the later patterns of gene expression to understand how the limb primordia is sequentially divided into morphologically distinct regions.

389. Gene Expression from DNA Injected into Embryos of the Amphipod Crustacean *Parhyale hawaiensis*. Edward Jay Rehm and Nipam H. Patel. Howard Hughes Medical Institute, University of California-Berkeley.

We have developed a simple procedure for expressing genes of interest in Parhyale embryos through the microinjection of supercoiled DNA. We have designed a cell autonomous and ubiquitous marker consisting of a modified DsRed gene under the control of a constitutive promoter/enhancer element from a *Parhyale* Ef1α gene. Embryos injected with this construct at the one- or two-cell stage begin expressing DsRed protein by early gastrulation. Expression is seen in all tissues and persists until hatching. We presume that the injected DNA remains extrachromosomal and replicates throughout embryogenesis. Our ubiquitous marker is useful for tracing cell fate patterns: clones produced from the progeny of individually injected blastomeres reveal distinct cell lineages characteristic of *Parhyale*. Transient expression of injected DNA will allow us to quickly analyze developmentally significant *cis*-regulatory elements and to generate new cell and tissue-specific markers. Microinjection of DNA will also provide a useful method to study gene function by ectopically expressing proteins of interest. The simplicity and efficiency of transient expression of injected DNA in *Parhyale* make it an ideal complement to the recently developed protocol for genetic transformation.

390. The Role of Ectodermal Stem Cells in *Porcellio scaber*. Mario A. Vargas-Vila and Nipam H. Patel. University of California, Berkeley/HHMI.

In order to understand the evolution of segmentation, we must gain a better understanding of the variety of molecular mechanisms amongst the extant segmented species. For example, while we understand many of the details governing segmentation in *Drosophila*, it is clear that extensive modifications to this process exist even in other insects. Within the arthropods, the malacostracan crustaceans (which include lobsters, crabs, etc.) represent one of the most diverse yet poorly represented groups in current molecular studies. Interestingly, most malacostracans also possess one of the most inherently simply cell lineage patterns that leads to body segments; individual parasegments are produced through the highly ordered division of single precursor rows of ectoderm cells (called Roman numeral rows), and for isopod crustaceans, each Roman numeral row is formed through a single round of division of a well-organized row of ectodermal stem cells. Using the terrestrial isopod *Porcellio scaber*, we are investigating the extent to which the ectodermal stem cells impart patterning information (at the level of Hox and pair-rule gene expression) to their Roman numeral row progeny. We are also using the *Porcellio* system to understand the evolution of the pattern seen in amphipod crustaceans where Roman numeral rows self-organize without the help of stem cell precursors. The experimental accessibility of *P. scaber*, along with its straightforward cell lineage, should make molecular segmentation studies fruitful in this species.

391. Hedgehog Signaling is Required at Multiple Stages of Zebrafish Tooth Development. William R. Jackman and David W. Stock. University of Colorado, Boulder, CO, USA.

Hedgehog signaling is essential for proper morphogenesis during mammalian tooth development, but it is unclear whether it also plays a role in tooth initiation. We have used the zebrafish pharyngeal dentition to test this possibility, as well as to examine whether the use of the hedgehog pathway differs between teeth in different body regions or has changed as a result of genome duplication. Zebrafish *shh* and *twhh*, paralogs which arose by genome duplication in ray-finned fishes, are expressed widely in the zebrafish pharyngeal epithelium, including that of the developing tooth germs. In contrast, expression of *Shh*, their single ortholog in the mouse, is restricted from its onset to the dental epithelium, and is absent from surrounding oral ectoderm. This difference may reflect evolutionary divergence in dental patterning mechanisms between mammals and fishes, between oral and pharyngeal regions, or both. Knocking down zebrafish *shh* and *twhh* expression with morpholinos singly and in combination has no specific effect on tooth morphology, unlike the strong effects on the dental epithelium seen when *Shh* is inhibited in mice. However, inhibiting all zebrafish hedgehog signaling with cyclopamine subsequent to tooth initiation severely disrupts mineralized tooth morphology. We are investigating whether the dental expression of other hedgehog ligands can explain these results. Furthermore, cyclopamine application at stages before morphological signs of tooth formation abolishes any gross sign of epithelial morphogenesis or tooth mineralization, suggesting that the hedgehog pathway is necessary for tooth initiation, at least in zebrafish.

392. Expression of a Presumptive *Pax1* Ortholog in *Xenopus laevis*: Insights into the Evolution and Development of the Anuran Axial Skeleton. Gregory R. Handrigan and Richard J. Wassersug. Dalhousie University, Halifax, Canada.

Pax genes encode transcription factors that regulate organogenesis in vertebrates. *Pax1*, for example, controls the development of pharyngeal arches and the axial skeleton. We have identified a full-length cDNA clone that represents a presumptive ortholog of *Pax1* in *Xenopus laevis* (*XlPax1*). The putative protein (XlPax1) bears an octapeptide sequence and a highly conserved paired box domain, which shares 98% and 96% sequence identity with that of other Pax1 and Pax9 orthologs, respectively. The putative c-terminus is far less conserved (~50%). Sequence alignment and phylogenetic analysis consistently group XlPax1 with other vertebrate Pax1 orthologs to the exclusion of Pax9, strongly suggesting that it represents the definitive *X. laevis* ortholog. We have characterized the expression profile of *XlPax1* by PCR and in situ hybridization (ISH) analysis. RT-PCR reveals that transcripts are up-regulated during the neural-fold stage, when somite segregation commences. Whole-mount ISH shows strong *XlPax1* expression in the pharyngeal pouches (I–V) and in the somites. Cross-sectional ISH pinpoints transcripts to the pharyngeal endoderm and to somitic cells immediately adjacent to the notochord, representing the sclerotome. These data corroborate a role for *XlPax1* in the development of the axial skeleton of *X. laevis*. Preliminary mRNA and morpholino injection trials are underway to elucidate its precise role. We suggest that *Pax1* may contribute to the lack of caudal vertebrae in anurans. A heterochronic shift in *Pax1* expression may account for the re-appearance of caudal vertebrae in a few derived anuran taxa.

393. *Xenopus tropicalis* ParaHox Genes: Conserved Roles in Posterior Development and Neurogenesis. Harry V. Isaacs, Jean C. Illes, and Laura Faas. University of York, York, YO10 5YW, UK.

The Hox and ParaHox gene clusters are believed to have arisen as the result of duplication of an ancestral ProtoHox cluster. Here we characterise the ParaHox gene cluster from the amphibian *Xenopus tropicalis*. In common with the basal chordate amphioxus amphibians have a single ParaHox cluster containing three genes of the gsh, pdx and cdx classes. We show that genes of the amphibian ParaHox cluster exhibit spatial co-linearity of expression, with gsh1 having the most anterior boundary of expression and cdx2 having the most posterior boundary. gsh1 and gsh2 represent homologues of the *Drosophila* ind gene, which is involved in the specification of intermediate column neurons during primary neurogenesis. Here we show that *Xenopus* gsh2 is also expressed in a restricted pattern within the primary neurogenic region of amphibians. We present data indicating that gsh2 has a role in the differentiation and specification of neurons in the primary amphibian nervous system. The three amphibian Cdx genes are expressed with different boundaries in the posterior axis. Single and compound knockdowns of amphibian cdx1, cdx2 and cdx4 demonstrate Cdx autoregulation and a requirement for Cdx function in the regulation of Hox gene expression in the developing posterior embryo. Our

data indicate that Cdx and Hox genes form a complex regulatory network, which reflects their common evolutionary origin.

394. Cloning, Expression and Evolution of Skeletogenic Genes (BMP-2/noggin) from the Turtle *Trachemys scripta*. Elizabeth E. LeClair, Carly A. Bien, and Farah A. Merchant. DePaul University, Chicago, IL 60614.

The turtle shell is a derived skeletal structure remarkable for its excessive bony growth. Shell development involves redirection of the ribs with respect to the limb girdles, expansion and fusion of adjacent ribs, and extensive ossification of the overlying dermis (Gilbert, 2001). How turtles regulate this novel skeletal pattern is poorly understood. Comparative studies in other vertebrates show that two molecular partners – bone morphogenetic proteins (BMPs) and their antagonists (e.g., noggin) – affect the growth, differentiation and mineralization of skeletal elements. Using total RNA from embryonic turtle shells (*Trachemys scripta*) at different stages (2–5 weeks post-laying) we established cDNA pools from the carapace ridge, cartilaginous shell, and early bony shell. 5′/3′ RACE was used to clone the turtle homologs of both BMP-2 and noggin. To understand the evolution and expression of these genes we have (1) analyzed the turtle sequences for phylogenetic relationships to homologous sequences in other vertebrates and (2) investigated the tissue-specific expression of these genes in the context of shell development at multiple stages. Other sequences retrieved from shell cDNAs include sonic hedgehog (*Shh*), decorin (an extracellular component of cartilage) and the cell signaling molecule NCK-AP (NCK-associated protein). By comparing the sequences and developmental expression of these genes in turtles with those of other vertebrates we hope to understand how modified gene expression operates both in evolutionary adaptations and in diseases of bone. Support: DePaul URC. eleclair@depaul.edu.

395. Gene Expression Patterns in the Development of the Skeletogenic Mesenchyme in the Red-Eared Slider Turtle, *Trachemys scripta*. Jacqueline E. Moustakas. University of California, Berkeley.

Developmental genetics has provided us new ways to investigate the evolution of molecules and morphology. Comparative studies, typological and phylogenetic, can allow us to more fully understand the evolution of homologous features. In my research, I investigate the developmental regulation of postcranial skeletogenic mesenchyme in the red-eared slider, *Trachemys scripta*. By studying the expression of developmental regulatory genes in these animals, I adopt a macroevolutionary approach to ask how differences in some basic features of the body plan evolved.

The dermal bones of both extinct and extant vertebrates are composed of cellular bone. The bone-forming osteoblasts in these tissues differentiate directly from mesenchymal precursor cells in the dermis of the skin, unlike the axial skeleton, which develops through a cartilage model from paraxial mesoderm. Testudines form postcranial dermal bone as shells dorsally and ventrally. The mesenchymal cells that form the dermal bones of the skull (the dermatocranium and viscerocranium) are derived embryonically from the cranial portion of the neural crest. This study aims to describe the expression of markers for specific populations of mesodermal and ectodermal cell lineages, and the expression of genes known to be regulated in mesenchymal stem cells with skeletogenic potential. The goal of this study is to gain an understanding of the patterning, organogenesis, and homology of these tissues.

396. The Role of FGF Signaling in the Formation of the Turtle Carapace. Judith A. Cebra-Thomas, Paul N. Riccio, Jacqueline C. Simonet, and Scott F. Gilbert. Swarthmore College, Swarthmore, PA 19081.

The turtle shell is the morphological innovation that defines the order Chelonia. The dorsal carapace develops as a result of the formation of a pair of carapacial ridges in the dorsolateral ectoderm. These ridges and the underlying mesenchyme alter the migration of the somite derivatives such that the sclerotome-derived rib precursors fail to migrate ventrally to form the rib cage. Instead, the ribs grow laterally, out towards the carapacial ridges, and as a result end up positioned between the dermis and the myotome. When the ribs undergo endochondral ossification, they induce the surrounding and overlying dermis to become bone. Our data suggest that the key event, the formation of the carapacial ridges and the attraction of the rib precursors into them, is mediated by members of the fibroblast growth factor (FGF) family in the red eared slider *Trachemys scripta*. FGF10, which is expressed in the mesenchyme underlying the carapacial ridges, is a prime candidate for both maintenance of the ectodermal ridges and attraction of the sclerotome cells. Treatment of chick embryo explants with beads containing FGF10 results in alterations in rib formation. In contrast, treatment of trunk explants in organ culture with specific inhibitors of FGF signaling blocks the maintenance of the carapacial ridges and the allows the somite derivates to develop in a manner similar to other vertebrates. This developmental sequence might explain the rapid emergence of turtles in the fossil record.

397. Plasticity and Constraint During Cranial Skeletogenesis. Brian F. Eames and Richard A. Schneider. Orthopaedic Surgery, UCSF, San Francisco, CA 94143.

The ability to adapt during morphological evolution can be augmented by plasticity in ontogenetic processes, but also be limited by entrenched cellular and molecular mechanisms that serve as constraints. Using a quail–duck chimeric system, we assess plasticity and constraint as factors in cranial skeletal development and evolution. Quail and duck are anatomically distinct and have divergent maturation rates. We transplant neural crest cells, which are progenitors of the jaw skeleton, between quail and duck embryos, and employ histological and molecular analyses to identify processes that control initiation and patterning of bone and cartilage in resultant chimeras. Pointing to a high degree of plasticity in developmental mechanisms underlying bone formation, we find that Runx2 and Col1 expression, as well as matrix deposition, are regulated cell-autonomously and follow spatiotemporal programs of donor neural crest. In contrast, chondrogenesis appears constrained by the host environment. Even though transcripts for Col2 are expressed according to the donor timetable, deposition of a histologically identifiable cartilage matrix depends upon the host embryonic stage. After initiation of overt chondrogenesis, however, chimeric cartilage elements seem more morphologically advanced and display donor-specific size and shape. Thus, clear differences exist in the way osteogenic and chondrogenic programs respond to spatiotemporal changes introduced via the chimeric system. Such differences reflect non-equivalent levels of plasticity and constraint that define each skeletogenic program, and likely bias the way bony and cartilaginous skeletons evolve when variations arise by natural means.

398. Origins of the Cardiac Neural Crest. Max Ezin and Marianne Bronner-Fraser. California Institute of Technology.

The prospective cardiac neural crest, a cell population that contributes to the great arteries of the heart, resides in the dorsal aspect of the hindbrain at neural tube stages in chick. However, no information is available on the origins and of the cardiac crest cells in the epiblast before neural fold formation. To address this question, we are generating a fate map of the cardiac neural crest in gastrula stage embryos. Our method consists of labeling small populations of cells with a lipophilic dye or by electroporation at gastrula stages, and culturing the embryo until neural tube stages, to determine the rostrocaudal distribution of the cells along the neural tube; at neural tube stages 10 to 12, cardiac neural

crest cells lie in the dorsal neural tube, at the level of the hindbrain rhombomeres 6 to 8. Our results show that the cardiac neural crest precursors are not yet segregated from other crest at gastrula stage 4. By stage 7, however, the cardiac neural crest forms a segregated population along the rostrocaudal axis. Indeed, by neural tube stages, cell populations that were labeled at stage 7 contribute to discrete streams of cells migrating towards forming pharyngeal arches 3, 4 and 6. Our work furthers our understanding of the formation of the cardiac neural crest and paves the way to a molecular characterization of the commitment and induction of cardiac crest.

399. Casein Kinase I Phosphorylates the Forkhead Transcription Factor FoxG1 and Promotes its Nuclear Localisation. Tarik Regad and Nancy Papalopulu. [1]The Wellcome Trust/Cancer research UK Gurdon Institute, Tennis court Road, Cambridge, CB2 1QR, UK; [2]Department of Anatomy, Downing site, University of Cambridge, UK.

Foxg1, a conserved forkhead transcription factor, controls neuronal differentiation in the vertebrate telencephalon. Foxg1 acts as a transcriptional repressor but how it is regulated is not known. Here we use deletions, site-specific mutagenesis and kinase assays to show that FoxG1 is directly phosphorylated in the N-terminus by Casein kinase I (CKI). This phosphorylation is necessary for the nuclear localisation of FoxG1 and its activity in the embryo. Inhibiting CKI blocks the nuclear localisation of FoxG1 in vitro and in vivo. The CKI phosphorylation site is perfectly conserved in all vertebrate FoxG1 homologues, suggesting a novel evolutionary conserved mechanism of regulation by CKI.

400. Cilia of the Early Rabbit Embryo Point to Non-Conserved Modes for Breaking Symmetry in the Mammalian Embryo. Anja Fischer,[1] Kerstin Muders,[1] Olaf Selchow,[2] Henny W. van Straaten,[3] Christoph Viebahn,[4] and Martin Blum[1]. [1]University of Hohenheim, 70593 Stuttgart, Germany; [2]University of Stuttgart, 70569 Stuttgart, Germany; [3]University of Maastricht, Maastricht, The Netherlands; [4]University of Göttingen, 37075 Göttingen, Germany.

Molecular symmetry breakage in the early vertebrate embryo leads to asymmetric development of inner organs like heart and stomach. In mouse this process was shown to be initiated by a leftward fluid flow caused by rotating 9 + 0 monocilia on ventral node cells at the anterior end of the primitive streak ('nodal flow'). Absent cilia or altered motility in mutants led to laterality defects while artificial fluid flow applied to cultured embryos reversed laterality in wild type and rescued laterality in mutant embryos. 'Nodal flow' was hence proposed to transport an extracellular compound or to be sensed by a subset of nodal cilia. As other vertebrates like frog and chick use different mechanisms for symmetry breakage, we analysed if 'nodal flow' was conserved in an archetypic mammal, the rabbit. Here we show that, unexpectedly, monocilia were not found on the ventral side of the node. Instead, the notochordal plate displays three types of motile cilia (9 + 0, 9 + 2, 9 + 4), which, however, produce only undirected fluid motions prior to molecular symmetry breakage and therefore suggest that multiple modes for breaking symmetry exist in mammals.

401. BMP Signals Control Interdigital Programmed Cell Death by Regulating FGF Signaling. Sangeeta P. Underwood,[1] Catherine Wilson Wilson,[1] Yuji Mishina,[2] and Mark Lewandoski[1]. [1]CCR, NCI-Frederick/NIH; [2]NIES/NIH.

In vertebrates lacking webbed limbs, the embryonic interdigit region is normally removed by programmed cell death (PCD). Numerous experiments have indicated that BMPs may act as direct PCD triggers in the interdigit region. BMPs may also regulate the initiation and regression of the apical ectodermal ridge (AER). We and other have previously shown that the AER is a source of FGF signaling that is required for normal limb bud patterning as demonstrated by hypodactyly (loss of digits) caused by the AER-specific inactivation of Fgf8. Here we disrupt BMP signals to the AER by Cre-mediated inactivation of a conditional mouse allele encoding a BMP receptor (*BmPR1a*). Early inactivation in the hindlimb prevents AER formation. Later *BmPR1a* inactivation in the forelimb causes an upregulation of two AER-FGFs, *Fgf4* and *Fgf8*, and a loss of interdigital PCD and thus webbed limbs. To determine whether excess FGF signaling inhibits interdigit PCD, we performed double and triple tissue-specific inactivations of *BmPR1a*, *Fgf4* and *Fgf8*. Webbing persist in AER-specific inactivations of *BmPR1a* and *Fgf8* which causes a further *Fgf4* upregulation such that loss-of-*Fgf8* limb defects are rescued. Inactivation of *BmPR1a*, *Fgf8* and one copy of *Fgf4* restores hypodactyly and eliminates webbing. Thus, during normal embryogenesis BMPs indirectly regulate interdigit PCD by regulating AER-FGFs, which act as survival factors for the interdigit mesenchyme. This work has implications for understanding human limb congenital defects such as that found in Apert's syndrome as well as the evolution of webbing in vertebrates.

402. Loss of the Alpha7 Integrin Affects Vascular Development and Integrity. Jennifer V. Welser, Nichole L. Flintoff-Dye, and Dean J. Burkin. University of Nevada, Reno, NV 89557.

The $\alpha7\beta1$ integrin is a heterodimeric laminin receptor present in skeletal, cardiac, and vascular smooth muscle. This integrin forms a transmembrane linkage system that regulates cell growth and survival. To investigate the function of the $\alpha7\beta1$ integrin in vascular development we have created $\alpha7$ integrin null mice using a LacZ knockin strategy. Expression of the $\alpha7$ integrin chain was shown by β-galactosidase staining. Loss of the $\alpha7$ integrin chain results in partial embryonic lethality. Analysis of embryos revealed cerebral vascular hemorrhaging starting at embryonic day (ED) 11.5. ED 13.5 $\alpha7$ null embryos exhibited reduced numbers of vascular smooth muscle cells. A 1.8-fold decrease in vascular smooth muscle cells in non-hemorrhaging embryos compared to the wild-type embryos was observed. In addition, hemorrhaging embryos exhibited a 4.4-fold decrease in vascular smooth muscle cells compared to wild-type embryos. In contrast, $\alpha7$ null mice that survived to birth showed a 1.8-fold increase in vascular smooth muscle cells, which may protect blood vessels from rupture. An investigation of vascular smooth muscle cells from $\alpha7$ null mice showed altered cytoskeletal organization and hypertrophy compared to wild-type animals. It appears that adult $\alpha7$ null mice are able to survive because they compensate for the lack of $\alpha7$ integrin by increasing the amount of vascular smooth muscle cells. Our results suggest for the first time a role for the $\alpha7\beta1$ integrin in vascular development and integrity.

403. Purified Wnt Proteins Control Cell Proliferation and Diffe-rentiation During Chondrogenesis in Limb Bud Mesenchyme Cultures. Derk ten Berge and Roel Nusse. Howard Hughes Medical Institute, Stanford University Medical School, Stanford, CA 94305.

Throughout embryonic development, extra-cellular signaling molecules influence the behavior of cells, guiding their proliferation and differentiation into tissues and organs. Members of the Wnt family of secreted lipoproteins are such molecules, and they control processes as diverse as embryonic axis formation, patterning of the central nervous system, and expansion of progenitor cell populations. We are developing ways to use purified Wnt proteins as tools to control the proliferation and differentiation of progenitor cells in vitro. Limb bud mesenchyme cells have the potential to differentiate into cartilage when cultured at high density. We show that purified Wnt3A protein added to limb bud mesenchyme cultures stimulates cell proliferation and inhibits differentiation into cartilage. Using microarrays we found several genes that are induced by Wnt3A in these cells. These targets where also induced when a bead soaked in Wnt3a protein was implanted in the limb bud, and a reporter construct containing promoter

sequences of one such target gene was Wnt3a-responsive in vitro. Overexpression of this target gene in limb bud cells regulates cell proliferation but does not block differentiation. This suggests that Wnt signaling employs several effector genes that independently regulate proliferation and differentiation of progenitor cells.

404. Drawing a Line in the *Arabidopsis* fruit. Adrienne Roeder,[1] Sarah Liljegren,[2] Cristina Ferrándiz,[3] and Martin Yanofsky[1]. [1]UCSD, 9500 Gilman Dr. DEPT 0116, La Jolla, CA 92093, USA; [2]Dept. of Biology, UNC at Chapel Hill, Chapel Hill, NC 27599, USA; [3]Instituto de Biologia Molecular y Celular de Plantas, Av de los Naranjos s/n, Valencia 46022, Spain.

The fruit protects the seeds while they develop and disperses the seeds at maturity. Although increased seed dispersal is beneficial for the survival and spread of natural plant species, controlling seed dispersal is of great agricultural interest in several crops such as soybean and canola. Canola is closely related to the model plant *Arabidopsis*. Both form seedpods that open at the valve margins, specialized bands of tissue that run the length of the fruit attaching the valves (seedpod walls) to the replum (central ridge). Cells within the valve margin physically separate, allowing the valves to detach, releasing the seeds. In *Arabidopsis*, the *SHATTERPROOF* (*SHP*) MADS-box and the *INDEHISCENT* (*IND*) and *ALCATRAZ* (*ALC*) bHLH transcription factors specify valve margin identity. How is the valve margin formed at the border between the valve and the replum? In *fruitfull* (*ful*) mutants, *SHP*, *IND*, and *ALC* are ectopically expressed in the valves and consequently valve margin tissue forms throughout the valves. The *FUL MADS*-box gene negatively regulates *SHP*, *IND*, and *ALC* limiting their expression to the edge of the valve. Likewise, in *replumless* (*rpl*) mutants, *SHP*, *IND*, and *ALC* are ectopically expressed in the replum, which differentiates as valve margin tissue. The *RPL* homeobox gene negatively regulates *SHP*, *IND*, and *ALC* limiting their expression to the edge of the replum. Thus, *FUL* and *RPL* position the valve margin between the valve and replum. See the poster by José Dinneny for upstream factors.

405. Leaf Polarity and Growth Factors Play Important Roles in Patterning the Fruit in *Arabidopsis*. José R. Dinneny,[1] Detlef Weigel,[2] and Martin F. Yanofsky[1]. [1]University of California, San Diego, La Jolla, CA 92093-0116; [2]Max Planck Institute for Dev. Biol., Tübingen D-72076, Germany.

Fruits of the model plant, *Arabidopsis thaliana*, are complex organs designed to protect seeds as they develop and disperse them upon maturation. We have found that the correct patterning of fruit-specific tissues requires spatial information provided by two groups of genes that regulate general developmental processes common to all lateral organs. *FILAMENTOUS FLOWER* (*FIL*) and *YABBY3* (*YAB3*), which help impart organ polarity and *JAGGED* (*JAG*), which regulates growth, function together to provide positional information necessary for the correct spatial activation of *FRUITFULL* (*FUL*) and *SHATTERPROOF* (*SHP*). *FUL* and *SHP* genes are necessary to promote the formation of the valve margins, stripes of tissue that allow the fruit to shatter along two defined borders. We also find that the formation of two distinct stripes of valve margin is promoted through the division of *FIL/YAB3/JAG* activity into two separate domains by *REPLUMLESS* (*RPL*). The observation that all floral organs are mis-patterned in *fil yab3 jag* triple mutants suggests that these genes may represent a patterning mechanism common to other organs, as well. (For more on RPL, see poster by Roeder et al.)

406. *Mesp* Separately Regulates Heart Specification and Migration in the *Ciona* tadpole. Brad J. Davidson, Weiyang Shi, and Michael S. Levine. University of California, Berkeley.

Directed migration of cardiac precursors cells is critical during early stages of vertebrate cardiogenesis. However, the genetic basis of this migration and how it relates to the earliest steps of cardiac specification remain obscure. Here we present a novel mechanism for differential regulation of cardiac migration and specification by a single gene. In both mice and *Ciona* (a primitive chordate), the bHLH factor *Mesp* has a critical, but poorly defined role in heart cell migration. Targeted expression of a constitutively activated form of *Mesp* (*Mesp-VP16*) in the emerging heart field of *Ciona* embryos induces cardiac specification independent of migration. Occasionally, this treatment produces juveniles with ectopic beating heart tissue in the resorbed tail. Conversely, a constitutive repressor form of *Mesp* (*Mesp-WRPW*) induces migration but blocks specification. These experiments reveal an aspect of *Mesp* function not exposed by simple gene disruption assays. Namely, *Mesp* functions as both a transcriptional activator and repressor. It activates target genes required for heart specification but also represses one or more inhibitors of cell migration. These results provide a foundation for characterizing distinct pathways underlying specification and directed migration in the primary heart field.

407. Understanding Expression Noise in Pattern Formation with the *Drosophila* Segmentation Network. David M. Holloway,[1] Carlos E. Vanario-Alonso,[2] John Reinitz,[2] and Alexander V. Spirov[2]. [1]British Columbia Institute of Tech., Burnaby, Canada; [2]SUNY Stony Brook, NY, 11794, USA.

Multicellular eukaryotes have complex developmental processes, in which genes must be expressed at precise times and positions for normal development to proceed. We use *Drosophila* segmentation to quantify temporal and spatial variability in gene expression, aiming to understand how gene regulation achieves such precision. We have whole-embryo expression patterns for 12 segmentation genes (maternal, gap, pair-rule, and segment-polarity), from over 1000 wild-type and mutant embryos. Each is stained for three different gene products, from the beginning of syncytial blastoderm (nuclear cleavage cycle 10) to the onset of gastrulation (end of cycle 14A). These have the temporal (~6.5 min) and spatial (single nucleus) resolution necessary for studying expression noise. We see increasing precision and coordination with developmental age, both between disparate expression domains of a single gene and between patterns of different genes. This trend appears to reverse at the end of cycle 14A. Then, precision appears to decrease, as pair-rule stripes change from symmetric (bell shaped) to sawtooth forms. At gastrulation, high pair-rule variability appears to be at odds with precise specification of downstream pattern. Downstream error reduction has been noted at the maternal to gap level (Houchmandzadeh et al., 2002; Spirov and Holloway, 2003). Our observations suggest that such downstream error reduction occurs at further levels of the segmentation hierarchy.

408. The EGF Receptor Affects Selector Gene Expression Through Wingless, and Affects Cell Affinity During *Drosophila* Eye-Antennal Imaginal Disc Patterning. Jennifer R. Curtiss and Marek Mlodzik. Mount Sinai School of Medicine, New York, NY 10029.

Most of the *Drosophila* head develops from a contiguous epithelial sheet called the eye-antennal imaginal disc, which serves as an excellent paradigm for specification and patterning of adjacent tissues. Current models suggest that the *Drosophila* EGF receptor (Egfr) promotes expression of the antennal selector Distal-less (Dll) and prevents *Drosophila* Pax6, which specifies eye, from being expressed in antennal precursors. Here, we find that Egfr is required but not sufficient for Dll expression. Moreover, Egfr is not required to prevent expression of Pax6 expression in antennal precursors. Thus, Egfr plays a permissive role in eye-antennal disc subdivision. However, Egfr participates in the development of two subfields: it interacts with the *Drosophila* Wnt, Wingless to promote Dll expression in the distal antenna, and to repress Pax6 expression in the vertex. Furthermore, by affecting cell

affinity, Egfr contributes to the formation and maintenance of multiple developmental fields in the eye-antennal disc.

409. Using Salivary Glands as a Model System to Study the Cell Biology of Hh Signaling in *Drosophila*. Barbara E. Sisson, Megan L. Killingsworth, Kelly K. McCabe, and Robert A. Holmgren. Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL.

The Hedgehog (Hh) signal transduction pathway plays a significant role in the development of invertebrates and vertebrates. While much is known about the pathway, cell biological studies have been limited. In order to investigate the subcellular distribution of the transcription factor of the pathway, cubitus interruptus (Ci), and its signaling complex we have looked to the large cells of the *Drosophila* salivary glands to serve as a model system. Our goal is to follow the subcellular distribution of Ci before and after Hh signaling and determine whether different Ci signaling complexes exist with distinct distributions. In addition, fluorescently tagged proteins will be used to visualize Hh signal transduction in living cells. While the increased cell size of salivary gland cells provide an excellent system to study changes in the cellular distribution of Hh pathway members, the presence of polytene chromosomes within these cells provides an assay with which to study the binding of transcription factors to DNA. We have examined the binding of Ci and the mammalian GLI proteins to polytene chromosomes using endogenous targets and a multimerized artificial enhancer with 320 Ci binding sites. As in previous studies, we find that the repressor form of Ci, Ci75 avidly binds its DNA targets. On the other hand, full-length Ci and full-length GLI3 do not readily bind polytene chromosomes. These differences are the subject of ongoing studies.

410. Regulation of Cubitus Interruptus by a Conserved Domain. Suzanne L. Ziegenhorn, Jennifer A. Croker, and Robert A. Holmgren. Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL.

The Hedgehog (Hh) signaling pathway is a vital developmental program that regulates many aspects of patterning in vertebrates and invertebrates. Much of our understanding of the pathway is derived from studies in *Drosophila*. Hh acts on responsive cells through an undefined transduction mechanism which controls the transcription of specific target genes via regulation of a cytoplasmic complex of proteins containing, among others, the zinc finger transcription factor cubitus interruptus (Ci). In *Drosophila*, Hh patterns embryonic epidermis and larval imaginal discs by regulating Ci on three levels: proteolytic cleavage into a repressor form, nuclear import, and activation. In this report, we characterize a highly conserved domain of *Drosophila* Ci identified by sequence alignment with the vertebrate homologues, GLI1, GLI2, and GLI3. This domain, which we term NR for "N-terminal Regulatory," is located in the center of the amino half of the protein. Deletion of this region generates a Ci molecule that is not properly sequestered in the cytoplasm. The number of conserved potential phosphorylation sites in this domain is particularly striking, as analysis of the amino acid sequence of this domain identifies a cluster of twelve perfectly conserved serines and one tyrosine. We propose that this region may be modified, possibly by phosphorylation, to regulate Ci in response to Hh signaling.

411. A Novel Conserved Gene, *paladin*, is Dynamically Expressed in the Midline and Paraxial Mesoderm of Zebrafish Embryos. Xiao Xu, Tina M. Han, and Sharon L. Amacher. University of California, Berkeley.

We isolated *paladin*, a novel conserved gene, in microarray assays designed to identify genes regulated by the T-box transcription factor, *no tail* (*ntl*)*/Brachyury*. Prior to gastrula stages, *paladin* is expressed fairly ubiquitously, but by the beginning of gastrulation, *paladin* expression is restricted to the developing midline. Near the end of gastrulation, midline expression disappears and instead *paladin* is expressed in paraxial tissue. To begin to define upstream regulatory inputs, as well as to better characterize the regulatory switch from axial to paraxial expression, we are examining *paladin* expression in midline-defective *no tail* (*ntl*) and *cyclops* (*cyc*)*/ndr2/znr1* mutant embryos, in paraxial mesoderm-deficient *spadetail* (*spt*)*/VegT/tbx16* mutant embryos, and in *smoothened* (*smu*) mutant embryos that cannot perceive Hedgehog signals. Although *paladin* is well conserved among vertebrate genomes, its sequence shares little homology with known protein domains. We are overexpressing *paladin* in zebrafish embryos to gain insight into its potential role during axial and paraxial development.

412. Withdrawn.

413. Zebrafish *foxd3* is Selectively Required for Neural Crest Sublineage Determination, Migration and Survival. Rodney A. Stewart,[1] Brigitte L. Arduini,[2] Cicely A. Jette,[1] John P. Kanki,[1] Paul D. Henion,[2] and A. Thomas Look[1]. [1]Dept. of Pediatric Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA; [2]Dept. of Neuroscience, Ohio State Univ., Columbus, OH.

The neural crest is a pluripotent cell population that generates a large variety of cell types, including neurons, cartilage and pigment cells. The genetic mechanisms underlying diversification of the neural crest to specific cell fates remain incompletely understood. Zebrafish homozygous for the *sympathetic mutation 1* (*sym1*) display defects in a subset of neural crest derivatives including neurons, glia and the craniofacial skeleton but undergo normal development of chromatophore sublineages. We identified the *sym1* mutation as a deletion within the *foxd3* gene, a highly conserved winged-helix transcription factor transiently expressed at the neural plate border and by premigratory neural crest cells. Consistent with the selective phenotypic defects in neural crest sublineages exhibited by *sym1* mutants, analysis of lineage-specific markers revealed loss of neuronal and cartilage progenitor cells, but not chromatophore progenitors. Examination of early neural crest development in *sym1* mutants revealed differential requirements for *foxd3* in the expression of other critical neural crest genes, such as *tfap2a*, *snail1b* and *sox10* in neural plate border and/or premigratory neural crest. In addition, TUNEL labeling showed that *foxd3* is required for the survival of a subpopulation of hindbrain neural crest that gives rise to many of the cell types disrupted in *sym1* mutants. The selective role for *foxd3* function in neural crest patterning and migration, as well as survival will be discussed.

414. AP-2 Transcription Factors Function at Multiple Steps of Zebrafish Neural Crest. Wei Li and Robert A. Cornell. University of Iowa, Iowa City, IA 52242.

Activator protein 2 (AP-2) is a family of transcription factors that in mammals has five members (alpha, beta, delta, epsilon, and gamma); all appear to bind to a consensus DNA motif. In all vertebrates thus far analyzed, AP-2alpha is expressed in premigratory neural crest. Loss of function studies in zebrafish and other animals have shown that AP-2alpha is necessary for development of melanophores and other neural crest derivatives. In order to clarify the role of AP-2 transcription factors in early neural crest development; we constructed a dominant-negative AP-2 (dnAP-2). Forced expression of this variant eliminates all premigratory neural crest, and this effect appears to be cell autonomous. We are testing the effect of driving expression of dnAP-2 in melanophores and Rohon–Beard lineages. To assess the dynamic distribution of AP-2 activity in early embryogenesis, we built a reporter

of AP-2 activity by fusing three consensus AP-2 binding sites upstream of a minimal promoter and the gene encoding destabilized EGFP. In transient transgenic embryos, GFP expression is seen only in cell types in which AP-2alpha is also detected. We report expression patterns of remaining zebrafish AP-2 family members. This work is supported by NIH grant GM 067841.

415. Molecular Mechanisms Specifying Separate Hindbrain and Spinal Cord Regions from the Neural Plate. Isaac Skromne,[1] Dean Thorsen,[2] Melina E. Hale,[2] Victoria Prince,[3] and Robert K. Ho[1]. [1,2,3]Deparment Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637, USA.

Classic and modern embryological experiments have shown that the posterior neural plate gives rise to the hindbrain and spinal cord portions of the central nervous system. Subsequently, the hindbrain intrinsically subdivides into rhombomeres, while the spinal cord remains unsegmented. What are the molecular mechanisms involved in the determination of the hindbrain and spinal cord regions from a common neural plate territory? We noticed that in various vertebrate species the anterior limit of *cdx* gene expression corresponds to the hindbrain/spinal cord transition point. *cdx* genes encode homeobox transcription factors required for posterior body formation; we have now examined their role in zebrafish spinal cord development. We find that loss of zebrafish *cdx4* function causes posterior shifts in the distribution of spinal cord neuronal populations and expansion of the most posterior rhombomeres. Loss of a second *cdx* gene, *cdx1a*, causes no apparent defects. However, double mutants fail to express any spinal cord markers, and the entire posterior neural plate exhibits characteristics of a greatly expanded hindbrain. Gene expression analysis reveals additional rhombomere-like segments, arranged in a mirror image duplicated pattern. We show this mirror image duplication depends on the same signaling molecules normally involved in hindbrain patterning. Based on our results we propose that in vertebrates, spinal cord specification involves repression of hindbrain characteristics in the most posterior neural plate by *cdx* transcription factors.

416. Insertional Mutagenesis in Zebrafish Identifies Novel Genes Involved in Forebrain Development. Eric C. Swindell,[1] Adam Amsterdam,[2] Nancy Hopkins,[2] Milan Jamrich[1]. [1]Baylor College of Medicine, Houston, TX; [2]Massachusetts Institute of Technology, Cambridge, MA.

The early vertebrate brain arises from a sheet of ectoderm called the neural plate that is located at the anterior of the early embryo. This sheet of ectoderm eventually forms the neural tube and is partitioned into three vesicles representing the forebrain, midbrain, and hindbrain. These vesicles are then patterned further to give rise to sub-regions that generate diverse but specific neuronal cell types. The embryonic forebrain consists of two major components, the diencephalon and the telencephalon. The diencephalon gives rise to the thalamus and the hypothalamus, while the telencephalon gives rise to the cerebral cortex, the basal ganglia, the hippocampus and the amygdaloid nuclei. The early pattering and regionalization of the forebrain is essential for the proper formation and maintenance of the neuronal circuits that make up these structures. A large scale insertional mutagenesis screen in zebrafish was carried out to identify genes essential for embryonic development. 525 insertional mutant lines representing at least 390 different loci were isolated. To identify mutants that effect early brain formation, the entire insertional mutant collection was screened by whole mount in situ hybridization using several marker genes. 35 mutants showed a disruption of *dlx2* expression in the forebrain. 5 of these mutants show a complete loss of *dlx2* expression in the forebrain. Functional analysis of the mutants that show disruptions in *dlx2* expression will concentrate on their roles in early regionalization of the forebrain and in the development of forebrain neurons.

417. Nlz, a NET Zinc Finger Protein has Repressor Function During Zebrafish Hindbrain Development. Mako O. Nakamura, Alexander P. Runko, and Charles G. Sagerstrom. University of Massachusetts Medical School, Worcester, MA 01605.

Nlz was cloned by subtraction screening from zebrafish and is a member of the NET family of zinc finger proteins. NET family proteins share SP, buttonhead (Btd) and zinc finger domains with SP family transcription factors. However, the NET family possesses only one zinc finger domain compared with the SP family that has three domains. Therefore NET family members are unlikely to bind DNA by themselves. Nlz starts expressing at shield stage (6phf) and expression stays in the hindbrain and tail bud at the segmentation stage. Misexpression of Nlz causes a disruption in expression of r2 and r3 makers such as krox20 and hoxa2 suggesting that Nlz acts as a repressor. To examine Nlz function, we used reporter assays based on the GAL4-UAS system. Co-transfection of Nlz fused to the Gal4 DNA binding domain with UAS-luciferase reporter plasmid showed transcriptional repression in HeLa cells. We have demonstrated that Nlz binds HDAC and Groucho co-repressors via the Btd domain and adjacent sequences. We find that deleting this domain disrupts the repression activity of Nlz. Further, the HDAC inhibitor TrichostatinA also relieves repression in the cells. These data suggest that Nlz suppresses transcription by recruiting HDAC. Further analysis using HDAC assay and co-immunoprecipitation are underway to confirm if HDAC activity is required for the repressor function.

418. Zebrafish Lmx1b.1 and Lmx1b.2 are Necessary to Maintain the Isthmic Organizer. Patrick O'Hara,[1] Lauren K. Barr,[2] and Daniel S. Kessler[1]. [1]Univ. of Pennsylvania, Philadelphia, PA 19104; [2]Harvard Medical School, Boston, MA 02115.

The mesencephalic and metencephalic region (MMR) of the vertebrate central nervous system develops in response to signals produced by the isthmic organizer (IsO). In the chick, the LIM homeobox transcription factor *Lmx1b* is expressed within the IsO where it is sufficient to maintain expression of the secreted factor *wnt1*. Here we show that zebrafish express two *Lmx1b* orthologs, *lmx1b.1* and *lmx1b.2*, in the rostral IsO, and demonstrate that these genes are necessary for key aspects of MMR development. Simultaneous knockdown of Lmx1b.1 and Lmx1b.2 using morpholino antisense oligos results in a loss of *wnt1*, *wnt3a*, *pax8*, and *fgf8* expression at the IsO, leading ultimately to programmed cell death and the loss of the isthmic constriction and cerebellum. Single morpholino knockdown of either Lmx1b.1 or Lmx1b.2 has no discernible effect on MMR development. Transient misexpression of Lmx1b.1 or Lmx1b.2 during early MMR development induces ectopic *wnt1* and *fgf8* expression in the MMR, as well as throughout much of the embryo. We propose that Lmx1b.1 and Lmx1b.2 regulation of *wnt1*, *wnt3a*, *pax8*, and *fgf8* maintain cell survival in the isthmocerebellar region.

419. Genetic Pathways Regulating Rhombomere 4/5 Formation. Charles Sagerstrom, Seong-Kyu Choe, and Nicholas Hirsch. Univ. of Massachusetts Medical School, Worcester, MA.

Subdivision of the embryonic vertebrate hindbrain into an array of rhombomeres (r) requires at least three functions—induction of a specific rhombomere fate, repression of alternative fates and appropriate cell sorting into rhombomeres. We have used misexpression experiments in wild-type and mutant zebrafish embryos to establish genetic pathways for r4 and r5 formation and we find that these various functions may be mediated by separate pathways. For instance, vhnf1, valentino, krox20 and PG3 hox genes all act in r5, but they appear to occupy at least two pathways. Specifically, valentino, krox20 and PG3 hox gene expression can be induced independently of vhnf1, but krox20 and PG3 hox expression is dependent on valentino. This suggests that valentino, krox20 and PG3 hox

genes act in one pathway, while vhnf1 appears to act in a second pathway. While valentino, krox20 and PG3 hox genes reportedly control r5 fate, vhnf1 appears to act primarily to repress non-r5 fates, suggesting that induction of r5 fates and repression of alternative fates are mediated by separable genetic pathways. A similar, although somewhat less clear, situation appears to operate in r4. Specifically, hoxb1a reportedly controls r4 fate, but we have recently identified two nlz genes that encode transcriptional repressors and that appear to act by repressing r5 fates. Thus, separate genetic pathways may control different aspects of rhombomere formation.

420. Endodermal Patterning in Zebrafish: Pancreas Development. Kristen M. Alexa, Philip diIorio, Letitiah Etheridge, and Charles Sagerström. Univ. of Massachusetts Medical School, Worcester, MA 01605.

The pancreas is an endocrine gland and an exocrine gland that produces both hormones and digestive enzymes. Molecular and genetic studies have provided insights into the intercellular signals that regulate pancreas development. These include transforming growth factor-α (TGF-α), Notch, Hedgehog, fibroblast growth factor (FGF), and epidermal growth factor (EGF) pathways. We find that Pbx and Meis, two homeodomain transcription factors (TFs), are involved in patterning the zebrafish endoderm. For example, pbx4 mutants (lazarus) display ectopic anterior expression of genes such as pdx1, insulin (endocrine pancreas), carboxypeptidase A (exocrine pancreas), iggy4 (liver), and transferrin (liver). We observe a similar phenotype in zebrafish embryos injected with a dominant-negative meis (dnMeis) construct. Also, blocking sonic hedgehog and ectopic retinoic acid results in anterior ectopic expression of insulin. To determine if pbx, meis and the signaling pathways act together, lazarus embryos will be treated with inhibitors of sonic hedgehog and retinoic acid to see if ectopic anterior expression of endoderm markers occurs. In addition, pbx and meis function as cofactors to other homeodomain and non-homeodomain TFs, giving the possibility of other transcription factors acting to pattern the endoderm. Currently, a zebrafish genetic screen is being performed to discover potential partners. Next, we would like detect which germ layer these endoderm patterning signals act. A zebrafish line will be created expressing endoderm promoter: :Gal4 and crossed with a UAS: :dnMeis line to determine if endoderm markers are ectopically expressed.

421. Activation of Goosecoid Transcription by Siamois and Twin. Christine D. Reid, Sangwoo Bae, and Daniel S. Kessler. Univ. of Pennsylvania, Philadelphia, PA 19104.

In early *Xenopus laevis* development, the Spemann organizer regulates the patterning of mesoderm at the marginal zone. The transcription factors *siamois* (Sia) and *twin* (Twn) are induced in a dorsal domain in response to stabilized β-catenin and are thought to be key regulators of organizer gene expression. Sia and Twn are among the earliest organizer genes expressed following the midblastula transition and have been shown to be essential for organizer formation and induction of *goosecoid* (Gsc) in the organizer. To investigate the transcriptional mechanisms of Sia and Twn, we performed in vitro biochemical and in vivo transcriptional activation analyses. EMSA and DNase footprinting reveal that Sia and Twn bind as homo- or heterodimers to the proximal element of the Gsc promoter and the homeodomain is necessary for this dimerization. In Sia, amino acids 1–75 are critical for the transcriptional activation function; in Twn, the activation domain maps to an identical position. Ongoing experiments include chromatin immunoprecipitation assays to confirm binding of Sia and Twn to the Gsc promoter in vivo as well as the identification and characterization of co-factors that may cooperate with Sia and Twn in the establishment of the organizer. Defining the mechanism by which Sia and Twn activate transcription of Gsc will further elucidate how the temporal and spatial regulatory strategies establish and maintain the Spemann organizer.

422. A Role for Notch Signaling in Mesoderm Patterning of *Xenopus laevis*. Carolyn M. Gaydos and Kelly A. McLaughlin. Department of Biology, Tufts University, Medford, MA 02155.

Proper cell–cell communication during the formation and patterning of tissues and organs is necessary to ensure the regulation of complex cellular behaviors such as proliferation, migration, death and differentiation. Members of the Notch signaling family of proteins have been shown to mediate a wide array of important cell fate decisions during the development of numerous organisms ranging from worms to humans. We have recently demonstrated a role for Notch signaling in establishing and maintaining boundaries between endodermal and mesodermal tissue types early in development, in particular during gastrulation. We therefore decided to extend these studies to explore potential roles for Notch signaling during mesoderm specification and patterning, more specifically during cardiogenesis. Previous work demonstrated that signaling through Notch was necessary for proper differentiation and morphogenesis of cardiac tissue after the heart field was already specified. However, a role for Notch signaling earlier in cardiac development still remains poorly characterized. In order to address this gap, we utilized temporally inducible forms of members of the Notch pathway in order to perturb signaling during the desired stage of heart development and subsequently examined resultant tadpoles. Our findings suggest that the perturbation of Notch signaling early in embryogenesis, specifically during the time when the cardiac field is specified, results in a profound disruption of cardiac development, providing evidence for an earlier role for Notch signaling during heart development than described previously.

423. A Genomic Screen for zic1 Target Genes in Early Neural Development. Shuzhao Li,[1] Ken W. Cho,[2] and Christa S. Merzdorf[1]. [1]Department of Cell Biology and Neuroscience, Montana State University, Bozeman; [2]Department of Developmental and Cell Biology, University of California, Irvine.

During embryonic development in vertebrates, the prospective nervous system forms distinct regions of specialized function. One of the earliest regulators in this patterning process is the transcription factor *zic1*. In addition to its role in neural patterning, *zic1* functions in the generation of neural crest cells and in other developmental processes. To search for genes regulated by *zic1*, we misexpressed *zic1* in animal caps of *Xenopus* embryos and examined the subsequent changes in gene expression using both cDNA microarrays and Affymetrix Genechips. Among the resulting candidate genes that were up- or downregulated by *zic1*, we have confirmed that several are direct target genes of *zic1* using real-time PCR experiments. The expression of these genes in embryonic development was characterized by in situ hybridization. One novel gene that is directly upregulated by *zic1* is Xl.15703. We tentatively named it *feb* due to its strong homology to a 17-kDa human fetal brain protein with no known function. The Feb protein contains five repeated SCP-like domains, which are present in a wide variety of proteins and are usually extracellular. *feb* expression initiates at early gastrula stage 10, shortly after *zic1* expression begins. *feb* is expressed widely in the neural plate and along the neural folds, extensively overlapping with *zic1* expression. Functional studies of *feb* are underway. We will also report on other *zic1* target genes identified in our screen. Our findings propose new regulatory relationships in early neural development.

424. Circadian Genes are Expressed During Early Development in *Xenopus laevis*. Kristen Curran,[1] Silvia LaRue,[2] and Carla Green[2]. [1]University of Wisconsin-Whitewater; [2]Univ. of Virginia, Charlottesville, VA 22904.

Circadian oscillators are endogenous time-keeping mechanisms that drive 24 h rhythmic changes in gene expression, metabolism, hormone levels and physical activity. We have begun to characterize the

developmental expression of genes known to regulate circadian rhythms in order to better understand the ontogeny of the circadian clock in a vertebrate. We examined the expression of genes known to be part of the core circadian oscillator mechanism (*Per1*, *Per2*, and *Bmal1*) as well as a rhythmic, clock-controlled gene (*Nocturnin*) by in situ hybridization of similarly staged embryos (from neurula to late tailbud stages) at two circadian time points (dawn and dusk). All genes examined were present in the developing nervous system such as the brain, eye, otic vesicle, olfactory bulb, and decreasing levels in the spinal cord. Surprisingly, these genes were also expressed in the developing somites. We did not observe any obvious differences in expression pattern or level of expression of any of the genes between the two circadian times. This indicates that at these stages of development these mRNAs are not expressed in a circadian fashion. Although it is known that melatonin synthesis is rhythmic by stage 26 in the eye (Green et al., 1996), these results suggest that circadian regulation may occur only in localized cell types (undetectable by in situ) or may occur at the level of protein synthesis. Of particular interest is the role of Nocturnin, a deadenylase thought to be involved in circadian regulation of mRNA stability (Baggs and Green, 2003), in somitogenesis, heart, and kidney development.

425. Nkx6 Genes in Patterning of Frog Neural Plate. Darwin S. Dichmann and Richard M. Harland. University of California, Berkeley, 571 Life Sciences Addition, Berkeley, CA 94720.

To investigate if mediolateral patterning of the frog neural plate is governed by the same mechanisms as in other vertebrates we have cloned two Nkx6 genes in *X. laevis* and studied their role during development. Nkx6.2 is expressed strongly from mid-gastrula stages in the medial neural plate and later in the ventral neural tube. Nkx6.1 on the other hand is not expressed until stage 13 and is restricted to two narrow stripes in the neural plate where the motor neuron progenitors form. Overexpression of these proteins show that they have overlapping activity: both inhibit primary neuron formation without quenching pan-neuronal markers suggesting that they keep cells in an undifferentiated state. In addition, they are able to repress Dbx, En1, and Pax2, which are expressed in the intermediate part of the neural plate, confirming a common vertebrate function in specifying medial cell fates by repressing more lateral ones. Interestingly, both Nkx6 genes are also able to repress Irx3 and Pax6, which are also expressed in the intermediate part of the neural plate but is not repressed by Nkx6 genes in other vertebrates. Using a morpholino knockdown approach we have tested the role of Nkx6 genes in neural plate development and show that these genes are necessary for proper patterning. Together, these experiments suggest that Nkx6.2 plays a prominent role as a patterning gene of neural plate in frogs, whereas Nkx6.1 function seems mainly confined to motor neuron differentiation. Therefore, although the main functions of the Nkx6 genes are similar between vertebrates, there are important differences.

426. Six3 Functions in Anterior Neural Plate Specification by Promoting Cell Proliferation and Inhibiting BMP4 Expression. Massimiliano Andreazzoli,[1] Gaia Gestri,[1] Matthias Carl,[2] Irene Appolloni,[1] Steve W. Wilson,[2] and Giuseppina Barsacchi[1]. [1]Laboratori di Biologia Cellulare e dello Sviluppo, Universita' di Pisa, Via Carducci 13, 56010 Ghezzano (Pisa), Italy; [2]Department of Anatomy and Developmental Biology, University College of London, Gower Street, London WC1E 6BT.

Although it is well established that Six3 is a critical regulator of vertebrate eye and forebrain development, it is unknown if this homeodomain protein has a role in the initial specification of the anterior neural plate. In this study we show that exogenous Six3 can expand the anterior neural plate in both *Xenopus* and zebrafish and that this occurs in part through Six3-dependent transcriptional regulation of the cell cycle regulators cyclinD1 and p27Xic1 as well as the anti-neurogenic genes Zic2 and Xhairy2. However, Six3 can still expand the neural plate in the presence of cell cycle inhibitors and we show that this is likely due to its ability to repress the expression of BMP4 in ectoderm adjacent to the anterior neural plate. Furthermore, exogenous Six3 is able to restore the size of the anterior neural plate in chordino mutant zebrafish indicating that it has the ability to promote anterior neural development by antagonizing the activity of the BMP pathway. On its own, Six3 is unable to induce neural tissue in animal caps but can do so in combination with Otx2. These results suggest a very early role for Six3 in specification of the anterior neural plate through the regulation of cell proliferation and the inhibition of BMP signalling.

427. Regulation of Sox Group E Activity During Neural Crest Development. Kim Taylor and Carole LaBonne. Dept. Biochemistry, Molecular Biology, and Cell Biology Northwestern University, Evanston, IL 60208.

Neural crest cells are proliferative, migratory, tissue-invasive stem cells that originate in the ectoderm of vertebrate embryos. These cells give rise to a diverse set of derivatives that includes most of the neurons and glia of the peripheral nervous system, melanocytes and the elements of the craniofacial skeleton. Formation of neural crest cells is regulated by the combined activities of a number of gene products including the Sox Group E transcription factors, Sox 8, 9 and 10. This family of proteins is also implicated in the development of non-neural crest-derived tissues, such as the ear.

428. The Avian Lateroseptal Organ and the Molecular Striatopallidal Border. Sylvia Bardet,[1] Inmaculada Cobos,[2] Eduardo Puelles,[3] Margaret Martínez-de-la-Torre,[1] and Luis Puelles[1]. [1]Univ. of Murcia Medical School, E30071 Murcia, Spain; [2]Univ. of California San Francisco, Med. School, N. Ireland Lab. Devel. Neurobiol., SF 94143; [3]Univ. of Miguel Hernández, Ins. of Neurociencias-CSIC, E3550 San Juan, Alicante, Spain.

The lateral septal organ (LSO) is a circumventricular organ described in the telencephalon of birds (Kuenzel and van Tienhoven 1982). Published data support the LSO is implicated in extraretinal photoreception. It is composed of cerebrospinal fluid-contacting neurons. Only its pars medialis is septal; its larger pars lateralis lies in the lateral wall subpallium. We investigated with molecular markers the position of the chick LSO relative to subpallial molecular borders. All the subpallium expresses Dlx genes. A nested domain within this area that expresses Nkx2.1 marks the pallidal primordium, the anterior entopeduncular area and the anterior preoptic area. The latter two areas selectively express Shh. Our immunocytochemical and in situ hybridization results using various markers for the LSO suggest that it forms in a fixed relationship to these subpallial domains. The organ extends under the lateral ventricle into the septum, like the ST, PA and AEP domains do. Moreover, an analogous circumventricular formation continues the LSO into the preoptic subregions of the subpallial complex. We conclude the LSO represents an integral part of the molecularly specified subpallial structural pattern. Work supported by Spanish MCYT grant BFI2002-03668. S.B. is a fellow of the MCYT associated to this project. E.P. benefits of a Ramón y Cajal MCYT contract at the Alicante Institute of Neuroscience.

429. Regulation of Motor Neuron Progenitor Differentiation by a Network of HLH Proteins. Bennett G. Novitch,[1] Steven E. Weicksel,[1] and Thomas M. Jessell[2]. [1]University of Michigan Medical School, Ann Arbor, MI; [2]HHMI, Columbia University, New York, NY.

The establishment of neural circuits requires that distinct cell types arise in the correct places, times, and cell numbers in developing embryos. Central

to this process is the commitment of neural progenitor cells to the production of a particular cell type, followed by their regulated division and expansion, and eventual exit from the cell cycle and differentiation. This process has been best described for spinal motor neurons (MNs), which arise from a set of rapidly differentiating progenitors in the ventral spinal cord. Previously, we have identified the bHLH transcription factor Olig2 as a critical regulator of MN progenitor cell differentiation. Olig2 repressor activity is both necessary and sufficient for the acquisition of MN identity and for pan-neuronal differentiation. Here, we provide evidence that Olig2 controls the characteristic differentiation of MN progenitors by promoting the expression and activity of the proneural bHLH proteins Neurogenin2 (Ngn2) and Math3/NeuroM at two levels. First, Olig2 promotes high-level expression of Ngn2 in MN progenitors through its ability to extinguish the expression of the anti-neural bHLH proteins Hes1 and Hes5 that, in turn, repress Ngn2 expression. Second, Olig2 represses the expression of members of the Id family of inhibitory HLH proteins in MN progenitors, and thus facilitates Ngn2 protein activity and the upregulation of Math3/NeuroM and MN differentiation markers. Together, these findings indicate that Olig2 regulates an intricate network of bHLH expression that leads to the appropriate early and rapid production of MNs.

430. Tbx5 Transcriptional Activity is Mediated by Sub-Cellular Localization. Troy Camarata and Hans-Georg Simon. Northwestern University, Feinberg School of Medicine, Children's Memorial Research Center, Chicago, IL 60614.

The T-box transcription factor Tbx5 has a vital role in both vertebrate limb and heart development. Perturbation of Tbx5 activity leads to severe developmental defects, which in humans, results in the genetic disease Holt–Oram syndrome. In an effort to elucidate Tbx5 function during development, we have identified a novel Tbx5 interacting protein called Lmp4. Lmp4 is a PDZ-LIM protein that localizes to actin filaments unlike the expected nuclear localization of Tbx5. However, when co-expressed in cells, Lmp4 binds to Tbx5 and localizes the transcription factor to actin filaments. This unique sub-cellular localization of Tbx5 only occurs in the presence of Lmp4 and uncovers a yet unknown regulatory mechanism for transcriptional activity. The withdrawal of Tbx5 from the nucleus and tethering to the actin cytoskeleton by Lmp4 results in repression of its transcriptional activity on the *Fgf-10* and *ANF* promoters. This transcriptional repression appears to be dependent on an intact actin cytoskeleton. Disruption of filamentous actin causes Lmp4/Tbx5 dissociation and re-localization of Tbx5 to the nucleus, despite the co-expression of the proteins within the same cell. Together, the data point towards a novel and sophisticated mechanism of transcription factor regulation during vertebrate development.

431. Hedgehog Signaling, Intraflagellar Transport Protein and Skeletal Development. Buer Song, Bradley K. Yoder, and Rosa Serra. University of Alabama at Birmingham.

Intraflagellar transport (IFT) proteins were initially found to be essential for the growth and maintenance of cilia and flagella. Recent studies showed that they also have the function of signal transduction. Studies in mice showed that defects in IFT affect the cilia in several organs, which leads to a pleiotropic phenotype including polydactyly and other skeletal defects. Moreover, it has been shown that IFT proteins are required in the vertebrate Hedgehog signal transduction at a step downstream of Patched1 and upstream of direct targets of Hedgehog signaling. As Hedgehog signaling plays a key role in the development and maintenance of normal skeleton, we propose to use a conditional IFT protein knockout mice model to further study the role of Hedgehog and IFT protein in cartilage and bone development. Mice with typeII collagen promoter-driven Cre have been crossed with mice in which both alleles of Kif3a (a subunit of Kinesin-II IFT motor) have been floxed. Skeletal defects were not observed in these mice with heterozygous Kif3a knockout (Cre^{+}/Kif3aLoxP$^{+/-}$) in chondrocytes. Mice with Kif3a homozygous conditional knockout (Cre^{+}/Kif3aLoxP$^{-/-}$) in chondrocytes have not yet been characterized. Further analysis of the skeleton in the developing embryos and possible newborn of these mice will reveal more insight into the mechanism of how Hedgehog and IFT proteins regulate skeletal development. Our study will also help to explain certain human disorders such as Jeune's syndrome, Kartagener's syndrome, Bardet–Bieldl syndrome and other disorders caused by defects in cilia with skeletal abnormalities.

432. The Role of Cilia/IFT in Shh Signaling and Limb Patterning. Courtney J. Haycraft, Qihong Zhang, Boglarka Banizs, and Bradley K. Yoder. Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.

Cilia are projections extending from the surface of many eukaryotic cells that can be either motile or immotile and are constructed through a process termed intraflagellar transport (IFT). Tg737 is an IFT/ciliogenic protein that when disrupted in $Tg737^{orpk}$ or $Tg737^{\Delta 2\text{-}3\beta\text{-}Gal}$ mutants results in six or 8–10 unpatterned digits on the limb, respectively, without ectopic expression of *Shh*. The lack of ectopic *Shh* expression in $Tg737^{\Delta 2\text{-}3\beta\text{-}Gal}$ mutants along with the formation of multiple non-patterned digits is reminiscent of the phenotype seen in $Shh^{-/-}$;$Gli3^{-/-}$ embryos. Recent work has shown a genetic link between IFT/polaris and Shh signaling. In agreement with this, we have shown that primary cells isolated from the limbs of $Tg737^{\Delta 2\text{-}3\beta\text{-}Gal}$ mutant embryos do not induce transcription of *Ptch1* or *Gli1* when cultured with *ShhN*-conditioned media. This suggests that *Tg737* is required for Shh pathway activation possibly by affecting the function of the Gli transcription factors. Indeed, there is an increase in the relative amount of unprocessed Gli3 in $Tg737^{\Delta 2\text{-}3\beta\text{-}Gal}$ mutants, and the function of both Gli2 and Gli3 are disrupted. In support of a direct role for cilia in Shh signaling, all three Gli proteins and Sufu, a negative regulator of Gli function, localize to the distal tip of cilia in primary limb bud cultures. Using conditional mutants we are currently investigating whether cilia/IFT function in the mesenchyme, ectoderm or both is required for proper patterning of the limb.

433. Establishment of an Lmx1b DorsalVentral Sharp Boundary is Coordinately Linked to AER Formation During Mouse Limb Development. Qiong Qiu and Randy L. Johnson. Univ. of Texas, M.D. Anderson cancer center, Houston, TX 77030.

Lmx1b is a LIM-homeodomain transcription factor required for dorsal–ventral patterning in vertebrates. How Lmx1b specifies dorsal fates of the mouse distal limb is not understood yet. In this study, we found Lmx1b expression was initially throughout the distal dorsal and ventral side, then limited to the dorsal mesenchyme of the forming limb bud after AER formation and built up a sharp dorsal–ventral boundary. This dynamic expression pattern of Lmx1b coordinately linked to AER formation is further confirmed by a CreERT2/loxP-based fate mapping approach. We used Lmx1b-CreERT2:R26R mice to determine the fate of Lmx1b cells at different time points by injection of tamoxifen: 9.0dpc(pre AER), 10.5dpc(AER), 11.5dpc(after AER). The initially marked cells at 9.0 dpc spanned throughout dorsal and ventral distal limb bud even if AER has already formed. However, cells marked after AER formation were restricted to dorsal side of limb bud. It suggested that Lmx1b gene initially expressed in ventral side of limb bud is turned off after AER formation. We also used this inducible CreERT2/loxP fate mapping approach to test whether an Lmx1b-independent boundary exists to limit Lmx1b expression to dorsal side of limb. A point mutation is introduced in Lmx1b homeodomain in addition that CreERT2 inserted in 3′UTR of Lmx1b. We used Lmx1bPM-CreERT2:R26R mice to determine the fate of Lmx1b mutant cells. Mutant cells spanned throughout dorsal and ventral limb bud even though we injected tamoxifen after AER formation. This indicated that establishment of Lmx1b expression boundary is Lmx1b dependent.

434. Temporal Requirements of Sonic Hedgehog Expression for Specifying Different Digits. Jianjian Zhu, Minh-Thanh Nguyen, and Susan Mackem. Laboratory of Pathology, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892, USA.

The zone of polarizing activity in the posterior limb bud regulates digit identity through the morphogen Sonic Hedgehog (Shh). There are several hypotheses addressing this patterning mechanism, including the classical spatial morphogen gradient model and a more recent expansion-based temporal Shh gradient model, which both propose that the highest cumulative levels of Shh signaling specify the most posterior digit. Yet another recent study showed that the response of posterior cells to Shh signaling appears to be lowered over time. To determine the temporal sensitivity of digits to the loss of Shh signaling, we have used a transgenic Hoxb6-CreER line expressing a tamoxifen-dependent Cre recombinase in the lateral plate mesoderm to evaluate the temporal requirements for Shh signaling by recombination of a floxed, conditional null allele of Shh. Unexpectedly, our preliminary results indicate that the most posterior digit is not the most sensitive to late loss of Shh and that some of the current models of Shh dosage dependence in digit formation may need to be reassessed.

435. The Role of Sox6 in Muscle Development. Nobuko Hagiwara, Betty Ma, Alice Ly, Quan Nguyen, and Michael Yeh. Univ. of California, Davis, CA95616.

We have previously shown that p^{100H} mutant mice which lack a functional Sox6 gene exhibit skeletal and cardiac muscle degeneration and develop cardiac conduction abnormalities soon after birth, never surviving beyond 2 weeks (Hagiwara et al. PNAS 97:4180). Although Sox6 is expressed in a wide variety of tissues, only its function in bone formation has been well characterized. The muscle defects observed in the p^{100H} mutant motivated us to investigate the role of Sox6 in muscle development. We identified muscle specific genes differentially expressed between wild-type and p^{100H} mutant skeletal muscles using microarray analysis and further investigated their temporal expression in the mutant muscle using real time PCR. We have found that in the mutant skeletal muscle, the expression of slow fiber isoform and cardiac isoform genes is significantly increased, whereas the expression of fast fiber isoform genes is significantly decreased. Onset of this aberrant fiber type-specific gene expression in the mutant skeletal muscle (embryonic days 15–16) coincides with the beginning of the secondary myotube formation. In addition, we have found that the fetal isoform sarcomeric protein genes fail to be suppressed in the mutant postnatal cardiac muscle, even though the adult isoform genes are normally induced. These results indicate that Sox6 is required for terminal differentiation of skeletal and cardiac muscles and may have a critical role for their functional maturation.

436. Identification of *Foxl1* Target Genes in the Mesenchyme of the Gastrointestinal Tract. Sara D. Sackett and Klaus H. Kaestner. Univ. of Pennsylvania, Philadelphia, PA 19104.

Members of the Wnt family of secreted glycoproteins play diverse roles in cell fate, polarity, death and proliferation. Wnt proteins in the canonical pathway activate expression of their target genes through the stabilization of cytoplasmic β-catenin. Constitutive activation of the Wnt/APC/β-catenin pathway is a frequent initiating event in gastrointestinal carcinogenesis. We have shown that the loss of the mesenchymal transcription factor *Foxl1* leads to increased nuclear β-catenin and a marked increase in proliferation. In addition, the loss of *Foxl1* on an *ApcMin* background causes an increase in tumor multiplicity in the colon and development of gastric tumors which are not normally found in *Apc*Min mice. These effects are the result of earlier tumor initiation due to accelerated loss of heterozygosity (LOH) at the *Apc* locus. We hypothesize that *Foxl1* is involved in the regulation of Wnt expression and thereby affects canonical Wnt signaling in the gastrointestinal tract. In order to elucidate the signaling molecules controlled by *Foxl1* in the gastrointestinal mesenchyme we are isolating mesenchyme of the gastrointestinal tract of E12.5 embryos. Isolation of these tissues from *Foxl1* null and control embryos will allow us to analyze the expression profiles of the Wnt family members and other signaling molecules in a tissue specific and temporal manner.

437. Understanding the Role of Gata Transcription Factors in the Developing Mouse Pancreas. Kimberly J. Decker and Lori Sussel. Univ. of Colorado Health Sciences Center, Aurora, CO 80045.

The pancreas regulates glucose homeostasis by fulfilling two important functions: the endocrine pancreas produces and secretes hormones into the bloodstream, while the exocrine pancreas produces and secretes digestive enzymes into the intestine. In addition, the ductal network of the pancreas provides the conduit that allows exocrine cells to secrete enzymes into the gut and may serve as a repository of progenitor cells for both the endocrine and exocrine pancreas. We have shown that Gata factors – specifically Gata4 and Gata6 – are expressed in the pancreas. Their expression is global at the onset of pancreas formation but becomes restricted to specific pancreatic domains during later developmental stages. Gata6 becomes restricted to endocrine cell types, including the beta cells, as well as the ducts, and is colocalized with other endocrine factors such as Nkx2.2 and Ngn3. Further, Gata6 interacts with Nkx2.2 in vitro. Gata4 becomes restricted to the exocrine pancreas, is coexpressed with the exocrine factor ptf1a, and interacts with ptf1a in vitro. We hypothesize that Gata transcription factors play a role in differentiation events during pancreas organogenesis, potentially in the terminal differentiation of various pancreatic cell types, including beta cells. We have used the Cre-LoxP system to generate pancreas-specific knockout mice for both Gata4 and Gata6. We are using these mice to determine the role of Gata factors in the complex regulatory network of the developing mouse pancreas.

438. Analysis of Baf250 and the Functional Distinction Between Mammalian Swi/Snf Complexes. Jennifer Brennan and Terry Magnuson. Univ. of North Carolina-Chapel Hill.

Regulation of gene expression in eukaryotes is inherently complicated by the higher order packaging of DNA into chromatin. This structure must be tightly regulated to maintain the flexibility to activate or repress transcriptional programmes during development and maintain specific expression patterns throughout cellular lifespans. Epigenetic mechanisms that regulate higher order chromatin structure include DNA methylation, histone modifications, and nucleosome remodeling. SWI2/SNF2 proteins are members of a protein family that utilizes the energy of ATP hydrolysis to alter nucleosome position, thereby affecting access to *cis*-regulatory elements of DNA and contributing to transcriptional regulation of genes. BRG1 and BRM are mammalian homologs of the yeast SWI2/SNF2 catalytic subunits and function in large, biochemically distinct complexes. Although Brg and Brm are 75% identical, the phenotypes of mouse null mutants revealed an essential role for Brg1 in early development, while Brm/animals were normal. BRG1 is the catalytic subunit in the two general types of SWI/SNF complexes, BAF and pBAF, whereas BRM is only present in the BAF complex. Baf250 is a unique component of BAF and not the pBAF complex, so analysis of its function should provide knowledge of the relative functional importance of BRG1 in these two similar complexes. Our lab maintains a cryopreserved library of ENU mutagenized ES cells that we are screening for point mutations in Baf250. The isolated mutant ES cells are being used to generate an allelic series of mutations in Baf250

to study its in vivo role and dissect the distinct functions of BRG1-dependent complexes throughout development.

439. Phenotypic Recovery of TGFbeta2 Null Embryos by Down-regulated Pax3. C. Shekhar K. Mayanil,[1] Hiromichi Nakazaki,[1] Beth A. Stahl,[1] Barbara Mania-Farnell,[2] David George,[1] Eric G. Bremer,[1] and David G. McLone[1]. [1]CMRC and Northwestern University Feinberg School of Medicine, Chicago, IL 610614; [2]Purdue University Hammond, IN 46343.

TGFβ2 null mice display spina bifida and die at birth. Similarly, Pax3 null embryos display neural tube closure defects and die early during development (E9.0 to E10.0). In our in vitro studies using Pax3 transfectants, we observed a reciprocal relationship between the canonical TGFβ2 signaling pathway and Pax3 downstream target genes. In the absence of TGFβ2 signaling pathway, as displayed by TGFβ2 null embryos, there was no negative regulation of the Pax3 downstream target genes. Similarly, in Pax3 null embryos there was no negative regulation of genes regulated by canonical TGFβ2 signaling pathway. We therefore asked the question whether downregulating Pax3 can rescue a TGFβ2 null phenotype. We made TGFβ2 and Pax3 double heterozygous mice and mated the males and females to obtained embryos of different genotypes. We observed that the embryos null to TGFβ2 and heterozygous to Pax3 showed rescue of TGFβ2 null phenotype. The expression of candidate downstream target genes of TGFβ2 signaling and Pax3, namely Sox9, and Neurogenin2 were studied using TGFβ2−/−Pax3+/− embryos. These studies showed that downregulated Pax3 can reverse the TGFβ2 null phenotype.

440. The Transcriptional Repressor *Snai1* is Required for Left–Right Asymmetry Determination in the Mouse. Stephen A. Murray, Ethan A. Carver, Kathleen F. Oram, and Thomas Gridley. The Jackson Laboratory, Bar Harbor, ME 04609.

Generation of left–right asymmetry is an essential component of pattern formation in the vertebrate embryo. This process requires tight coordination of the expression of genes such as *Nodal*, *Lefty1/2*, and *Pitx2* in the early mouse embryo to differentiate left from right. Previous work in the chick has demonstrated the important role of the *Snai1* gene in defining the right side of the embryo by repression of the left side determinant Pitx2. To test the role of *Snai1* in establishment of the left–right axis in the mouse, we have generated a conditional *Snai1* allele, which rescues the early lethality of the *Snai1*-null mice to E9.5 when crossed to the Meox2-Cre line. The Meox2-Cre; $Snai1^{flox/flox}$ embryos exhibit multiple laterality defects, including randomization of heart looping and embryonic turning. The left determinant genes *Nodal*, *Lefty2*, and *Pitx2* display bilateral expression patterns which are particularly prominent in the posterior region, overlapping the normal *Snai1* expression domain. These changes are independent of gross structural defects at the midline and in the node. Because of *Snai1*'s demonstrated function as a transcription repressor, we propose a model in which loss of *Snai1* results in depression of critical left side determinants and disruption of normal left–right axis formation.

441. Elucidating the Timing of the Endothelin-A Receptor Function During Craniofacial Development. Louis-Bruno Ruest,[1] Tom Brock,[2] and David E. Clouthier[1]. [1]Department of Molecular, Cellular and Craniofacial Biology, University of Louisville, Louisville, KY 40292; [2]Encysive Pharmaceuticals, Houston, TX 77030.

Lower jaw development requires the interaction of numerous cells types. One cell type, cephalic neural crest cells (NCCs), migrates from the posterior midbrain/hindbrain to the mandibular pharyngeal arch from which the lower jaw and middle ear arise. Once there, NCCs are patterned by numerous factors organized into hierarchical signaling pathways. One or more of these pathways are regulated by signaling from the endothelin-A receptor (Ednra). Targeted deletion of Ednra results in severe craniofacial defects, including transformation of mandibular structures into maxillary-like structures. However, the timing of Ednra function during craniofacial development is poorly understood. To address this issue, we administered an Ednra-specific antagonist to pregnant wild-type mice between embryonic day (E) 8.0 and E10.0 at one or two consecutive time points separated by 12 hours. Analysis of E18.5 skeletons revealed that lower jaw defects occurred when pregnant mice were treated between E8.5 and E9.5, with defects peaking around E9.0. These defects included shortening of the mandible and malformation or absence of Meckel's cartilage, malleus, incus and tympanic and gonial bones. This timing of Ednra action was also reflected in disrupted expression of Dlx3, Dlx5, Dlx6, Hand1 and Hand2, though the timing of disruption differed slightly between these genes. Taken together, our results indicate that Ednra function is essential for establishing NCC patterning within the mandibular arch around E9.0, soon after NCCs reach the arch.

442. Role of the PDGFRs on the Migratory Ability of Cardiac Neural Crest Cells. Alicia M. Richarte and Michelle D. Tallquist. UT Southwestern Medical Center.

Migration is one of the central cellular functions of neural crest cells (NCCs). Two subpopulations of NCCs, cranial and cardiac, are essential components of the craniofacial mesenchyme and aortic arch smooth muscle, respectively. Because many common human birth defects occur as a result of a defect in these cells, there is much interest in determining the factors involved in their development. Recent findings in our lab demonstrate that signaling through both the platelet-derived growth factor receptors (PDGFR) is required for cranial and cardiac NCCs. When the PDGFRα is defective, aortic arch defects are incompletely penetrant, but, when both PDGFR α and PDGFRβ are disrupted, persistent truncus arteriosus occurs with 100% penetrance. Through NCC lineage tracing, we observed a decrease in the number of cells available to septate the outflow tract of the aortic arch. No changes in proliferation nor apoptosis were observed. Therefore, we believe that both receptors regulate the migratory ability of these cells through the aortic arch region. In vitro analysis of primary NCC cultures will be used to verify these observations. In addition, we will analyze the expression profiles of various transcription factors required for NCC migration that may be downstream of these receptors and thus being affected by loss of these receptors in that population. Finally, we want to determine if there is an extracellular matrix (ECM) defect that affects the migratory ability of NCCs. These data will define a growth factor family involved in NCC migration and opens an avenue of investigation for studying signal transduction control of NCC migration.

443. *Tbx4* rRegulates Endothelial Cell Fate Decisions in the Allantois. Virgina E. Papaioannou and L.A. Naiche. Dept. of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA.

Blood vessel formation takes place via two distinct processes: vasculogenesis, the de novo differentiation of endothelial cells from undifferentiated mesenchyme, and angiogenesis, the elongation of existing vessels. The precursor to the umbilical cord, the allantois, is a vasculogenic tissue, but little is known about vasculogenic regulation in this tissue as compared to the well-studied yolk sac. *Tbx4* is expressed throughout the allantois during its development. Mutation of *Tbx4* causes multiple defects in the allantois, including aberrant vasculogenesis. Previous work has shown that endothelial cells successfully differentiate in the mutant allantois but do not form an organized network of vessels. We have now further analyzed this phenotype by examining the expression of genes in key vasculogenesis pathways. We have also made wild-type-*Tbx4*-null chimeric embryos, which reveal a startling phenotype. Despite the fact that *Tbx4* mutant cells are capable of

forming endothelial tissue, in chimeras mutant cells are specifically excluded from the allantoic vasculature. The allantoic vasculature is disorganized, but overall numbers of endothelial cells remain constant. The exclusion of competent cells from endothelial cell fates suggests that *Tbx4* controls a lateral inhibitory process that regulates endothelial differentiation from the mesenchyme. We have observed that *Jagged1*, a Notch ligand, is normally expressed in the allantois but absent in *Tbx4* mutants, further suggesting that the lateral inhibition may be regulated by the Notch pathway.

444. Analysis of Eye Development in Mice with a Targeted Mutation of the *Tbx2* Gene. Hourinaz Behesti,[1] Jane C. Sowden,[1] and Virginia E. Papaioannou[2]. [1]Developmental Biology Unit, Institute of Child Health, UCL, London; [2]Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, NYC.

Tbx2 belongs to the evolutionarily conserved family of T-box transcription factor genes. Analysis of mice containing a targeted mutation of *Tbx2* revealed that it is essential for heart development*. We have analysed these mice to investigate the role of *Tbx2* in eye development. *Tbx2* is expressed in the distal optic vesicle (OV) at 9.5 days post coitum (dpc). The OV invaginates to form the optic cup (OC) by 10.5 dpc and *Tbx2* becomes restricted to the dorsal-periphery of the OC. *Tbx2* is also expressed in extra-ocular mesenchyme. In $Tbx2^{-/-}$ embryos the OC forms, but is smaller than in wild-type littermates, and shows several morphological defects. Analysis of gene expression shows that the dorsal expression domain of T-box gene *Tbx5* is reduced, while that of *Ephrin-B2* is expanded. By contrast, the expression of other dorsal genes (*Bmp4*, *Msx2*) is normal. The expression domain of *Vax2* in the ventral retina is also reduced. Thus, *Tbx2* is not essential for specification of the dorso-ventral axis of the OC, although without it certain gene expression domains are altered. Expression of the retinal pigment epithelium (RPE) gene *Silver* is normal in the absence of *Tbx2*, indicating that specification of the RPE occurs without it. Our data show that *Tbx2* is not essential for OV morphogenesis and early specification events. It is however important for normal growth and morphology of the OC. This study sheds light on the position of *Tbx2* in the genetic hierarchy of eye development. *Harrelson et al. Development 2004, 131(20): 5041–52.

445. Signaling Downstream of Pitx2 is Required for Normal Development of the Optic Stalk. Amanda L. Evans and Philip J. Gage. Univ. of Michigan, Ann Arbor, MI 48105.

The *Pitx2* homeobox gene is expressed in neural crest and mesoderm during eye development. Global loss of *Pitx2* results in severe eye defects in the cornea, iris and extra-ocular muscles, which are chimeric and derived from both embryonic lineages. We generated mice with tissue-specific deletion of *Pitx2* in neural crest in order to understand the essential functions of this gene in this embryonic precursor pool. Eye primordia in resulting mutants develop normally up to day e10.5, when *Pitx2* is normally activated in neural crest. By e12.5, the optic stalk (OS) fails to extend, and the eyes are displaced towards the midline until they are ultimately attached directly to the ventral hypothalamus by day e14.5. This phenotype is not due to a failure in OS specification since *Pax2* is expressed normally, indicating that expression of *Pax2* is not sufficient for morphogenetic extension of the OS and *Pitx2*-dependent signaling from the neural crest is required. We plan to identify the signal(s) downstream of *Pitx2*. These findings suggest a stepwise model for OS development in which ventral hypothalamic Shh specifies the OS through activation of *Pax2*, and *Pitx2* activates signals that induce OS extension. The shortened OS in these mice also exposes the optic cup to higher levels of midline Shh, leading to an expansion of *Pax2*, and a parallel decrease in pigment and RPE markers in the proximal optic cup. Specific derivatives of the ocular neural crest are also affected in the mutant animals. These findings indicate *Pitx2* expression in the ocular mesenchyme plays a novel role in OS development by activating signaling that patterns the neuroepithelium. Funding provided by: EY014126.

446. Identification of Gli Target Genes Using Chromatin Immunoprecipitation with a Genetically Inducible System on Genomic Arrays. Steven A. Vokes,[1] Hongkai Ji,[2] Wing H. Wong,[2] and Andrew P. McMahon[1]. [1]Harvard University, Cambridge, MA 02138; [2]Stanford University, Palo Alto, CA 94305.

The hedgehog (Hh) signaling pathway performs multiple essential roles in patterning the embryo. These seemingly diverse signals are mediated by the Gli transcription factors (Gli1-3), but little is known about which targets are directly activated or repressed by Gli signaling. This knowledge is essential for a deeper understanding of how Hh signals are translated into cellular outputs in different embryonic regions. We have utilized chromatin immunoprecipitation (ChIP) in an effort to determine the direct targets of Hh signaling. In our approach we generated ES cells and mice containing inducible, epitope-tagged Gli proteins in forms that act either as a constitutive transcriptional activator or as a repressor. Then, using ChIP on embryoid bodies containing induced or uninduced (control) Gli proteins, we have been able to obtain enriched samples of genes known to be directly targeted by Hh signaling. These samples have also been used to probe custom genomic arrays containing approximately 30 kilobases of tiled sequence flanking the transcriptional start site of approximately 250 genes that were previously identified as Hh responsive. In addition to identifying control Gli binding sites, we identify additional targets that represent putative Gli enhancer sites. Furthermore, these arrays allow us to narrow the region of the Gli binding site to approximately 500 base pairs. Future experiments are aimed at characterizing these enhancer sites and at applying this technique in vivo to identify Gli targets in the developing limb bud.

447. Multiple Roles of Gli3 in Mouse Inner Ear Patterning. Jinwoong Bok and Doris K. Wu. National Institute on Deafness and Other Communication Disorders, Rockville, MD 20850.

Previously, we have shown that Sonic Hedgehog (Shh) from the notochord and floor plate is required for inner ear patterning in both mouse and chicken. To address the mechanisms whereby Shh mediates its effect on otic epithelial cells, we investigated the role of Gli3, a potential mediator of Shh signaling in inner ear development. Gli3 is a zinc finger transcriptional regulator that can act either as an activator or repressor in other systems depending on the level of Shh signaling. Inner ears of *Gli3−/−* mutants lack the lateral semicircular canal, and the anterior canal is often missing or truncated. However, all ventral inner ear structures are apparently normal, and various sensory patches are present including the lateral crista, the sensory organ for the lateral canal. In contrast, inner ears from *Shh−/−* mutants show a complete absence of ventral structures such as the saccule and cochlear duct. In addition, the lateral canal and its sensory organ the lateral crista are also absent in the *Shh−/−* mutants. The absence of ventral structures in *Shh−/−* mutants are partially rescued in *Shh−/−;Gli3−/−* double mutants, suggesting that Gli3 normally acts as a repressor in that region of the inner ear. Dorsally, canal and crista defects observed in *Shh−/−* mutants are rescued in *Shh−/−;Gli3+/−* mutants, suggesting a normal repressor function of Gli3 in canal formation as well. However, the inner ears of *Shh−/−;Gli3−/−* double mutants display anterior and lateral canal defects similar to the *Gli3−/−* single mutants. These results suggest that there is an additional requirement for *Gli3* function in canal formation that is independent of *Shh*.

448. A Requirement for Gbx1 in Normal Locomotion. Samuel T. Waters, Catherine Wilson, and Mark Lewandoski. Cancer and Developmental Biology Laboratory, National Cancer Institute-Frederick, National Institute of Health, Frederick, MD 21702.

Recent investigations aimed at establishing the mammalian neural mechanisms that control the basic rhythm of stepping during locomotion have focused on development of the embryonic spinal cord. We have recently

reported the complete mouse *Gbx1* coding region and expression analysis of *Gbx1* during murine development. In the developing central nervous system *Gbx1* is expressed in a dynamic pattern, that includes the forebrain medial ganglionic eminence, and regions of the ventricular zone of the neural tube. To investigate the functional role of *Gbx1* in development of the CNS and potential genetic redundancy with its sole family member, *Gbx2*, we targeted the *Gbx1* locus using the allelogenic strategy. With this genetic strategy, both Cre- and Flp-mediated DNA recombination are used to generate a series of alleles including a null allele, a potential hypomorphic allele and a conditional allele, which is suitable to study *Gbx1* in a tissue-specific manner. Our analysis demonstrates that *Gbx1* null homozygotes are viable, unlike mice that carry two *Gbx2* null alleles. Interestingly, *Gbx1* null homozygotes display a severe locomotor defect that specifically affects hindlimb motion. We have begun molecular and genetic analyses using in situ hybridization and tissue-specific inactivation of *Gbx1* (via the Cre/loxP system). Our initial tissue-specific gene inactivation studies suggest that *Gbx1* is required in the caudal neural tube for normal locomotion.

449. A Role for the vHNF1 Homeodomain Protein in Establishing Early Rhombomere Identity by Direct Regulation of Kreisler Expression. Sabine P. Cordes,[1] Florence A. Kim,[1] Angela Sing,[2] Tomomi Kaneko,[1] Melissa Biemann,[1] Nicole Stallwood,[1] and Virginia S. Sadl[1]. [1]Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, ON, Canada M5G 1x5; [2]Department of Medical Genetics and Microbiology, University of Toronto, Toronto, ON, Canada M5G 1X5.

The early transcriptional hierarchy that subdivides the vertebrate hindbrain into seven to eight segments, the rhombomeres, is largely unknown. The Kreisler (MafB, Krml1, Val) gene is required for development of the fifth and sixth rhombomeres (r5, r6). We have identified a regulatory element that directs early Kreisler expression in the r5/r6 domain. A binding site for variant hepatocyte nuclear factor 1 (vHNF1/HNF1beta/LF-3B) within this element is essential but not sufficient for r5/r6-specific expression. This site and some of the surrounding sequences are evolutionarily conserved in the genomic DNA upstream of the Kreisler gene among species as divergent as mouse, humans, and chickens. This provides the first evidence of a direct requirement for vHNF1 in initiation of Kreisler expression, suggests that the role of vHNF1 is conserved across species, and indicates that vHNF1 collaborates with other transcription factors to establish.

450. Role of the Fgf and Wnt Genes in Forebrain Patterning. Ugo Borello and John L. Rubenstein. University of California San Francisco, CA 94158.

What are the genetic and developmental processes that produce the diversity of cortical regions of the forebrain? Recently, several experiments provided evidence that intrinsic genetic mechanisms have fundamental roles in regulating regionalization of the cerebral cortex. Cortical regionalization appears, in fact, to be regulated in part by gradients of homeobox gene expression in cortical progenitor cells. However, little is known about what regulates the expression of these transcription factors. It was postulated that there are dorsal, rostral and ventral patterning centers. Midline organizing centers, expressing Fgf and Wnt genes, have important roles in regulating brain development. My hypothesis is that the cross-regulation between Fgf and Wnt genes have a central role in controlling prosencephalic growth and regionalization. It was demonstrated that Fgf8, expressed in the rostral midline, is a key factor in the patterning of the rostral forebrain. My data show that Fgf8 regulates the expression of the other Fgfs, Fgf15 and Fgf17, expressed in the rostral midline. On the other hand, Fgf8 mutants show overexpression of the Wnt genes in the dorsal midline. In addition, my preliminary data suggest that Fgf15 is an important factor in the regulation of the crosstalk between Fgf8 and Wnt in forebrain patterning.

451. Genetic Dissection of the Role of *En2* During Cerebellum Development. Yulan Cheng, Sema K. Sgaier, Gina Rocco, Melissa Villanueva, Frada Berenshteyn, and Alexandra L. Joyner. HHMI and Skirball Institute, Dept. of Cell Biology, NYUMC.

The molecular mechanisms underlying cerebellum morphogenesis are largely unknown. The *Engrailed 2* (*En2*) homeobox gene, the expression patterns of which partially overlap with *En1* in space and time, is expressed before the onset of the cerebellar anlage within its presumptive territory and accompanying cerebellar development with a dynamic expression pattern. Loss of En2 function results in cerebellar size reduction and altered foliation. In order to resume or remove En2 function at different time points, we made two conditional *En2* alleles, one that is silenced at the locus until Cre is expressed and the other a floxed allele. To address when foliation and the size of cerebellum are regulated, and whether these processes are inter-dependent, the *En* alleles were bred with an inducible Cre ($Rosa26^{CreERT2}$) line. Activation of En2 function during formation of the cerebellar primordia (E11–E12) fully rescued the foliation defects and the major size reduction, suggesting that before ~E11 *En2* is not required for foliation or size regulation. Inactivation of En2 after ~E11 resulted in a null mutant phenotype, arguing that *En2* plays a separable role from *En1* at later stages. Partial activation or ablation of En2 function due to less efficient recombination generated intermediate phenotypes, with a general correlation between the size reduction and extent of the foliation defects, showing that the percentage of cells expressing *En2* is also critical. Alteration of En2 function at later stages and within critical cell types using different CreER lines will be performed to further address the issues.

452. The Role of the Roof Plate in Cell Fate Specification and Dorsal CNS Development. Tanya E. Borsuk[1] and James H. Millonig[2]. [1]Graduate School of Biomedical Sciences-UMDNJ; [2]University of Medicine and Dentistry of NJ-RWJMS; [3]CABM, NJ 08854.

Dorsal CNS development is coordinated by a transitory signaling center called the roof plate, which secretes Bmps and Wnts. We have previously shown that the roof plate and all roof plate-specific Bmps are absent in the spontaneous neurological mouse mutant *dreher* (*dr*). Dorsal midline Wnt expression remains unperturbed. This is due to mutations in the Lim homeobox gene Lmx1a, which is expressed only in the roof plate. Thus, the *dr* mutation is an important in vivo system for studying roof plate formation and function. In this study we use the *dr* mutant to further elucidate the in vivo role of the roof plate and roof plate-specific Bmps during dorsal spinal cord development. We have demonstrated that a subset of dorsal progenitor and post-mitotic populations are mis-specified to a more ventral fate in E10.5 *dr/dr* embryos. A more global spinal cord defect was observed in E12.5 *dr/dr* embryos. An increase in the number of dorsal ventricular cells is observed at the expense of dorsal post-mitotic cells. This increase in the number of dorsal ventricular zone cells is due in part to an increase in the number of replicating dorsal progenitors. Together these results suggest that cell cycle exit is disrupted in the *dreher* mouse. These data provide further proof that the roof plate is instrumental for normal dorsal spinal cord development and identifies a new possible role for the roof plate in coordinating cell cycle exit with cell fate specification.

453. Genome-Based Approach Reveals Transcriptional Hierarchy Regulated by Hoxb1 in the Developing Central Nervous System. Gary O. Gaufo,[1] Benjamin R. Arenkiel,[2] and Mario R. Capecchi[3]. [1]Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249; [2]Department of Neurobiology, Duke University Medical Center, Durham, NC 27710; [3]Department of Human Genetics and Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT 84112.

The Hox genes encode a family of homeodomain-containing transcription factors necessary for generating the unique segmental identities of organs and tissues along the major axes of the vertebrate body. How the Hox genes orchestrate this highly conserved phenomenon remains unclear. To reveal the molecular network(s) influencing this process, a genome-based approach was undertaken in the mouse. Using the hindbrain as a model, we compared rhombomere 4 (r4) mRNA expression profiles between control and Hoxb1-mutant embryos. While this analysis confirms a previously described transcriptional cascade governing facial motoneuron specification, it also reveals a novel, non-colinear regulation of Hoxa3 in the differentiation of gustatory sensory interneurons. Furthermore, the loss of Hoxa3 expression in r4 of Hoxb1 mutant embryos, observed through a Hoxa3YFP reporter, suggests a cross regulatory mechanism inherent amongst Hox genes within a given segment. Together, these findings expose a complex genetic hierarchy integrating both Hox and non-Hox encoded homeodomain-containing transcription factors that ultimately contribute to the signature of a unique segment and neuronal identities in the vertebrate hindbrain.

454. Changing the Functional Specificity of Hoxa1 in Vivo Results in Multiple Homeotic Transformations in Mouse. Nathalie Wéry,[1] Sophie Remacle,[1] Olivier De Backer,[2] Nathalie Pacico,[1] Jacques J. Picard,[1] and René Rezsohazy[1]. [1]UCL, Louvain-la-Neuve 1348, Belgium; [2]FUNDP, Namur 5000, Belgium.

While the role of Hoxa1 in development has been largely investigated, the mode of action of this protein remains poorly understood. The specificity of action of Hox proteins can be related to their expression pattern, the presence of cofactors or the regulation of their activity but also to intrinsic properties of their homeodomain. While the amino acid residues involved in protein-DNA contacts are extensively conserved among Hox proteins, Hoxa1 shows atypical asparagine (N) and alanine (A) at positions 2 and 3 of its homeodomain. In order to determine the importance of these residues in the specificity of action of Hoxa1, we generated a recombinant mouse in which they were substituted by the canonical lysine (K) and arginine (R). This resulted in a phenotype overlapping with homeotic transformations obtained upon the inactivation of distinct other Hox proteins including Hoxa4, Hoxd4 or Hoxb7. This suggests that the mutant Hoxa1 acts as a dominant-negative against those homologues. The interference exerted by the Hoxa1 (NA-KR) mutant on the Hox code might result from the induced misregulation of Hox genes or from misregulation of some of their targets. These distinct hypotheses are currently under investigation. In situ hybridization will allow to examine to what extent Hox genes expression are disturbed in mutant mice. In parallel, competitions between Hoxa1 (NA-KR) and Hoxa4, -d4 and -b7 will be assayed in vitro to evaluate a possible interference between the mutant Hoxa1 and other proteins for their ability to activate target genes.

455. *Six2* is a Downstream Target of Hoxa2 in the Second Branchial Arch. Eva Kutejova, Bettina Engist, and Nicoletta Bobola. Max-Planck-Institute of Immunobiology, 79108 Freiburg, Germany.

Genetic analyses in flies and vertebrates have identified *Hox* genes as key developmental regulators required to specify segmental identity in the developing embryo. In mouse, disruption of the *Hoxa2* gene affects development of the second branchial arch, its major domain of expression. The skeletal elements that in the wild-type form in the second arch are lost and replaced by first branchial arch-specific elements, arranged in a mirror image disposition. Despite the clear phenotype observed in the *Hoxa2* mutant, the molecular mechanisms underlying Hoxa2 action remain still unclear. Using a genomic approach to compare the mRNA content of wild-type and $Hoxa2^{-/-}$ second branchial arches, we identified *Six2* as a gene upregulated in the mutant second arch. Gain of function of *Six2* in the second arch (using a *Hoxa2* enhancer) results in increased cartilage formation, mimicking the $Hoxa2^{-/-}$ phenotype. We identified a fragment of *Six2* promoter that retains Hoxa2-dependent regulation in vivo and is specifically recognized by Hoxa2 in vitro. These data, together with the observation that Hoxa2 is sufficient to repress *Six2* endogenous expression in the craniofacial mesenchyme in vivo, strongly suggest that the regulation of Six2 might represent one of the mechanisms employed by *Hoxa2* (and possibly other *Hox* genes) to pattern the developing embryo. Further experiments are being conducted in order to precisely characterize the contribution of *Six2* to the $Hoxa2^{-/-}$ phenotype.

456. Correlating HOXA13's DNA Binding Specificity with Loss of Function Phenotypes. Wendy M. Knosp,[2] Hans Peter Bächinger,[1,3] and H. Scott Stadler[1,2]. [1]Shriners Hospital for Children Research Division, Portland, Oregon 97239; [2]Department of Molecular and Medical Genetics, OHSU, Portland, Oregon 97239; [3]Department of Biochemistry and Molecular Biology, OHSU, Portland, Oregon 97239.

The homeobox (*Hox*) genes encode a highly conserved family of transcription factors involved in patterning the developing embryo. In humans and mice, a loss of HOXA13 function causes defects in the embryonic growth and patterning of the limbs, genital tubercle, and umbilical arteries, presumably through the loss of target gene transcriptional regulation. To date few target genes of HOXA13 have been identified, however using a combination of biochemical, genetic and developmental approaches, we have identified several direct transcriptional targets of HOXA13 including *Bmp2* and *Bmp7*. Analysis of *Bmp2* and *Bmp7* genomic DNA sequences identified multiple HOXA13 DNA-binding sites. Sequence analysis of these HOXA13 DNA-binding sites revealed a canonical "TAAT" core motif. Interestingly the nucleotides flanking this core varied greatly from sequences bound by other HOX proteins, suggesting that HOXA13 binds unique DNA sequences to facilitate its tissue-specific regulation of gene expression. A biochemical survey of the DNA sequences revealed several DNA-binding motifs that were bound by HOXA13 with high affinity. Interestingly, HOXD13, the closest paralog of HOXA13, did not exhibit high affinity for these same sites, suggesting the sequences identified by this analysis may be unique motifs bound by HOXA13. These analyses have exciting implications in broadening our understanding of how HOX proteins direct the formation of complex three-dimensional structures.

457. Hox11 Paralogous Genes Interact with the Pax-Eya-Six Regulatory Network in Kidney Development. Deneen Wellik, Nancy Gong, Gregory R. Dressler, and Sabita Rakshit. University of Michigan Medical Center.

Hox genes are fundamental regulators of the vertebrate body plan and have been shown to control major aspects of patterning and organogenesis. Functional redundancy has been retained among paralogous Hox genes and often their basic roles have only been revealed by eliminating the function of all paralogous members. We previously demonstrated that by removing Hox11 paralogous gene function, kidney initiation was blocked. In triple mutant embryos, the ureteric bud is not induced and the metanephric mesenchyme undergoes apoptosis. In the early metanephric mesenchyme, Pax2 Wt1 and Eya1 are expressed normally but there is a specific loss of Six2 and Gdnf expression. Further work has demonstrated genetic interaction between the Hox11 paralogs and Eya1. Further, Pax2, Eya1 and the Hox11 proteins synergistically activate Six2 in vitro. Together, this work suggests that the Hox11 paralogous genes interact with the conserved Pax-Eya-Six regulatory network to control mammalian kidney development.

458. Osr1 is Necessary for Metanephric Kidney Formation. Caramai N. Kamei,[1] Richard G. James,[1] Qingru Wang,[2] Rulang Jiang,[2] and Thomas M. Schultheiss[1]. [1]Molecular Medicine Unit, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215; [2]Center for Oral Biology, Department of Biomedical Genetics, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Odd-skipped related 1 (Osr1) is a vertebrate homolog to the *Drosophila* odd-skipped family of genes. Osr1 is the earliest known marker of intermediate mesoderm (IM), which gives rise to the aorta, gonads, definitive blood and kidneys. At early stages of development Osr1 is expressed throughout the IM and medial edges of the lateral plate but is later confined to undifferentiated mesenchymal cells. It is also down-regulated as kidney-specific markers such as pax2 are simultaneously up-regulated. Mice lacking Osr1 die at about E11.5 and exhibit a partial defect in nephric duct formation and complete absence of metanephric mesenchyme, characterized by loss of markers such as pax2 and six2, with an accompanying increase in apoptosis as evidenced by increased TUNEL staining. This leads to the hypothesis that Osr1 is involved in patterning of the kidney and possibly maintenance of a population of undifferentiated, proliferating cells that will contribute to the formation of developing kidney structures.

459. Role of the *Drosophila melanogaster* Gene *bubblegum* in Nervous System Patterning and Embryogenesis. Anna J. Sivatchenko, Aloisia Schmid, and Anthea Letsou. Department of Human Genetics, University of Utah, Salt Lake City, UT 84112.

The *Drosophila melanogaster* gene *bubblegum* (*bgm*) codes for a very-long-chain fatty-acid (VLCFA) acyl-CoA synthetase that is required for degradation of VLCFAs. It has been previously shown that mutation of *bgm* leads to neurodegeneration, characterized by a bubbly appearance of the optic lobe, a reduced life span, and visual impairment (Min, K. T. & Benzer, S. 1999. *Science* 284, 1985–8). Our analysis of the *bgm* spatial expression profile in *Drosophila* embryos reveals it to be ventrally restricted: the transcript is evident in presumptive mesoderm in the early embryo and in the developing nervous system during later embryonic stages. A computational screen for regulatory elements in the *bubblegum* promoter revealed consensus binding sites for the Twist transcription factor, which is essential for specification of ventral cell fates during dorsoventral patterning in *Drosophila* embryos. We are currently testing the hypothesis that the *bubblegum* gene product, which has a defined enzymatic function, is a biological effector of ventral fate determination and nervous system development in *Drosophila*. Data acquired in this study are expected to provide significant insight both into the role of fatty acyl CoA synthetases in embryogenesis and into the pathogenesis of neurodegeneration in *Drosophila melanogaster*.

460. *Zed* is Required for Anterior Neural Tube Closure and Development of Branchial Arches in the Mouse Embryo. Joshua E. Bloomekatz and Kathryn V. Anderson. [1]Sloan Kettering Institute, 1275 York Avenue, New York, NY 10021; [2]Weill Graduate School of Medical Sciences, Cornell University, 445 69th St. New York, NY 10021.

To identify novel genes that direct embryogenesis during mouse development we have undertaken an ENU mutagenesis screen for recessive mutations. Here we describe the characterization of *Zeushead* (*Zed*), a mutant identified in the screen. *Zed* mutants are first morphologically distinguishable at E9.0 by the presence of an open anterior neural tube and smaller body size. At E9.5 defects are also apparent in the formation of the 2nd and 3rd branchial arches, heart looping and the curvature of the body axis. Mutants that survive to E10.5 show severe morphological defects including improperly shaped somites. The position and timing of the morphological defects in the *Zed* mutants are strikingly similar to defects in the *Bmp5*, *7* double mutants. For example, in *Zed* mutants the neural tube is open from the anterior forebrain through the hindbrain, which corresponds to the position of the open neural tube in *Bmp5*, *7* double mutants (Solloway, M et al., 1999). Further, both *Bmp5*, *7* and *Zed* mutants display reduced expression of AP-2 in the neural tube at E9.5, while other expression domains of AP-2 are normal. These similarities suggest that the *Zed* mutation could be involved in establishing specificity within the Bmp pathway for the 60A subgroup. Analysis of the phosphorylation state of the Bmp-specific Smad genes in *Zed* mutants as well as the expression of downstream effectors of the Bmp pathway is in progress. The *Zed* mutation maps to a 10-cM region on chr. 2. Work towards the positional cloning of this mutation is in progress.

461. Noggin Plays Different Roles in Mammalian Neurulation, Depending on Axial Level. John Klingensmith, Mark Berrong, and Rolf Stottmann. Duke University Medical Center, Durham, NC.

Despite the clinical significance of neural tube defects, the molecular and genetic regulation of neurulation is poorly understood. Many lines of evidence suggest bone morphogenetic protein (BMP) signaling regulates neural tube closure. To further explore the role of BMP signaling in mouse neurulation, we have characterized the consequences of elevated BMP signaling on neural tube development, using mice lacking the BMP antagonist, Noggin. Noggin−/− mice are born with spina bifida and exencephaly (open brain). We find the exencephaly is due to a failure of midbrain neural plate bending at the dorso-lateral hinge points, likely due to increased activity of the Sonic hedgehog pathway. The spina bifida defect is the result of a different mechanism: increased BMP signaling in the mesoderm between the limb buds leads to a somite differentiation defect. This improper somite differentiation leads to axial skeletal defects and lack of mechanical support for the developing neural tube, resulting in spina bifida. We show genetically that this defect is due to elevated BMP4 signaling. Thus, BMP antagonism is required for mammalian neurulation in at least two contexts, dependent on position along the rostrocaudal axis.

462. Regulation of Zebrafish Brain and Visceral LeftRight Asymmetry by Brachyury. Michael R. Rebagliati and Nadira Ahmad. Univ. of Iowa, Iowa City, IA 52242

Zebrafish notail (*ntl*) mutations are lesions in the *brachyury* (*bra*) gene. *ntl* mutants show severe disruptions in visceral and early diencephalic left–right (LR) asymmetry. The nodal-related gene *southpaw* (*spaw*) is normally expressed on the left side, and this expression is required for both brain and visceral organ LR asymmetry (1). In *ntl* mutants, the asymmetry of *spaw* expression is lost, leading to the aforementioned global defects in LR patterning. We have used *ntl* mutants and *ntl* morpholino dosage titration experiments to dissect the role of *ntl* in regulating the LR asymmetry of *spaw* transcripts. Partial inhibition of *ntl* does not disrupt Ntl functions in the notochord, but causes LR defects and down-regulation of a non-ciliary, LR regulatory factor in the tailbud. We summarize evidence suggesting this effect may be direct, i.e., reflecting the loss of transcriptional activation by Bra. Stronger inhibition of *ntl* causes the down-regulation of an additional LR factor within the posterior notochord. These effects are separate from any putative changes in ciliary-driven nodal flow and either lesion is sufficient to cause loss of *spaw* asymmetry. Finally, *ntl* mutations lead to a delay in the onset of perinodal spaw expression in the tailbud. This observation provides a way to reconcile why asymmetric *nodal* expression domains are affected in different ways in zebrafish and mouse *bra* mutants. We discuss the implications of these results for nodal flow models of LR patterning. (1) Long, S., Ahmad, N. and Rebagliati, M.R. (2003) Development 130: 2303–2316.

463. The Aristaless-Related Homeobox (Arx) Gene Product is Essential for Normal Forebrain Development. Heithem M. El-Hodiri and Lisa E. Kelly. Center for Molecular and Human Genetics, Columbus Children's Research Institute, Columbus, OH.

The aristaless-related homeobox (Arx) gene is expressed in the developing vertebrate rostral forebrain. *Xenopus* Arx expression initiates in the presumptive forebrain beginning at neural plate stages. In older embryos, it is expressed primarily in ventral and medial regions of the telencephalon. Human ARX is mutated in patients with some forms of inherited mental retardation. Some of these patients exhibit lissencephaly, suggesting an intact ARX gene is necessary for normal forebrain development. We have been characterizing the role of Arx in *Xenopus laevis* forebrain development. Overexpression of Arx results in development of a shortened forebrain that lacks rostral midline structures. We have shown that expression of many markers of forebrain development is absent in neurula embryos and abnormal in tailbud embryos. We have analyzed the effect of Arx overexpression on molecular markers in tadpole brains. We find that markers of telencephalic development are expressed, albeit in a disorganized and compressed pattern. Antisense morpholino-mediated knockdown of one of the two *Xenopus laevis* Arx gene products results in deficiencies in forebrain development. We observed both reductions and abnormalities in expression of telencephalic markers. These results suggest that partial loss of Arx function is sufficient to impair telencephalon development, confirming the important role Arx plays in normal forebrain development.

464. The *Alien* Gene is Required for Normal Forebrain Development in the Mouse. Rolf W. Stottmann, Pam Tran, Annick Turbe-Doan, and David R. Beier. Brigham and Women's Hospital, Boston, MA 02115.

The *alien* locus was previously identified in our laboratory as part of an ENU mutagenesis screen (Herron et al., 2002 *Nat. Genetics*). This screen was designed to identify recessive mutations causing organogenesis defects at embryonic day (E) 18 in the mouse. *Alien* mutant embryos have several severe defects including preaxial polydactyly, craniofacial abnormalities, as well as defects in the vertebrae and eye. A particularly striking defect is in the nervous system where we observe overgrowth of the forebrain and the neural tube. The forebrain overgrowth is visible as early as E11 and persists to birth. Histological analysis reveals a layering defect in the cerebral cortex detected by E15 and we are currently conducting analysis of *alien* forebrain dorsal–ventral patterning. To understand the developmental mechanism behind the *alien* phenotypes, we have used whole mount RNA in situ hybridization analysis to show dysregulation of the sonic hedgehog (*Shh*) pathway. The *Shh* ligand is expressed at elevated levels as early as E9. *Shh* target genes are similarly overexpressed at early stages of forebrain development. We have also documented a genetic interaction between the *alien* locus and the *Shh* pathway. The gene mutated in the *alien* mouse has been identified with its predicted structure suggesting a role in the formation of intramolecular complexes. Further biochemical analysis of the *alien* gene product is ongoing. The *alien* gene is a novel gene important in development of the forebrain which acts, at least in part, by modulating *Shh* activity.

465. Retinoic Acid is Unnecessary for Early Forebrain Development but Functions Later in the Striatum. Natalia Molotkova, Andrei Molotkov, and Gregg Duester. The Burnham Institute, La Jolla, CA 92037.

During vertebrate development the vitamin A metabolite retinoic acid (RA) functions as a cell–cell signaling molecule by binding to nuclear RA receptors that directly regulate transcription. RA is synthesized by three enzymes of the retinaldehyde dehydrogenase (RALDH) family expressed in unique spatiotemporal patterns. Genetic studies have implicated Raldh2 in hindbrain rhombomere formation and spinal cord motor neuron generation. Raldh1 and Raldh3 function in eye and nasal pit development. In mouse and chick, Raldh3 is initially expressed in the surface ectoderm adjacent to forebrain tissue that gives rise to the eye field, and then later in the retina. In mouse, but not chick, Raldh2 expression in the optic vesicles also contributes to early forebrain RA. Studies in chick using RA receptor antagonists have suggested that RA synthesized by Raldh3 is necessary for early forebrain/frontonasal expression of Shh and Fgf8, and later for Meis2 expression in the intermediate region of the telencephalon. However, in the mouse we find that Raldh2−/− and Raldh3−/− single and double mutants all retain normal expression of Shh, Fgf8, and Meis2 in the head. Furthermore, analysis of a lacZ RA-reporter transgene shows that the forebrain does not exhibit RA activity from E7.5 to E10.5, even in wild-type embryos. Instead we find that forebrain RA activity is first observed at E12.5–E13.5 in the lateral ganglionic eminence (future striatum) coinciding with initial forebrain expression of Raldh3. Our findings suggest that RA does not play a role in early forebrain development, but that it may play a later role in striatal neuron differentiation.

466. Generation of Neuronal Diversity in the Chick Tectum and the Development of Tectal-Hindbrain Projections. Natalia Fedtsova and Eric E. Turner. Univ. of California, San Diego, CA 92093; VA Medical Center, San Diego, CA 92122.

The mature chicken tectum consists of numerous cell types organized into a laminar structure. Previously we have demonstrated the heterogeneity of postmitotic tectal neurons which is characterized by the specific expression of homeodomain transcription factors of the POU, LIM and PAX families (Fedtsova and Turner, 2001). Here we show that in the adult chicken brain Pax7, Brn3a and LIM1/2 transcription factors continue to be expressed in multiple tectal layers, with distinct patterns of laminar distribution. In the stratum griseum centrale (layer 13) large "ganglion cells" express the POU-domain factor Brn3a, whereas Pax7 and Lim were found only in the small neurons. Neurons from layer 13 form major tectal projections to hypothalamus and hindbrain. We used staged electroporation of a construct constitutively expressing GFP to further investigate the development of these classes of neurons. Because only neurons that exit the cell cycle shortly after electroporation are strongly labeled by GFP, we were able to trace the distant projections of neurons which developed at specific stages. The majority of the large Brn3a-positive ganglion cells from layer 13 exit the cell cycle on days E3 and E4. We found that ipsilateral and contralateral hindbrain projections of tectum are formed predominantly by the neurons that exit cell cycle between days E3 and E4.

467. Induction of Functional Ectopic Eyes in a Vertebrate. Andrea S. Viczian, Eduardo Solessio, and Michael E. Zuber. SUNY Upstate Medical University, Syracuse, NY 13210.

Molecular and genetic evidence suggests that vertebrate eye organogenesis is driven by the coordinated action of eye field transcription factors (*ET*, *Rx*, *Pax6*, *Six3*, *Lhx2*, *tll* and *Optx2*). Following the specification of retinal stem/progenitor cells, extrinsic and intrinsic cues direct their differentiation into mature retinal cell types. Ectopic vertebrate eyes can be experimentally generated by either transplantation of eye primordia or by misexpression of gene products required for normal eye formation. Eyes induced by the ectopic expression of one or more gene product(s) have the tri-layered structure of a normal eye and express some retinal cell-specific markers. However, one obvious but crucial question remains—can these eyes see? We have induced ectopic vertebrate eyes by misexpressing eye field transcription factors. Induced eyes are molecularly and structurally identical to endogenous eyes. Using the electroretinogram (ERG), we show that induced eyes contain the functional retinal cell types and neural circuits required for sight. To our knowledge, this is the first evidence that induced

ectopic vertebrate eyes can detect and process a light stimulus. Our results also demonstrate that the combined expression of (at most) seven transcription factors is sufficient to form a vertebrate eye that is not only structurally, but functionally normal. These results suggest that it may be possible to redirect pluripotent, non-retinal embryonic tissue into retinal stem/progenitor cells. Current experiments are underway to test this hypothesis.

468. Six3 is a Key Regulator in Mammalian Eye Development. Wei Liu, Oleg V. Lagutin, and Guillermo Oliver. Dept. of Genetics, St. Jude Children's Research Hospital, 332 North Lauderdale Street, TN 38105.

In mouse embryos, the homeobox Six3 is initially expressed in the anterior neuroectoderm and developing retina field. Later on, its expression is observed in the developing lens, retina and optic stalk. Targeted inactivation of Six3 in mice results in a severely truncated prosencephalon, and the expression of Wnt1 is rostrally expanded indicating that the mutant head was posteriorized. To address the role of Six3 during visual system development, we have now generated a conditional Six3 knock-out mouse strain. This approach revealed that Six3 activity is also necessary for lens and retina development. We used an available Pax6 lens-Cre mouse line to study the role of Six3 during lens development. We determined that Six3 activity is efficiently deleted from the surface ectoderm as early as E9.5. Conditional removal of Six3 activity from the lens results in different degrees of lens malformations. These defects range from smaller but relatively normal lens, to some other ones in which the mutant lens is quite abnormal, degenerated, and maintaining a persistent connection between the lens and cornea. Despite these defects, lens differentiation is normal as indicated by the normal expression of β-crystallin, γ-crystallin. In the most severely affected cases, we found that lens were missing from the mutant eye. A detailed characterization of the mutant lens phenotype will be presented.

469. Distinct Developmental Programs that Require Different Levels of Bmp Signaling During Mouse Retinal Development. Deepa Murali,[1] Chuxia Deng,[2] and Yasuhide Furuta[1]. [1]Dept. of Biochemistry & Mol. Biol., Univ. of Texas MD Anderson Cancer Center and Program in Genes and Development, Graduate School Biomedical Sciences, Univ. of Texas Health Science Center, Houston, TX; [2]National Institute of Diabetes and Digestive and Kidney disease, NIH, Bethesda, MD.

We are interested in the extracellular signaling events that mediate communication between cells during retinal development. The bone morphogenetic proteins constitute secreted signaling ligands of the TGF-beta superfamily. In order to test the autonomous requirement of Bmp signaling within the embryonic mouse retina, we generated conditional mutants for *BmprIa* and *Bmpr1b* in the retina using a retina-specific cre/loxp system (Six3-Cre). These genes encode putative type I transmembrane receptors for Bmp ligands in the embryonic retina. Our studies reveal that a graded reduction in the available Bmp receptors affects different aspects of retinal development, including cell survival and dorso-ventral patterning. In attempting to further elucidate the mechanisms by which Bmp signal transduction controls diverse cellular events, we generated retina-specific conditional mutants for *Smad4*, a common downstream transcriptional mediator thought to be essential for all TGF-beta signaling. Intriguingly, these mutants display less severe phenotypes compared to the Bmp receptor compound mutants, with only a patterning defect similar to that seen in $BmprIa^{-/fx}$; $BmprIb^{-/+}$; *Six3-Cre* mice. Thus in the context of retinal development, Bmp type I receptors may require Smad4 only for a subset of downstream smad1/5/8-transcriptional events. Alternatively, they may regulate certain processes in a Smad-independent manner.

470. β-Catenin is Required for Pattern Formation but not Cell Differentiation in Mouse Retinal Development. Xueyao Fu, Hongxia Sun, William H. Klein, and Xiuqian Mu. The University of Texas, M.D. Anderson Cancer Center, Houston Texas, 77030.

β-Catenin functions as both a cell adhesion molecule and nuclear effecter for the canonical Wnt signaling pathway in various biological processes. However the role of β-catenin in vertebrate retinal development has yet to be determined. To address this question, we conditionally deleted β-catenin in the retina. Deletion of β-catenin led to reduced retina size. Histological analysis showed that in the β-catenin-null retina, the laminar structure was completely disrupted. Further analysis by in situ hybridization and immunofluorescence staining showed that all seven retinal cell types differentiated but they did not migrate to their correct position and therefore resulted in their abnormal localization in the mature retina. Immunofluorescence staining for cell adhesion molecules such as N-cadherin and actin showed that their localization in early retinal development was disrupted. BrdU labeling showed that cell proliferation is not affected in the β-catenin null retina although enhanced apoptosis is observed. These results indicated that β-catenin plays an important role in pattern formation in retinal development, and that patterning and cell differentiation are two independent processes. To further distinguish β-catenin's function in cell adhesion and Wnt signaling pathway, we conditionally overexpressed Axin to inhibit the canonical Wnt pathway, and a stabilized version of β-catenin to activate the Wnt pathway in the developing retina. These experiments indicated that the canonical Wnt pathway may not be involved in this process and β-catenin functions as cell adhesion molecule in the retinal development.

471. Wnt2b/beta-Catenin Signaling Regulates Peripheral Retinal Development. Seo-Hee Cho and Constance L. Cepko. HHMI, Harvard Medical School, Boston, MA 02115.

Wnt signaling regulates cell proliferation, cell fate decision, cell migration, axon guidance, and stem cell maintenance and renewal. Recently, it has been reported that Wnt2b promotes retinal cell proliferation in in-vitro reaggregation culture. The expression pattern of genes involved in Wnt2b signaling and retinal progenitor genes, combined with a reporter assay for Wnt activity, suggest that Wnt2b signaling may also control the development of the peripheral retina, where the ciliary body and iris are derived. In contrast to a previous report, introduction of constitutively active (CA)-β-catenin inhibited the proliferation of retinal progenitor cells in explants and in ovo. In addition, expression of Wnt2b and CA-β-catenin in the central retina inhibited the expression of retina-specific markers, such as Chx10 and Notch1, and interrupted the normal differentiation of retinal neurons. Instead, central retinal cells with ectopic Wnt signaling displayed ectopic expression of the peripheral markers CollagenIX and BMP7, indicating the adoption of a peripheral fate at the expense of the central retinal fate. Furthermore, expression of Wnt antagonist or dn-Lef1 or Lef1 RNAi constructs in the peripheral retina interfered with normal ciliary/iris epithelial development. These results suggest that Wnt2b/β-catenin signaling is necessary and sufficient for determination of the peripheral fate of the optic cup. This signal is antagonistic to the acquisition of retinal fate, including an inhibition of retinal cell proliferation. These results also reveal evolutionary conservation of Wnt/*wg* signaling between vertebrate and invertebrate eye development.

472. Pygopus2 is Required for Lens Induction in Mouse. Ni Song,[2] Larry T. Patterson,[1] Lucas McClain,[1] Kristopher Schwab,[2] Dong Yan,[2] Xinhua Lin,[1] S.S. Potter,[1] and Richard A. Lang[1]. [1]Division of Dev. Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; [2]College of Medicine, University of Cincinnati, Cincinnati, OH.

Pygopus has recently been identified as a new nuclear component of the canonical Wnt signaling pathway in *Drosophila*. In vertebrates, the role of Pygopus remains unknown. Interestingly, we find that mouse Pygopus2 is required for lens development. In Pygopus2−/− mice, we find pronounced lens development defects ranging from small lenses to no lenses. Furthermore, the essential lens development marker Pax6 is down regulated at E9.5 in the presumptive lens ectoderm in Pygopus2−/− mice. To further understand the role of Pygopus2 in mouse lens development, we will determine which Pygopus2-expressing cell type is involved and whether lens defects in Pygopus2 mice are caused by defects in Wnt pathway signaling. We will also perform molecular epistasis analysis to determine the genetic relationships between Pygopus2 and other genes of the lens induction pathway.

473. Wnt Dependent Regulation of Inner Ear Morphogenesis is Balanced by the Opposing and Supporting Roles of Shh. Douglas J. Epstein,[1] Shinji M. Takada,[2] and Martin M. Riccomagno[3]. [1]University of Pennsylvania School of Medicine, Philadelphia, PA 19104; [2]Okazaki Institute for Integrative Biosciences, Okazaki, 444 8787, Japan; [3]University of Buenos Aires School of Medicine, Buenos Aires, Argentina.

Organization of the inner ear into auditory and vestibular components is dependent on localized patterns of gene expression within the otic vesicle. Surrounding tissues are known to influence the dorsoventral polarity of the otic epithelium, yet until recently the participating signals remained unclear. We previously identified Sonic hedgehog (Shh) secreted by the notochord as a primary regulator of auditory cell fates within the mouse inner ear. However, the nature of the extrinsic signals responsible for dorsal otic development remained unclear. In this study, we demonstrate that Wnt signaling is active in dorsal regions of the otic vesicle where it functions to regulate the expression of Dlx5, Dlx6 and Gbx2, three genes with essential roles during vestibular morphogenesis. We further show that the source of Wnt impacting on dorsal otic development emanates from the dorsal hindbrain and identify Wnt1 and Wnt3a as the specific ligands required for this function. Interestingly, the restriction of Wnt target genes to the dorsal otocyst is also influenced by Shh as the expression of Dlx5/6 and a Wnt responsive reporter (Topgal) are expanded ventrally in Shh mutant embryos. Thus, a balance between Wnt and Shh signaling activities is key in distinguishing between vestibular and auditory cell types (this work is supported by a grant from the NIH-NIDCD).

474. Genetic Specification and Neural Refinement of Vestibular Function in the Zebrafish Inner Ear. Bruce B. Riley,[1] Su-Jin Kwak,[1] Shruti Vemaraju,[1] and Stephen J. Moorman[2]. [1]Biology Department, Texas A&M University, College Station, TX 77843-3258; [2]Neuroscience and Cell Biology, Robert Wood Johnson Medical School, Piscataway, NJ 08845-5635.

The vertebrate inner ear is a morphologically complex structure containing 6–8 sensory patches, each of which transmits either vestibular (balance) or auditory signals. In tetrapods, functional specialization is tied to morphogenesis of surrounding nonsensory structures. However, in zebrafish the ear begins to function when still a simple ovoid vesicle containing only two sensory patches, one vestibular and one auditory. We show that mutations or manipulations that disrupt the rostral (utricular) macula permanently disable vestibular function and are invariably lethal. A promising candidate for a vestibular specifier is Pax5, which is induced in the rostral end of the ear by Fgf3 from adjacent hindbrain tissue. Pax5 marks cells in the utricular macula and adjacent neuroblasts before they delaminate to join the statoacoustic ganglion (SAG). Knockdown of Pax5 does not detectably alter morphogenesis or differentiation of the utricular macula or SAG, yet vestibular function is lost. SAG axons project normally into the hindbrain and inner ear but do not synapse properly with utricular hair cells. These axons subsequently fail to undergo activity-dependent remodeling in the hindbrain, mimicking the effects of other conditions that disrupt vestibular function. Thus, Pax5 could coordinate the identities and activities of the utricular macula and SAG neurons that innervate it.

475. BMP Signaling in Sympathetic Preganglionic Neuron Development. Ed Laufer, Daniel Vasiliauskas, and Josh Walonoski. Columbia University, New York, NY 10032.

Sympathetic preganglionic neurons (SPNs) are autonomic motor neurons located in thoracic spinal cord. They extend axons peripherally and synapse with sympathetic ganglionic neurons. Functionally, SPNs provide the primary CNS outflow to the peripheral sympathetic nervous system. Developmentally, SPNs and somatic motor neurons are derived from a common ventral progenitor pool. Nuclear phosphoSmad1/5/8 (PS1) marks cells transducing BMP family signals. We have identified a unique nuclear PS1+ postmitotic population in developing spinal cords. Developmental and lineage tracing markers identify these as SPNs, raising the possibility that BMP signals regulate postmitotic SPN development. Loss of BmprIa function in conditional mouse mutants or by ectopic exposure to BMP antagonists in the chick both lead to loss of PS1 immunoreactivity. SPN markers are also lost from conditional BmprIa mice. However lineage tracing and retrograde labeling show that SPN cell bodies are present in appropriate numbers, although there are settling defects. Thus a secreted BMP family signal is required for normal SPN differentiation, but not specification of SPN identity. We have explored the source of the signal using mouse mutants, chick explant cultures and gene misexpression. We find that peripheral targets and intact SPN axons are required for signal transduction, and paradoxically that required BMP ligands are also generated by spinal cord populations. These and other experiments suggest a complex combination of local and peripheral signaling components. Together our data define a novel postmitotic function for BMP signals in the development of a specific spinal cord neuron population.

476. The Role of Skeletal Muscle-Secreted Neurotrophic Factors in the Survival of Motor Neurons. Anne C. Belliveau and Boris Kablar. Dalhousie University, Halifax, NS, Canada B3H 4H7.

To determine what combination of skeletal-muscle secreted neurotrophic factors were relevant for the survival of precise subpopulations of developing spinal cord motor neurons, we employed mouse embryos containing differentially committed myogenic precursor cells (MPSc) and immunohistochemistry against several muscle-expressed neurotrophic factors and their receptors. At the peak of motor neuron cell death, skeletal muscle development is delayed in the back and body wall muscles of *Myf5*−/− embryos and in the limb muscles of *MyoD*−/− embryos. We hypothesized that, if the skeletal muscle was indeed an important source of survival factors for motor neurons, the back, the abdominal wall and the forelimb MPCs of *Myf5*−/− or *MyoD*−/− embryos should produce at least some neurotrophic factors necessary for the survival of motor neurons. We found that different subpopulations of MPCs express different combinations of neurotrophic factors required for the survival of the precise subpopulations of innervating motor neurons. This work is supported by the CIHR and CFI grants to BK.

477. Noggin Regulation of BMP Signaling During Pituitary Development. Shannon W. Davis and Sally A. Camper. University of Michigan, Ann Arbor, MI 48109-0638.

Bone morphogenetic protein (BMP) signaling is critical for the development and patterning of the pituitary. BMP4 expression in the ventral diencephalon is necessary for the early proliferation and growth of Rathke's Pouch. Later expression of BMP2 in the ventral domain of

Rathke's pouch and the adjacent mesenchyme is thought to establish a gradient of BMP that counteracts FGF signaling from the dorsal infundibulum. The ventral to dorsal BMP gradient in conjunction with the dorsal to ventral FGF gradient may establish the five specific cell types of the anterior pituitary in a spatial and temporal manner. BMP antagonists might help to establish this BMP gradient; therefore, our initial experiments have focused on determining the expression pattern of known BMP antagonists within and surrounding the pituitary. *Noggin*, a known BMP 2/4 antagonist, is expressed in the ventral diencephalon during Rathke's pouch induction and then in the underlying cartilage plate during cell specification. *Noggin* null embryos have a variable pituitary phenotype, which ranges from a developmentally delayed Rathke's pouch to an induction of an apparent secondary pituitary. Cell specification in *noggin* null embryos appears normal; therefore, the affect of Noggin may be to regulate BMP4 during the induction of Rathke's Pouch. Our working model is that in the absence of *noggin* increased BMP4 signaling results in an expanded Rathke's Pouch, including additional invaginations that could form a secondary pituitary. Future experiments will investigate this possible mechanism for Noggin activity during pituitary development.

478. Dpp Utilizes Two Distinct Mechanisms to Specify Nonoverlapping Subsets of Pericardial Cells in the *Drosophila* heart. Aaron N. Johnson and Stuart J. Newfeld. Arizona State University, Tempe, AZ 85287.

Early in embryogenesis, Dpp signals from the dorsal ectoderm to the mesoderm specify the heart precursor cells. These precursor cells will differentiate into two cell types: myocardial cells that form the linear heart tube and pericardial cells that surround the heart. A 3rd round of Dpp signaling from the dorsal ectoderm occurs later development and restricts the expression of the pericardial cell marker Zfh1. We will show that this round of Dpp signaling also restricts the number of cells comprising two distinct subsets of pericardial cells: one cell type expressing Odd-skipped and a non-overlapping cell type expressing Tinman. First, our analysis of the relationship among Dpp, Zfh1 and Odd identified a linear pathway in which Dpp signaling via Zfh1 maintains the correct number of Odd-expressing pericardial cells. Second, we will show that overexpressing an activated form of the Dpp receptor Tkv solely in pericardial cells, but not in cardial cells, reduces the number of Tinman-expressing cells. This result, in addition to our Dpp loss of function data, demonstrates the 3rd round of Dpp signaling specifically restricts the number of Tinman-expressing pericardial cells. Third, we found that Dpp regulation of Tinman-expressing pericardial cells is Zfh1 independent and may involve direct regulation of *tinman* expression. Previous studies showed that Dpp signals early in embryogenesis positively regulate Tinman expression in all heart precursor cells. In contrast, this study shows that later in development Dpp signals restrict the number of Tinman-expressing and Odd-expressing pericardial cells via distinct mechanisms.

479. Knockdown of Plakoglobin in Zebrafish Development. Eva D. Martin and Maura Grealy. Pharmacology Department and National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland.

Plakoglobin, or γ-catenin, is a cytoplasmic cadherin-associated cell adhesion molecule which also has a signalling role. We have previously mapped the expression of plakoglobin in normal zebrafish embryo development. Knockdown of the zebrafish plakoglobin protein was carried out using a morpholino antisense oligonucleotide. Plakoglobin morpholino, at one of three doses, or a 5-base-pair mismatch control morpholino was injected into zebrafish embryos at the 1-cell stage. Uninjected embryos and phenol red injected embryos were included for analysis. Embryos were examined morphologically at 24, 48, and 72 hpf and scored into four categories, unaffected, weak, intermediate, and severely affected. At 24 hpf, plakoglobin morpholino-injected embryos had a delay in border formation such as the midbrain–hindbrain boundary and at somite borders. At 48 hpf and 72 hpf morpholino-injected embryos showed a reduction in heart size and in blood circulation, pericardial edema, blood pooling, and a kinked tail. All doses were statistically different to control morpholino-injected embryos ($P = 0.01$). The severity of effect was similar across all three doses at 24 hpf. By 48 hpf, the 2.5-ng injected embryos had recovered whereas the 5 ng had only partially recovered and the 7.5-ng injected embryos did not show any recovery. At 72 hpf, 2.5-ng and 5-ng injected embryos did not show any further recovery but a partial recovery was seen in 7.5-ng injected embryos. These data indicate the importance of plakoglobin in heart and boundary formation. Funding: EMBARK from IRCSET, NCBES, and Millennium Fund NUI, Galway, Ireland.

480. Genetic and Molecular Analysis of Heart Valve Formation in Zebrafish. Thomas Bartman and Tyler Martin. Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229.

Our lab seeks to identify the genetic and epigenetic factors which regulate development of the atrio-ventricular (AV) heart valves. The zebrafish has proven to be informative for studying early steps of heart development but has been underutilized for studying late steps such as valve development. The desire to use zebrafish to study the effects of genes and pharmacologic agents on valve development is evident from a recent surge in publications, yet our basic understanding of how and when valves develop in zebrafish is lacking. As a foundation for further study, we performed detailed histological analysis of valve development in the fish and find that endocardial cushions form at 4 days post-fertilization (dpf) and mature valves later than 7 dpf at the earliest, well beyond the experimental timing of all published studies to date. This indicates that pericardial edema or regurgitation of blood from ventricle to atrium prior to 96 hpf cannot be due to structural cushion/valve defects. The composition of zebrafish AV valves is unique from other species—by 28 dpf there is no detectable collagen or elastin in the AV valves, although collagen is abundant in the outflow tract. However, at this stage the valves appear to be highly cellular and may contain significant amounts of muscle fiber. New data will be presented which aims to identify the provenance of the valvular cells and the molecular composition of the valves. Other research presented will include progress on positional cloning of a mutation which leads to AV regurgitation of blood at 48 hpf, which implies a defect in early AV boundary myocardial function or endocardial specification.

481. A Novel Component in Signaling by a Sphingosine 1-Phosphate Receptor. Nick Osborne,[1] Nathalia S. Glickman,[2] Jonathon Alexander,[1] Deborah Yelon,[2] and Didier Y. Stainier[1]. [1]UCSF; [2]NYU.

The phospholipid sphingosine 1-phosphate (S1P) is a key regulator of both developmental and physiological processes. In zebrafish, S1P regulates the morphogenesis of the heart, via its G-protein-coupled receptor (GPCR) Miles Apart (Mil). In *miles apart* (*mil*) mutant embryos, the precardiac mesoderm (PCM) fails to migrate to the embryonic midline, resulting in formation of bilateral heart structures. *mil* mutants also display epithelial blistering in their tails. These phenotypes are shared by the zebrafish mutant, *two of hearts* (*toh*). *mil* and *toh* both function in the morphogenesis of anterior endoderm, the migratory substrate for PCM. In wild-type embryos, injection of *mil* RNA disrupts gastrulation movements, while *toh* mutant embryos show no such effects. This rescue suggests *toh* is required for signaling via Mil. *toh* mutations do not globally affect GPCR signaling, as processes controlled by other GPCRs are not affected in these mutants. Interestingly, *toh* mutants are less susceptible to the effects of overexpression of certain constitutively active G protein α (Gα) subunits. The *toh* locus encodes a homologue of the *Drosophila spinster* and zebrafish *not really started* genes. We are currently investigating which Gα

subunits *toh* mutations affect and whether other signaling components downstream of GPCR signaling are affected. Furthermore, we are investigating whether *toh* mutations effect signaling by other S1P GPCRs. *toh* may represent a novel and specific signaling component for this important family of receptors.

482. Molecular Anatomy of the Embryonic Zebrafish Heart. Gillian R. Siegal,[1] I.I. Adameyko,[2] S.G. Tevosian,[2] and D. Yelon[1]. [1]Skirball Institute, NYU School of Medicine, New York, NY; [2]Dartmouth Medical School, Hanover, NH.

The embryonic zebrafish heart is composed of two major chambers, a ventricle and an atrium. During the process of chamber formation, morphological distinctions arise regionally over time and are accompanied by molecular changes that distinguish subpopulations of cells. Our goal is to characterize the molecular anatomy of the developing heart by identifying cardiac genes with spatially and/or temporally expression patterns. By screening a panel of >100 zebrafish ESTs and candidate genes, we have accrued a panel of >30 markers of heart development. These include markers of cardiac differentiation, cardiac maturation, chamber specification, and atrioventricular canal development. Our panel of markers facilitates detailed molecular analysis of cardiac mutant phenotypes. Additionally, these genes provide excellent candidates for functional studies as they may play key roles in cardiac maturation and regionalization. We are currently pursuing reverse genetic analysis of especially intriguing genes from our collection. One gene of particular interest, *serdin*, encodes a leucine-rich protein that appears to be highly conserved among vertebrates. Its expression is temporally restricted, initiating after zebrafish heart tube assembly is complete. Using antisense morpholino oligonucleotides, we are currently testing the hypothesis that Serdin plays a critical role during the functional maturation of the heart tube. Ongoing studies of *serdin* and additional genes from our panel should provide us with a broader understanding of the complex mechanisms controlling vertebrate heart development.

483. Genetic Analysis of Myocardial Differentiation and Heart Morphogenesis in the Zebrafish. Ian C. Scott, Leonard A. D'Amico, Herwig Baier, and Didier Y. Stainier. University of California, San Francisco.

Unlike what has been observed in skeletal muscle, "master regulators" of cardiac muscle differentiation have not been identified in vertebrates. We have undertaken a genetic screen in zebrafish to identify additional genes that regulate the initial specification and/or proper differentiation of the myocardium. A number of interesting mutations that appear to affect distinct phases of myocardial differentiation, including specifying the proper number of precursors, expansion of the atrial and ventricular chambers, and later maintenance of the myocardium, have been identified. Positional cloning efforts are currently well underway for 3 of these mutants. Results from the initial characterization of these mutants will be discussed, as well as a more detailed molecular analysis of a few selected mutants.

484. Hand2 Regulates Myocardial Differentiation Within the Lateral Plate Mesoderm. Jeffrey J. Schoenebeck and Deborah Yelon. Developmental Genetics Program and Department of Cell Biology, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY.

Cardiogenesis begins with the specification of cardiac progenitors during gastrulation. These cells are incorporated into the anterior lateral plate mesoderm (ALPM), where they await cues to differentiate. The regulatory mechanisms insuring correct spatiotemporal differentiation of cardiac progenitors remain undefined. By coupling double fluorescent in situ analysis with caged-fluorescein fate mapping, we have located zebrafish cardiac progenitors as a specific subset of ALPM that expresses *gata4*, *tbx20*, *hand2*, and *nkx2.5*. Furthermore, we find that ventricular and atrial progenitors are spatially organized within the ALPM preceding expression of ventricular and atrial-specific markers. Our data indicate that ventricular progenitors are located medial to and extend more anterior than atrial progenitors. These findings are compatible with our laboratory's previous studies of progenitor organization prior to gastrulation and regionalized expression of ventricular and atrial markers during myocardial differentiation. Using mutant analysis, we are investigating regulators of myocardial differentiation. In *hands off/hand2* (*han*) mutants, most cardiac progenitors fail to differentiate despite normal ALPM patterning. Fate mapping and double in situ analysis indicate that *han* myocardium is derived from a medial subset of *nkx2.5*-expressing precardiac mesoderm. The specific location of this subpopulation suggests that a regionally restricted mechanism can promote Hand2-independent myocardial differentiation; this mechanism is currently under investigation.

485. Hedgehog Signaling Promotes Cardiomyocyte Formation in Zebrafish. Natalie Thomas and Deborah Yelon. Developmental Genetics Program, Skirball Institute, NYU School of Medicine, New York, NY 10010.

The first key step in heart formation is the specification of the proper number of cardiac progenitors from a multipotential population. Progenitor selection depends upon a network of inductive and repressive signals. Here, we investigate the impact of hedgehog signaling on cardiac specification. Previous work in mouse *smoothened* mutants implicated hedgehog signals in cardiac differentiation and morphogenesis. However, this work did not address effects on progenitor number, or differential sensitivity of atrial and ventricular progenitors. We are examining how hedgehog signals promote cardiac specification in zebrafish by characterizing the cardiac phenotypes in *smoothened* (*smu*) mutants and cyclopamine (CyA, a smoothened antagonist)-treated embryos. *smu* embryos have small ventricles with abnormal morphology. CyA-treated embryos have a more severe phenotype, with a greater reduction in ventricular and atrial precursors; the milder *smu* phenotype is likely due to compensation by maternal *smo*. Thus, hedgehog signaling is needed to generate appropriate numbers of cardiomyocytes. This requirement may be due to hedgehog-dependent progenitor specification or to later effects on the differentiation and/or proliferation of cardiac precursors. To distinguish between these possibilities, we will determine the time window when hedgehog signals are required for cardiomyocyte formation. Also, by constructing a 40% epiboly cardiac fate map of CyA-treated embryos, we will address if hedgehog signals affect the distribution or density of cardiac progenitors.

486. The Role of p57kip2 in Mouse Heart Development. Sheila A. Haley and Lazaros K. Kochilas. Department of Pediatric Cardiology, Rhode Island Hospital, Providence, RI 02903.

p57kip2 is a member of the conserved CIP/KIP cyclin-dependent kinase inhibitor family. Unlike the other members of this family, p57kip2 is expressed primarily during embryonic development, and its expression subsequently diminishes during postnatal life. In addition, the majority of p57kip2 knockout mice die in utero, and none survive to adulthood. It is thought that p57kip2 may be involved in the terminal differentiation of cells. We are primarily interested in the role of p57kip2 in the control of the cell cycle and terminal cell differentiation in the heart. In particular, we are interested in p57kip2 involvement in the differentiation of ventricular myocardium. We have previously observed that p57kip2 is upregulated in the trabecular zone of the embryonic mouse heart at day E11.5, a time when the heart is undergoing enhanced cardiomyocyte differentiation. Therefore, we hypothesized that the cell cycle inhibitory activity of p57kip2 participates in cell cycle arrest and terminal differentiation of cardiomyocytes. We have now created a transgenic mouse that will constitutively

overexpress p57kip2 selectively in the ventricular tissue. The subsequent mouse hearts have undergone Langendorf functional analysis, as well as analysis of protein and mRNA localization and expression levels in the ventricular myocardium. In addition, we have analyzed embryoid bodies derived from mouse stem cells overexpressing p57kip2 for precocious differentiation as well as enhanced cell cycle arrest.

487. Fgf8 is Required for Proper Anterior Heart Development. Roger Ilagan,[1] Radwan Abu-Issa,[1] Doris Brown,[2] Robert Schwartz,[3] and Erik Meyers[1]. [1]Duke University Medical Center, Durham, NC 27710; [2]University of California-San Francisco 94143; [3]Baylor College of Medicine, Houston, TX 77030.

Recent work has demonstrated that the extracellular signaling molecule, Fgf8, is required for neural crest cell survival, pharyngeal arch artery development, and outflow tract septation. These events are all critical for proper cardiovascular patterning; however, no studies have revealed a direct role for Fgf8 in heart tube elongation and anterior heart development. Here, we employ a Cre/loxP genetic strategy to define the critical expression domains necessary for heart tube elongation. Our analysis suggests that *Fgf8* expression in the pharyngeal endoderm is necessary for the maintenance of the anterior heart field, which lies immediately ventral to the endoderm. Deletion of *Fgf8* in the *Nkx2.5-Cre* domain (which includes the ventral pharyngeal endoderm) results in an increase in apoptosis and a decrease in cell proliferation within the anterior heart field. The resulting population reduction in the anterior heart field translates into a severe truncation of the outflow tract and right ventricle later in development. The inflow tract and left ventricle remain unaffected, suggesting that Fgf8 function is specific to anterior heart development. Interestingly, cardiac-specific deletion of *Fgf8* results in an increase in apoptosis in migrating neural crest cells, suggesting a mechanism for coordination between the two cell populations.

488. A Complex Nuclear Receptor Response Element Controls Atrial Specificity of the SMyHC3 Promoter in Mice. Tatiana G. Freitas-Matos,[1] Allysson C. Sampaio,[1] Michelle Vasconcelos,[1] Tiago Sobreira,[1] Paulo de Oliveira,[1] Esfir Slonimski,[2] Nadia Rosenthal,[2] and José Xavier-Neto[1]. [1]InCor - HC.FMUSP, São Paulo 05403900, Brazil; [2]EMBL, Monterotondo-Scalo, Italy.

The SMyHC3 promoter is the earliest-activated regulatory sequence driving expression in mouse atria. Deletion of distal 72 bp in transgenic mice indicates that a basal cardiac activator lies within the proximal 768 bp, but that ventricular repressors and atrial activators are contained in the 72-bp fragment. The 72-bp fragment includes a purine-rich 30-bp harboring multiple binding sites for nuclear receptors. Computerized profiling and gel-shift analysis indicate it behaves as a complex nuclear receptor response element (CNRRE). The CNRRE displays three major nuclear binding sites, hexads A, B, and C, supporting three mutually-exclusive binding modes: distal (A + B), proximal (B + C), and spaced (A + C). Mutagenesis of hexad C uncovers a dual *cis*-element repressing expression in ventricles and stimulating it in atria. A further left ventricular/proximal outflow tract repressor was mapped to hexad B. Manipulation of RA signaling indicates that atrial-specific activation, but not ventricular repression is achieved via liganded RA receptors. Thus, atrial specificity of the SMyHC3 promoter is sculpted upon a basal cardiac activity by ventricular repression and further atrial activation by RA signaling.

489. *Tbx2* and *Tbx3* Display Functional Overlap During Atrioventricular Patterning in Mouse. Zachary Harrelson and Virginia E. Papaioannou. Columbia University, New York, NY 10032.

Tbx2 and *Tbx3* are closely related members of the T-box transcription factor gene family. Both genes are demonstrated transcriptional repressors which display expression overlap at many sites throughout embryonic development, including the atrioventricular canal (AVC). Targeted mutagenesis of *Tbx2* in mouse has shown that the gene is essential for the repression of cardiac chamber formation in myocardium of the AVC. Further investigation has revealed that the AVC is normally specified in *Tbx2* homozygous null mutant hearts at 9.5 dpc, and that the observed molecular defects therefore represent a failure to repress a chamber differentiation program in atrioventricular (AV) myocardium. Targeted mutagenesis of *Tbx3* has identified roles for the gene in the development of several structures, including the hindlimb, but defects in cardiac development have yet to be reported. To address the possibility of a genetic interaction, we have generated mice that are doubly heterozygous for the *Tbx2* and *Tbx3* null alleles. $Tbx2^{+/-}; Tbx3^{+/-}$ mice have compromised viability on a mixed 129/C57/ICR genetic background. A molecular analysis of chamber formation has identified a functional interaction between *Tbx2* and *Tbx3* during AV patterning at 9.5 dpc. A subset of chamber-specific markers which exhibit ectopic AV expression in *Tbx2* homozygous mutants are also abnormally expressed in the hearts of $Tbx2^{+/-}; Tbx3^{+/-}$ embryos. The quality of ectopic expression observed in $Tbx2^{+/-}; Tbx3^{+/-}$ hearts, however, was distinct from that identified in *Tbx2* homozygous mutant embryos, suggesting that the AV functions of the two genes are overlapping but non-redundant.

490. Hedgehog Signaling is Critical for Development of the Outflow Tract of the Heart. Matt Goddeeris and Erik Meyers. Duke Univ. Med. Cen, Durham, NC 27710.

The anterior heart field (AHF) and cardiac neural crest cells (CNCC) are two distinct cell populations critical to the development of the outflow tract of the heart. However, the regulation of their respective contributions to the heart is unclear. The AHF is a recently described population that contributes to the myocardium of the outflow tract and right ventricle, whereas the CNCC populate the cushions of the outflow tract and are essential for outflow tract septation. Hedgehog (Hh) signaling has been implicated in many aspects of development including heart formation. Expression patterns of Hh components and mouse knockout phenotypes of Sonic hedgehog and Smoothened implicate Hh signaling as a potential mediator of AHF and CNCC survival and organization. To address the role of Hh signaling in vertebrate heart development, we have conditionally ablated Hh signaling components from the AHF and neural crest cells using specific cre recombinase transgenic lines. We find that Sonic hedgehog signaling from the pharyngeal endoderm to both the AHF and CNCC is essential for normal outflow tract patterning. Furthermore, loss of Smoothened from either CNCC or AHF results in similar cardiac defects. Our data suggest a model where complex Hh signaling-dependent interactions between the AHF and CNCC contribute to the development of the outflow tract.

491. Filamin A is Required in Endothelial Cells for Cardiovascular Development. Yuanyi Feng,[1] Ming-Hui Chen,[2] Ashley M. Mendonza,[1] David J. Kwiatkowski,[3] and Christopher A. Walsh[1]. [1]HHMI, Dept. of Neurology, BIDMC, Harvard Medical School, Boston, MA 02115; [2]Cardiovascular Division, BWH, Harvard Medical School, Boston, MA 02115; [3]Hematology Division, BWH, Harvard Medical School, Boston, MA 02115.

Mutations in the actin-binding protein Filamin A (FlnA, also known as ABP280) in human are known to cause a wide variety of developmental defects involving brain, bone, and many other important organs. To understand the mechanism of FlnA in mammalian development, we generated null and conditional null mutations of FlnA in mice. Loss of FlnA protein results in embryonic lethality before E15 due to widespread systemic hemorrhage. FlnA null embryos also displayed severe congenital cardiac defects due to abnormal septation of the ventricules and cardiac outflow tract resulting in ventricular septal defect (VSD) and persistent

truncus arteriosus (PTA). Conditional knockout of FlnA in the neural crest lineage also results in PTA with 100% genetic penetrance, but interestingly the migration of neural crest cells appears to be normal. Examination of the vasculature of the FlnA null embryos suggests that loss of FlnA function is associated with abnormal angiogenesis and defects in vascular endothelial cell–cell contact, but the vascular endothelial cells showed very subtle changes in their cellular actin structures. As FlnA can potentially interact with multiple cell surface receptors that are required for vascular endothelial cell development and function, it may act as a downstream modulator for various receptor signaling pathways in the dynamic regulation of the actin cytoskeleton during the development of cardiovascular system.

492. A Hypomorphic Prep1 Mouse Shows Impairment of Angiogenesis and Hematopoietic Development. Elisabetta Ferretti,[1] Patrizia Di Rosa,[1] Juan Carlos Villaescusa-Ramirez,[2] Luis Fernandez,[1] Giuliana Ferrari,[1] and Francesco Blasi[1]. [1]Univ. Vita Salute San Raffaele, Milano, Italy; [2]IFOM (FIRC Institute of Molecular Oncology), Milano, Italy.

The Meinox protein Prep-1 cooperates with Pbx and anterior Hox proteins. A Prep-1 null mutation results in embryonic lethality at E7.5 (see abstract Fernandez et al.). The insertion of a retroviral enhancer trap vector in the first intron of the Prep1 gene results instead in a hypomorphic mutation (Prep1i/i), with about 3% of the mRNA and 5–10% of the protein produced. The phenotype of homozygous Prep1i/i mice is influenced by the genetic background and shows variable penetrance and expressivity. Heterozygous crosses of back-crossed (C57BL/6) mice show a variable lethality after E15.5 and before P0. The newborn Prep1i/i mice (6% instead of 25%) are apparently normal. Prep1i/i embryos display a greatly reduced level of all Pbx and Meis proteins, and an almost complete absence of DNA-binding activity. Also, the phenotypes show some variability. The most affected process in Prep1i/i embryos, is hematopoiesis. Prep-1 is expressed in the PAS/AGM, and in the more immature hematopoietic precursors. In vitro colony assays in methylcellulose and repopulation experiments in lethally irradiated mice show a very broad effect of the hypomorphy of the Prep1 gene on hematopoiesis. Both myeloid and lymphoid lineages are strongly affected, as well as the total number of Long-Term Repopulating Hematopoietic Stem Cells. A variety of important target genes in different lineages have been identified.

493. A Novel Zebrafish Ets Domain Protein Etsrp is a Master Regulator of Vasculogenesis. Saulius Sumanas and Shuo Lin. University of California, Los Angeles.

During embryonic development, multiple signaling pathways control specification, migration, and differentiation of the vascular endothelial cell precursors, angioblasts. No single gene responsible for the specification and differentiation of angioblasts has been described as yet. Here, we report isolation, characterization, and functional studies of a novel ets domain protein Etsrp. Etsrp embryonic expression is only restricted to vascular endothelial cells and their earliest precursors, preceding expression of all other known vascular-specific genes tested. Morpholino (MO) knockdown of Etsrp protein function resulted in the complete absence of circulation in zebrafish embryos. Angioblasts in etsrp-MO-injected embryos (morphants) failed to undergo migration and differentiation, and did not coalesce into functional blood vessels. Expression of all vascular endothelial molecular markers was severely reduced in etsrp morphants whereas hematopoietic markers appeared unaffected. Overexpression of etsrp RNA caused multiple cell types to adopt the vascular endothelial fate. These results argue that etsrp functions as a master regulatory gene in the specification and differentiation of angioblasts during vasculogenesis.

494. The role of *Xenopus* Disabled-2 in Embryonic Angiogenesis. Seong-Moon Cheong and Jin-Kwan Han. Division of Molecular and Life Sciences, POSTECH, San 31, Hyoja-Dong, Pohang, Kyungbuk, 790-784, Republic of Korea.

The signaling molecules that regulate the formation of embryonic blood vessels are poorly understood. In addition, what signaling molecules take part in the regulation of the vascular endotherial growth factor (VEGF) gene expression are not fully understood, although VEGF is a critical factor in blood vessel formation. Here, we show that Disabled-2 (Dab2), a cytosolic adaptor, has a pivotal role for the blood vessel formation in *Xenopus* and regulates the induction of VEGF via TGF-β signaling pathway. Using the EST database, we isolated cDNA for *Xenopus* Disabled-2 (xDab2) and examined its potential role in *Xenopus* embryogenesis. It is intriguingly expressed in blood vessels including pronephric sinus, vascular vitelline network, common cardinal vein, anterior cardinal vein, posterior cardinal vein, and intersomitic vein. Loss-of-function experiments using the morpholino oligonucleotides (MO) show that the formation of intersomitic veins is disrupted. In animal cap assay, we also find that TGF-β signaling induces VEGF expression and its induction is decreased by Dab2 MO. Furthermore, Dab2 knock-down embryos are rescued by VEGF. Taken together, these results suggest that xDab2 is essential for embryonic angiogenesis by regulating VEGF induction through TGF-β signaling pathway.

495. Lymphangiogenesis in *Xenopus laevis*. Roland E. Kälin, Martin P. Kretz, Irena Senn, and André W. Brändli. Instit of Pharmaceutical Sci, Swiss Fed Instit of Tech, Zürich, Switzerland CH-8093.

The lymphatic system is composed of a vascular network of thin-walled capillaries that drains protein-rich lymph from tissues and organs back to the blood vascular system. Moreover, it serves as a conduit for the dissemination of lymphocytes and, under pathological conditions, of tumor cells. Much of our understanding of the molecular events controlling lymphangiogenesis has been gained from the analysis of mouse mutants. In contrast, little is known about the lymphatic system in lower vertebrates. Here, we investigated whether the *Xenopus* embryo would represent a simple experimental model for studies on lymphangiogenesis. In a first step, we identified *Xenopus* cDNAs encoding key regulators and markers of mammalian lymphatic development, such as FoxC2, Prox1, VEGF-C, VEGF-D, and VEGFR3. Expression pattern analysis revealed that, similar to mammals, the *Xenopus* lymphatic system develops by sprouting from the posterior cardinal vein. Next, we developed a novel method for microlymphangiography, which exclusively labels the lymph vessels in the living *Xenopus* embryo. Finally, we show that antisense morpholino-mediated knockdown of VEGF-C function in the *Xenopus* embryo has no effect on normal development of the vascular system, but specifically disrupts lymphangiogenesis. These findings indicate that lymphangiogenesis in *Xenopus* and mammals appears to be controlled by the same evolutionary conserved VEGF-C-dependent pathway. Taken together, the present work establishes the *Xenopus* embryo as a novel attractive model for studies on the development and function of the lymphatic vascular system in vertebrates.

496. Gene Profiling of Early Hematopoietic Mesoderm in *Xenopus*. Donald A. Yergeau,[1] Joanne R. Doherty,[1] Emin Kuliyev,[1] Geoffery A. Neale,[2] and Paul E. Mead[1]. [1]Department of Pathology; [2]Hartwell Center for Bioinformatics and Biotechnology; [3]St. Jude Children's Research Hospital, Memphis TN 38105.

Blood is one of the first organ systems to develop in vertebrates. Characterization of defined blood lineages has been extensively studied in a number of vertebrate models. However, studies focusing on the early hematopoietic environment have been lacking. *Xenopus laevis* provides an ideal model to study the formation of early blood cell progenitors. In

order to understand the regulation of early blood cell development, we have analyzed global gene expression in explants at developmental stages 15, 25, and 35. Animal caps were treated with the TGFβ superfamily member Activin A (1 pg) to induce mesoderm or Activin A + BMP-4 (100 pg) to pattern the nascent mesoderm to a ventral fate. A third data set, untreated animal cap explants, was included as a control. Pilot studies showed increased levels of the early hematopoietic markers LMO2 and SCL in Activin A + BMP-treated caps as well as the down-regulation of a-actin, a marker for dorsal mesoderm. Comparison of RNA harvested from Activin A vs. Activin A + BMP-4 treated stage 15 caps applied to Affymetrix gene chips showed the up-regulation of a number of ventral mesoderm specific genes and down-regulation of dorsal mesoderm genes. We used ANOVA analysis to identify a list of 253 genes that changed significantly in the Activin A + BMP-4 samples at stage 15 relative to the controls. Northern blot, RT-PCR, and in situ hybridization studies have been used to confirm the Affymetrix data and have provided additional filters to narrow down the original list to a manageable size for further study.

497. Low Levels of BMP Signaling Promote Gene Expression in the Intermediate Mesoderm in a Cell Autonomous, but Indirect Manner. Richard G. James[1] and Thomas M. Schultheiss[2]. [1]Harvard University; [2]Beth Israel Deaconess Medical Center, Boston, MA 02215.

Bone morphogenic protein (Bmp) signaling regulates cell fate determination along the dorso-ventral axis of vertebrates. Although it is known that Bmp signals activate different response genes at low and high levels, the mechanisms by which this is accomplished are not well understood. The current study examines the mechanism of Bmp-dependant activation of gene expression of the avian trunk mesoderm. We find that low and high levels of Bmp ligand are necessary and sufficient to activate intermediate and ventral mesodermal gene expression, respectively, both in vivo and in vitro. Dose-dependent activation of intermediate and ventral mesodermal genes by Bmp signaling is cell autonomous, as demonstrated by electroporation of the avian embryo with constitutively active Bmp receptors driven by promoters of varying strengths. In explant cultures, activation of Osr-1, the earliest known gene expressed in the intermediate mesoderm, is blocked by cyclohexamide, indicating that activation of this low level Bmp responder by Bmp is indirect. The data from this study are integrated with that of other studies to generate a model for the role of Bmp signaling in trunk mesodermal patterning in which low levels of Bmp activate intermediate mesoderm gene expression by inhibition of repressors present in dorsal mesoderm, whereas high levels of Bmp repress both dorsal and intermediate mesoderm gene expression.

498. Inversin is Required for Normal Nodal Flow in Kupffer's Vesicle and Left–Right Development in Zebrafish. Patricia A. Sacayon, Jeffrey J. Essner, and H. J. Yost. Hunstman Cancer Institute, Center for Children, Department of Oncological Sciences, University of Utah.

Organogenesis in vertebrates is dependent on the establishment of left–right patterning. However, the mechanism by which left–right asymmetry is initiated in developing embryos remains elusive. It is proposed that monocilia establish left–right patterning by generating and perhaps sensing nodal flow in the mouse. Monocilia are found in the node in mouse and chick, in the dorsal organizer in *Xenopus* and in Kupffer's vesicle (KV) in zebrafish. *Inversin* (*inv*) mouse mutants have predominantly inverted left–right patterning and inv has been shown to be a component of monocilia in the mouse node. However, the role of inv in left–right patterning remains unclear. To determine how inv functions in left–right patterning, we used an *inv* morpholino to knock down expression of *inv* in zebrafish. Knock-down of *inv* resulted in inverted laterality of *lefty2*, *pitx2*, *cmlc2*, and *fkd2* similar to the reversed laterality observed in mouse *inv* mutants, suggesting that inv function is conserved in vertebrates. As in mice, *inv* is expressed ubiquitously during early zebrafish development. This raises the question of what cell lineages require inv for left–right patterning. Here, we show that inv is required specifically in Dorsal forerunner cells, the progenitors of ciliated KV, for left–right patterning. This is the first demonstration that inv functions in node/KV cells for LR development in any vertebrate. We further demonstrate that knock-down of *inv* leads to a decrease in *left–right dynein-related1* (*lrdr1*) expression, a reduced number of cilia in KV, and aberrant nodal flow.

499. Cited2 is Required for Heart Morphogenesis and Establishment of the Left–Right Axis in Mouse. Sally L. Dunwoodie,[1] Kylie Lopes Floro,[1] Wolfgang J. Weninger,[2] Jost I. Preis,[1] and Timothy J. Mohun[3]. [1]Victor Chang Cardiac Research Institute, Sydney 2010, Australia; [2]Medical University Vienna, A-1090 Austria; [3]National Institute for Medical Research, Mill Hill NW71aa, UK.

Establishment of the left–right body axis is a fundamental process of vertebrate embryogenesis. Failure to develop left–right asymmetry leads to incorrect positioning and morphogenesis of numerous internal organs, and is proposed to underlie the etiology of several common cardiac malformations. The transcriptional modulator Cited2 is essential for embryonic development; Cited2-null embryos die during gestation with profound developmental abnormalities including cardiac malformations, exencephaly, adrenal agenesis, and placental insufficiency. Cited2 is also required for normal establishment of the left–right axis; we demonstrate that abnormal heart looping and right atrial and pulmonary isomerism are consistent features of the left–right patterning defect. We show by gene expression analysis that Cited2 acts upstream of Nodal, Lefty2, and Pitx2 in the lateral mesoderm, and Lefty1 in the presumptive floor plate. The left–right patterning defect is not fully penetrant in Cited2-null embryos. Embryos with normal left–right patterning develop a distinct set of cardiac defects which include: muscular ventricular septal defects and reduced cell density in the atrioventricular endocardial cushions. We show that Cited2 expression correlates with these defects. We therefore conclude that Cited2 is required at two levels for normal heart morphogenesis: the first is early in embryonic development in tissues external to the heart; the second is within the heart itself.

500. A Mutagenesis Screen to Identify Genes Involved in *Drosophila* Early Gonad Formation. Jill J. Weyers, Allison B. Milutinovich, and Mark Van Doren. Johns Hopkins University, Dept. of Biology.

Organ development is a complex process involving numerous events including cell migration, cell signaling, cell–cell interactions (often between multiple cell types), and morphological changes. One ideal system in which to study this assortment of processes is the *Drosophila* gonad. The fly gonad is made up of two cell types – somatic cells called "somatic gonadal precursors (SGPs)" and germ cells – which must come together and interact to form a functional gonad. These cellular interactions will eventually result in one of two final structures (an ovary or a testis); however, the early stages of gonad formation are similar in both sexes. These early events can be divided into five distinct steps: SGP specification (where clusters of SGPs form from mesodermal tissue in three separate segments), germ cell migration (where germ cells migrate to the SGP clusters), SGP cluster fusion (where the SGP clusters begin to join together and make contacts between each other as well as with germ cells), ensheathment (where SGPs physically wrap themselves around individual germ cells), and compaction (where the cells condense into the proper gonad morphology). To better understand these early steps of gonad formation and the morphological changes and cell–cell interactions involved, we performed a mutagenesis screen focused on finding disruptions in gonad formation. The variety of

phenotypes from our resulting mutant lines suggests that all five steps of early gonad formation can be dissected out genetically. We will present a description of our mutant phenotypic classes, as well as some preliminary analysis of individual complementation groups.

501. Does FOXL2 Regulate Aromatase Expression During Avian Sex Determination? Quanah J. Hudson, Craig A. Smith, and Andrew H. Sinclair. Department of Paediatrics, University of Melbourne and Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia.

The forkhead transcription factor, FOXL2, is implicated in vertebrate ovary development. Mutations in the *FOXL2* gene are associated with premature ovarian failure in humans and mice. We cloned the chicken FOXL2 orthologue and found that it was highly conserved with the mammalian sequences. Chicken FOXL2 shows expression in the embryonic ovary, conserved with mammals. Chicken FOXL2 shows temporal and spatial co-localization with the key estrogen-synthesizing enzyme, aromatase, in the embryonic ovarian medulla, suggesting a potential interaction. Aromatase is required for ovarian differentiation in birds. The chicken aromatase promoter has three putative FOXL2 binding sites. It is hypothesized that FOXL2 regulates aromatase as a critical step in the initiation of female sex determination in the chicken embryo. We demonstrated that aromatase inhibition (resulting in female to male sex reversal in chicken embryos), reduced, but did not abolish, FOXL2 expression. This suggests that FOXL2 lies upstream of aromatase in avian sex determination, but it responds to depleted estrogen synthesis. The reduction in *FOXL2* expression may be accounted for by the interruption of a positive feedback loop via estrogen, or the influence of testis-promoting factors, such as SOX9, in the masculinized gonads. To further explore the role of FOXL2 in chicken gonadogenesis, we have cloned *FOXL2* into the avian retroviral vector, RCAS. We are conducting FOXL2 overexpression studies in vitro and in ovo, to assess the impact on aromatase expression and gonadal phenotype.

502. NHERF2/SIP-1 Interacts with Mouse SRY via a Different Recognition Mechanism than Human SRY. Laurie Thevenet,[1] Kenneth H. Albrecht,[2] Safia Malki,[1] Philippe Berta,[3] Brigitte Boizet-Bonhoure,[1] and Francis Poulat[1]. [1]Institut de Génétique Humaine, CNRS UPR 1142, Montpellier, France; [2]Boston University, School of Medicine, Boston, MA 02118 USA; [3]Université de Nîmes, Nîmes, France.

In mammals, male sex determination is controlled by the SRY protein, which differentiates the bipotential embryonic gonad into testis. The main role of SRY is to activate the Sertoli cell differentiation program. SRY is a transcription factor which belongs to the HMG box protein family. If the morphological effects of SRY are well described, its molecular mechanisms remain still unknown. Moreover, SRY proteins display high sequence variability among mammalian species, which makes protein motifs difficult to delineate. Our lab has previously isolated SIP-1/NHERF2, a PDZ protein, as a human SRY interacting protein. SIP-1/NHERF2 interacts with the C-terminal extremity of the human SRY protein. Here, we show that the interaction of SIP-1/NHERF2, via the PDZ1 domain, is conserved with mouse SRY, but surprisingly, involves a different recognition mechanism, via an internal sequence. This interaction was further confirmed by co-immunoprecipitation. Then, using immunofluorescence, we have shown that mouse and human SRY are able to induce a nuclear accumulation of the SIP-1/NHERF2 protein. Finally, a transgenic mouse line expressing GFP under the control of the mouse Sry promoter allowed us to show that SRY and SIP-1/NHERF2 are coexpressed in the nucleus of pre-Sertoli cells during testis determination. Taken together, our results suggest a conserved function for SIP-1/NHERF2, among human and mouse species, as a cofactor of SRY.

503. Identification of Genes Involved in Ovary Development Using Microarrays. Hyunjoo Lee and Kenneth Albrecht. Boston University School of Medicine, Boston, MA 02118.

Mammalian sex determination involves the differentiation of the bipotential genital ridges into either ovaries or testes. In XY mammals, expression of *Sry* in the supporting cell lineage directs these cells to differentiate into testicular Sertoli cells, rather than ovarian granulosa cells. The genes that direct granulosa cell differentiation, which are potentially critical for ovary determination, have not been identified. In fact, very few genes involved in early ovary development are known. Our study is thus aimed at identifying novel markers of ovarian somatic cell differentiation, which may lead to the identification of ovary determining genes. We have developed transgenic mice that express EGFP under the control of the *Sry* promoter. This EGFP transgene is expressed uniquely in the supporting cell lineage of both male and female gonads, and is the earliest known marker of these cells. Pre-granulosa cells, identified on the basis of EGFP expression, and other non-vascular somatic cells were isolated from E13.5 fetal ovaries by FACS. Gene expression differences between these two populations of cells were determined using Affymetrix GeneChip Mouse Expression Set 430 2.0 arrays. We have identified 471 genes whose expression in pre-granulosa cells and other somatic cells differs by at least 2-fold (FDR < 0.1). RT-PCR, immunohistochemistry, and in situ hybridization analyses reveal gene expression patterns that are consistent with the microarray results. We will present a preliminary analysis of a subset of the differentially expressed genes, some of which display sex-specific expression patterns.

504. FKBP52 is Required for Steroidal Regulation of External Genital Development and Fertility. Hanying Chen,[1] Irene M. Wolf,[2] Zuocheng Yang,[1] Sumudra Periyasamy,[2] Edwin R. Sanchez,[2] and Weinian Shou[1]. [1]Indiana Univ. School of Medicine, Indianapolis, IN 46202; [2]Medical College of Ohio, Toledo, OH 43614.

FKBP52 is the best known of four tetratricopeptide repeat (SRA-TPR) proteins that associate with steroid receptor via heat-shock protein 90 (Hsp90). Yet, the role of FKBP52 in SR-regulated physiology remains an enigma. Although FKBP52 is ubiquitously expressed, it has a unique gradient pattern of expression in urethral epithelium in embryonic penis, suggesting a function in external genital development. Here, we report that FKBP52-deficient mice have altered steroid regulation of genital development and fertility. Male FKBP52 (¯C/¯C) mice are infertile due to development of hypopadias (failure of ventral urethral tube closure in the developing penis), the most common birth defect in boys. The hypopadias phenotype in FKBP52-deficient males was partially reversed by administration of testosterone, suggesting a defect of androgen receptor (AR) action. Female FKBP52 (¯C/¯C) mice are sterile due to inactivity in the uterus by the progesterone receptor A (PR-A) isoform, leading to a failure of implantation, while ductal development in the mammary gland, a PR-B-mediated phenotype, was normal. Biochemical analysis showed preferential association of FKBP52 with PR-A, as compared to PR-B. While the hormone-binding function of FKBP52-deficient SR was unimpaired, the steroid-induced transcriptional activity of FKBP52-deficient SR was greatly affected. Our results demonstrate a novel and a new fundamental mechanism for regulating tissue-restricted steroid function and physiology via SRA-TPR proteins.

505. External Genitalia Formation; A Model System to Study Organogenesis. Gen Yamada. Center for Animal Resources and Development (CARD), Kumamoto Univ., Japan.

We have been analyzing the molecular mechanisms of reproductive organ formation. The external genitalia constitute terminal appendage

organs with endodermally derived structures, i.e., initially, urethral plate, and later, tubular urethra. Developmental mechanisms of the genital tubercle (GT; the embryonic anlage of external genitalia) may possess some aspects of similarities with the developmental mechanisms of the limb bud formation. This has been noticed by some "similar" phenotypes in human birth defects displaying multiple appendage-hypoplasia such as in limbs and external genitalia. Recently, conserved expression and functions of signaling genes have been identified for limb and external genitalia development. Several growth factors, including fibroblast growth factors (FGFs), sonic hedgehog (Shh), and Bmps, have been implicated in the control of external genitalia development in mouse embryos. Our recent findings with regulatory genes, and growth factors–transcription factors interactions will be discussed. Ref. Suzuki, K. et al. Development. 131: 6209–6220 (2003). Yamada, G. et al. Differentiation. 71: 445–60 (2003). Haraguchi R. et al. Development 128. 4241–4250 (2001), Haraguchi, R. et al. Development 127, 2471–2479 (2000).

506. Genome-Wide Transcriptional Activity in Wild-Type and Diseased Mouse Placentas. Kirstin S. Knox and Julie C. Baker. Department of Genetics, Stanford University, Stanford, CA.

Many diseases of pregnancy are caused by placental defects, yet little is known about the transcriptional events that direct development of this unique organ. We have used whole genome mouse microarrays to construct a transcriptional profile of the developing mouse placenta from e8.0 (prior to chorioallantoic fusion) to P1. Placentas were manually dissected into maternal and fetal portions, yielding separate maternal and fetal samples for each of the ten stages of our time course. These profiles give us a genome-wide view of transcriptional activity in maternal and fetal tissues during development of the mouse placenta, and suggest novel roles for many genes in placental development and function. Although the placenta has taken on its definitive form and begun to transfer nutrients by e10, we have found that the majority of highly expressed genes experience their most dramatic expression changes after this time, between e10 and e17.0. In addition, we have extended our expression studies to search for placental factors that may be involved in initiating symptoms of pre-eclampsia. Fetuses lacking expression of Kip2 induce symptoms of pre-eclampsia in their mothers, most likely as a result of placental abnormalities. We have compared Kip2 mutant placentas to wild-type sibling placentas at e12, just prior to onset of symptoms of pre-eclampsia, as well as at several later time points, and are currently analyzing these results. Our wild-type and Kip2−/− transcriptional profiles will serve as a resource for scientists investigating placental development, function, and disease, and have defined the transcriptional changes that occur throughout placentation.

507. Uncovering a Role for *Patched1* in Epidermal Homeostasis: Interfollicular Epidermal Hyperplasia in $Ptc^{mes/mes}$ Mutants. Erica Nieuwenhuis and Chi-chung Hui. Program in Developmental Biology, The Hospital for Sick Children, Department of Molecular and Medical Genetics, University of Toronto, Canada.

Patched (*Ptc*) encodes a cell surface receptor for Sonic hedgehog, a secreted signaling molecule that regulates embryonic growth and patterning of various tissues, including epidermal structures such as skin and hair follicles. Humans and mouse models suggest *Ptc1* mutations are associated with the development of basal cell carcinomas. The precise role of *Ptc1* in normal epidermal homeostasis and maintenance remains unknown. The multilayered epidermis consists mainly of proliferating and terminally differentiated keratinocytes. Differentiated keratinocytes are derived from transit-amplifying cells originating from the bulge and within the basal cells of the interfollicular epidermis. Since $Ptc1^{lacz/lacz}$ mutants die prior to the development of skin structures, we are studying a *Ptc1* allele resulting from a spontaneous mutation, Ptc^{mes}, to determine the role of Ptc1 in the epidermis. $Ptc^{mes/mes}$ mice are viable with minor developmental anomalies, but do not develop skin tumors. Although hair follicle development appears to be normal, adult animals have severe epidermal hyperplasia affecting only the interfollicular region. The hyperplastic epidermis contains undifferentiated, basaloid-appearing cells of which the nuclei are vertically elongated. Immunohistochemical analysis of epidermal differentiation markers suggests that the basal layer is expanded, while stratification of suprabasal layers is normal. These results suggest that a role for Ptc1 in maintaining the adult interfollicular epidermis is uncovered in the $Ptc^{mes/mes}$ mutant.

508. Runx3 Plays a Role in Hair Structure Determination. Uri Gat,[1] Eli Raveh,[1] Shulamit Cohen,[1] Ditsa Levanon,[2] and Yoram Groner[2]. [1]Department of Cell and Animal Biology, Silberman Life Sciences Institute, The Hebrew University in Jerusalem, 91904, Israel; [2]Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel.

Members of the Runx family of transcriptional regulators play critical roles in normal development, and when mutated lead to genetic diseases and cancer. Based on the possible connections between the Wnt pathway effector, Lef-1, a key player in hair development, and Runx proteins, we studied the expression pattern of these factors in skin and its appendages during mouse development. Here, we focus on Runx3, which is predominantly expressed in the dermal compartment of the hair follicles, the dermal papilla as it forms, and throughout the hair cycle. Its expression is also observed in other skin appendages; in the nail mesenchyme, which corresponds to the dermal papilla of the hair follicle, and in the outflow tube of the sweat glands. Interestingly, Runx3 is also found in melanocytes, which populate the hair bulb during its growth phase and provide melanin to hair producing keratinocytes. Runx3-deficient mice display a perturbation of the normal hair coat, caused by hair type and hair shape changes, suggesting its possible role in regulating the formation of the structural hair by affecting dermal to epidermal interactions. Comparison of the phenotype of the Runx3 mutant mice to that of other mutations suggests that Runx3 may affect the Wnt and TGFb/BMP pathways, both of which are known to be essential for hair formation and differentiation. The roles of the other Runx family members are also being studied in order to define the principal mode of action of these factors in organ development.

509. Fetal Breathing-Like Movements are Essential for Lung Cell Differentiation. Mohammad R. Inanlou and Boris Kablar. Dalhousie University, Halifax, NS, Canada B3H 4H7.

The role of fetal breathing-like movements (FBMs) in fetal lung growth and cell differentiation was examined employing Myf5−/−:MyoD−/− (or amyogenic) mouse embryos. As previously found, amyogenic embryos had no skeletal muscle, including respiratory musculature. As a consequence of that, they suffered from pulmonary hypoplasia and died shortly after birth. The lung of amyogenic embryos was much smaller because of the significantly decreased cell proliferation and increased cell death. Development of their lung was arrested at the canalicular stage and it never reached the saccular stage of lung development. In addition, the number of lung cells expressing platelet-derived growth factor B and insulin growth factor I was decreased, while the gradient of the thyroid transcription factor 1 was not maintained at E17.5. Importantly, as revealed by transmission electron microscopy (TEM), Type II pneumocytes had a failure in surfactant storage and secretion, but were able to synthesize the surfactant-associated proteins. On the other hand, Type I pneumocytes were readily detectable employing an early differentiation marker but the late differentiation of type I pneumocytes never occurred, as revealed by TEM. Together, our findings suggest that pulmonary distension due to FBMs plays an important role not only in lung growth, but also in lung cell differentiation. This work is supported by the NSERC and CFI grants to BK, and a NSHRF fellowship to MRI.

510. Nmyc Plays an Essential Role During Lung Development as a Dosage-Sensitive Regulator of Progenitor Cell Proliferation and Differentiation. Tadshi Okubo,[1] Paul S. Knoepfler,[2] Robert N. Eisenman,[2] and Brigid L. Hogan[1]. [1]Department of Cell Biology, Duke University Medical Center; [2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center.

Understanding how lung progenitor cells balance proliferation against differentiation is relevant to clinical disorders such as bronchopulmonary dysplasia of premature babies and lung cancer. Previous studies established that lung development is severely disrupted in mouse mutants with reduced levels of the proto-oncogene Nmyc, but the precise mechanisms involved have not been explored. Nmyc expression in the embryonic lung is normally restricted to a distal population of undifferentiated epithelial cells; a high proportion of these cells are in the S phase of the cell cycle (E12.5). However, Nmyc expression dramatically declines at late stage (E18.5). To determine the role of Nmyc in lung development, we performed gain- and loss-of-function studies. Overexpression of Nmyc in the epithelium under the control of surfactant protein C (Sftpc) promoter expands the domain of S phase cells and upregulates numerous genes associated with growth and metabolism. In addition, there is marked inhibition of epithelial differentiation, coupled with an expanded domain of expression of Sox9 protein, which is also normally restricted to the distal epithelial compartment. By contrast, conditional deletion of Nmyc leads to reduced proliferation, high levels of apoptosis in the epithelium, and to premature differentiation. We propose a model in which Nmyc is essential in the developing lung as a dosage-sensitive regulator for maintaining a distal population of undifferentiated progenitor cells.

511. Mapping the Gene Expression Program of the Embryonic Mouse Lung. F. Hernán Espinoza and Mark A. Krasnow. Stanford University School of Medicine & Howard Hughes Medical Institute.

In order to obtain a comprehensive framework for understanding and investigating the genetic program that controls mammalian lung development, we are systematically mapping gene expression in the embryonic mouse lung using whole mount in situ hybridization. Our analysis of the expression patterns of ~1500 genes has given us insights into the patterning of the lung, the control of the tissue and cell differentiation within the lung, and numerous molecular markers for those processes. These studies will provide the first global view of the temporal and spatial gene expression program for the formation of the mammalian lung. Furthermore, these studies provide an analytical baseline and molecular makers to better understand changes to the lung caused by genetic defects, disease, toxins, and drugs.

512. Genomic Analysis of the Initiation of *Drosophila* Respiratory System Organogenesis by the Trachealess bHLH Transcription Factor. Elizabeth D. Chao and Mark A. Krasnow. Department of Biochemistry and HHMI, Stanford University School of Medicine, Stanford, CA 94305-5307.

We have investigated the gene expression program that mediates the initiation of *Drosophila melanogaster* tracheal system organogenesis. Examination of the embryonic gene expression patterns of a third of the genes in the genome allowed us to identify the percentage of genes selectively expressed during the earliest stage of tracheal development. To understand how early tracheal genes are regulated, we examined the role of Trachealess (Trh), a basic-helix–loop-helix–PAS transcription factor required for the initiation of tracheal organogenesis and able to initiate the process in ectopic positions. Whole-genome expression profiles of trh mutant embryos and validation of affected genes by whole mount in situ hybridization determined the fraction of early tracheal genes dependent on Trh for their expression. Many of the Trh-dependent genes contain conserved clusters of Trh consensus binding sites, implying that they are direct Trh targets. Trh target genes encode mostly signal transducers and transcription factors, but also specialized molecules associated with cell junctions, the cytoskeleton, secretion, and translation. Genetic characterization of one newly identified target gene that encodes a cell junction protein demonstrates that it has a novel function in tracheal development. Our study provides a global view of the circuitry underlying the initiation of an organogenesis program by a master regulator.

513. From Signalling Pathways to Cellular Reorganisation During Tracheal Morphogenesis in *Drosophila*. Véronique Brodu and Jordi Casanova. Institut de Biologia Molecular de Barcelona (IBMB), CSIC, Barcelona, Spain.

During epithelial morphogenesis, in response to signalling molecules, cell shape rearrangements and cytoskeletal modifications sculpt the body plan. However, the mechanisms that integrate cellular changes with signalling pathways are not yet well characterised. Cell invagination is a major initial process of epithelial morphogenesis that leads to the bending of the epithelial sheet. In *Drosophila*, the tracheal network provides a powerful model system for investigating the molecular and morphological basis of cell invagination and subsequently outgrowth. The tracheal system is composed by a highly stereotyped network of interconnected branches. Many signalling pathways have been identified as required for the determination of the tracheal cells and for the initial steps of tracheal morphogenesis. But invagination and early migration are poorly characterized at the cellular level. In addition, little is known about the contribution of signalling pathways to cell remodelling that accompanies invagination. Here, we will present data that illustrate the different cell shape changes that occur during tracheal morphogenesis and we will analyse the contribution of signalling pathways to these changes.

514. Patterns of Airway Branching During Mouse Lung Development. Ross J. Metzger and Mark A. Krasnow. Howard Hughes Medical Institute and Stanford University School of Medicine.

The elaborately branched airway tree of the lung has long fascinated scientists, and mathematical and molecular models have been proposed to explain how such a pattern could be generated. A major limitation of these models and of our understanding of the branching program, however, is that the pattern of airway branching is unknown. To determine the branching pattern in the mouse lung during embryogenesis, we visualized the developing airway tree by whole mount immunostaining of dissected lungs from embryos using an airway-specific antibody. The pattern is complex yet highly stereotyped, and follows specific patterning rules that change at specific times and specific positions during lung development. Our analysis provides a foundation for identifying the genes and signaling pathways that control and pattern this dynamic sequence of branching events.

515. Essential Function of FGF Signaling Pathway During Branching Morphogenesis of Mammary Gland. Pengfei Lu, Andrew Ewald, Gail Martin, and Zena Werb. Department of Anatomy, School of Medicine, University of California, San Francisco, CA 94143.

Fibroblast growth factor (FGF) signaling plays an essential role in a variety of developmental processes and its deregulation is known to be responsible for many human malignancies, including breast cancer. Previously, it has been shown that FGF signaling is required for the formation of the mammary gland during embryonic development. However, its role in branching morphogenesis during postnatal mammary gland development has remained unclear. Using a combination of in vivo

and novel three-dimensional (3D) in vitro culture systems, we have examined components of the FGF signaling pathway in mammary gland branching morphogenesis. We found that a loss of FGF signaling by removing FGF receptor 2 (Fgfr2) gene function in the mammary epithelium caused a severe retardation branching phenotype in virgin females. However, an opposite phenotype, precocious ductal invasion, was observed when Sprouty2, an antagonist of receptor tyrosine kinase signaling, function was inactivated in the mammary epithelium. Using a genetic mosaic analysis, we found that epithelial cells lacking Fgfr2 function failed to incorporate into the epithelium undergoing morphogenetic movements during mammary branching in vitro and were eliminated by programmed cell death. Together, our results demonstrate an essential role for FGF signaling in branching morphogenesis of the mammary gland.

516. Spry4 is an Antagonist of FGF8 and is Required for Proper AER Development. Benjamin D. Yu,[1] George Minowada,[2] and Gail R. Martin[3]. [1]Department of Dermatology, School of Medicine, University of California, San Francisco; [2]University Hospitals of Cleveland, Case Western Reserve University, Cleveland; [3]Department of Anatomy and Program in Developmental Biology, School of Medicine, University of California, San Francisco, San Francisco, CA 94158, USA.

Members of the SPROUTY family are negative feedback antagonists of receptor tyrosine kinase (RTK) signaling. In mice, *Spry1*, *Spry2*, and *Spry4* are known to be expressed in the mesenchyme of the developing limb bud. Whereas *Spry1* and *Spry2* null mice have normal limbs, we have found that animals lacking *Spry4* are viable and fertile but have forelimb-specific digit deformities. In some cases, digit 3 is missing, whereas in others digits are either bifurcated (split), fused, or abnormally spaced. These defects appear to result from abnormal gaps in the apical ectodermal ridge (AER). Since *Spry4* is thought to function intracellularly to inhibit the transduction of FGF signals, the defects in the *Spry4* null mice could be caused by a hypersensitivity of the limb bud mesenchyme to FGF signals from the AER. To test this hypothesis, we generated *Spry4* null mice that are heterozygous for *Fgf8* (*Spry4*−/−; *Fgf8*+/−), an FGF family member produced by the limb bud ectoderm at pre-AER stages and by the AER at later stages. We found this reduction in *Fgf8* dosage completely rescues the *Spry4* null limb phenotype. Thus, we conclude that the main function of *Spry4* in the limb bud is to regulate the response of limb bud mesenchyme to FGF signals from the AER, and that in its absence, the mesenchyme fails to provide signals necessary for normal AER development.

517. *Tbx4* is Dispensable for Post-Bud Hindlimb Outgrowth. L.A. Naiche and Virginia E. Papaioannou. Dept. of Genetics and Development, College of Physicians and Surgeons, Columbia Univ., New York, NY 10032, USA.

The growth of the vertebrate limb is governed by a signaling feedback loop between the mesenchyme, which expresses *Fgf10*, and the overlying epithelium, which expresses *Fgf8*. *Tbx4* and *Tbx5* are closely related T-box transcription factors expressed in the hindlimb and forelimb, respectively. Targeted mutagenesis of both *Tbx4* and *Tbx5* have shown that they are required for the outgrowth of their respective limbs and function to initiate or maintain *Fgf10* levels in the limb mesenchyme. This requirement is presumed to be due to direct regulation of *Fgf10*, as *Tbx5* has been shown to bind the *Fgf10* promoter and upregulate it in vitro. We here describe the use of a conditional allele of *Tbx4* to further elucidate the role of this gene in hindlimb development. Excision of *Tbx4* at E7.5 rescues allantois-related early embryonic lethality, but produces a limb phenotype indistinguishable from the null allele. Surprisingly, we have found that excision after brief expression of *Tbx4* in the early limb bud is sufficient to allow complete outgrowth of the hindlimb and results in only minor perturbation of the limb FGF feedback loop. Hindlimbs that have expressed *Tbx4* transiently are distally complete and show markers of normal molecular and morphological hindlimb identity. These hindlimbs exhibit relatively minor defects at all axial levels, including pelvic and fibula hypoplasia as well as aberrant digit patterning. This work suggests that *Tbx4* is probably not a direct regulator of *Fgf10*, but instead functions to regulate another gene required for the limb feedback loop.

518. Live Three-Dimensional OPT Imaging and Tomographic Reconstruction of Limb Development. Marit J. Boot,[1] Juanjo Sanz,[2] Miguel Torres,[2] and James Sharpe[1]. [1]MRC Human Genetics Unit, Edinburgh, UK; [2]Centro Nacional de Biotecnologia, Madrid, Spain.

Limb development comprises of a complex sequence of cell movements. Live three-dimensional (3D) imaging of the developmental processes that occur on the limb ectodermal surface combined with tomographic reconstruction of the processes that occur inside the living limb will give new insights into embryonic development. We extensively adapted the static Optical Projection Tomography (OPT) scanner to facilitate imaging of living organs and called this innovative technique 'live OPT'. Mouse embryo body segments containing the hind limbs were cultured submerged in DMEM or BGJb medium and rotated through 360° for imaging. Both the live OPT scanner and the organ culture technique have been successfully adapted to allow limb culture at embryonic days 11.5 and 12.5. Limb development was imaged in 3D for 36 h; subsequently, tomographic reconstructions allowed us to visualize virtual sections in all planes. The cultured limbs display significant expansion in the proximal–distal axis, formation of the autopod, and inter-digital cell death similar to hind limbs developed in utero; however, the growth rate in culture is slower. Cartilage formation was studied in time in the tomographic reconstructions. Further optimization of both the culture technique and the OPT scanner will be necessary to improve the growth rate of the cultured limbs. In conclusion, these results show that live 3D imaging and tomographic reconstruction of a living mouse limb is feasible and that the live OPT is a powerful technique to improve our understanding of developmental processes. (EU Research funding.)

519. How Thyroid Hormone Controls Limb Development During *Xenopus laevis* Metamorphosis. Liquan Cai, Biswaji Das, and Donald D. Brown. Department of Embryology, Carnegie Institution of Washington.

During metamorphosis of *Xenopus laevis*, thyroid hormone (TH) controls biological events as diverse as tail resorption and limb growth. Formation of the limb bud early in the tadpole does not require TH. However, its subsequent growth and differentiation including cell proliferation is dependent completely on the hormone. TH regulates cell proliferation and differentiation in all cell types of the developing limb, including the muscle and cartilage. The multiple TH-controlled programs in the limb can be distinguished by sperm-mediated transgenesis in which different tissue/cell-type-specific promoters (muscle, nerve, and cartilage) are targeted with a dominant-negative form of the TH receptor (mutated ligand binding domain in the TH receptor gene, TRDN). Transgenic animals develop paralyzed limbs and die at the climax of metamorphosis. But the limb defects exist in different tissue/cell types corresponding to the promoter used. We have placed the TRDN driven by a muscle promoter under the control of the Tet system. When the TRDN transgene is induced by doxycycline, limb muscle that has already formed degenerates completely. The expression of muscle-specific transcription factors and structural genes (such as actin, myogenin, myoD) is turned off after 1 month in the Tet inducer. This phenotype is reversible if the inducer, doxycyclin, is removed from the rearing water. Despite the complete loss of muscle, the patterning and size of the affected limb are normal. Therefore, the TH-induced muscle program is cell autonomous.

520. Dwarf Zebrafish Mutant for *trpm7* Exhibits Defective Skeletogenesis and Kidney Stone Formation. Michael R. Elizondo,[1] Erin L. MacDonald,[1] Brigitte L. Arduini,[2] Jennifer Paulsen,[3] Jaime L. Sabel,[3] Paul D. Henion,[2] Robert A. Cornell,[3] and David M. Parichy[1]. [1]Department of Biology, University of Washington; [2]Center for Molecular Neurobiology, Ohio State University; [3]Department of Anatomy and Cell Biology, University of Iowa.

Development of adult form requires coordinated growth and patterning of multiple traits in response to local gene activity as well as global endocrine and physiological effectors. Here, we analyze the zebrafish *nutria* mutant, which exhibits severe growth retardation and gross alterations in skeletal development. We show that *nutria* mutants have extensive reordering of ossification sequence compared to wild-type siblings, with accelerated ossification of endochondral bones and delayed ossification of intramembraneous bones. *nutria* mutants also exhibit other skeletal malformations, including vertebral column compression and bone malformations. We show that *nutria* is allelic to *touchtone*, and that mutant phenotypes result from mutations in *trpm7*, encoding a Mg^{2+} and Ca^{2+} channel and kinase. We find *trpm7* expression in kidney suggesting that mutant defects in growth and ossification timing reflect physiological consequences of altered cation homeostasis. Consistent with renal dysfunction, we show that mutants develop kidney stones. These results identify a requirement for *trpm7* in growth and skeletogenesis, and highlight the utility of zebrafish as a model for post-embryonic development and disease.

521. The Analysis of *Myf5−/−:MyoD−/−* Amyogenic Embryos Reveals Importance of Early Muscle Contractions in Mouse Skeletogenesis. Irena Rot-Nikcevic,* Tyler Reddy,* Kevin J. Downing,* and Boris Kablar. Dalhousie University, Halifax, NS, Canada B3H 4H7.

The mechanical loading of striated muscle is thought to play an important role in shaping bones and joints. Here, we examined skeletogenesis in late embryogenesis (E18.5) in *Myf5−/−:MyoD−/−* fetuses completely lacking striated muscle. The phenotype included enlarged and kyphotic cervical vertebrae and spinal linearization, a domed neurocranium and varius viscerocranial anomalies (e.g., especially of the mandible), long bone truncation and fusion, absent deltoid tuberosity of the humerus, scapular hypoplasia, deformities of the clavicle and cleft palate and sternum. While the magnitude of individual effects varied throughout the skeletal system, the results are consistent with skeletal development depending on functional muscles. Novel abnormalities in the amyogenic fetuses relative to less severe paralyzed phenotypes (e.g., in the chicken embryos and the mouse *muscular dysgenesis*, *mdg*) extend skeletogenic dependence to early embryonic muscle contraction and static loading of muscle on bone. This work is supported by the NSHRF and CFI grants to BK. TR and KJD are recipients of the NSERC Undergraduate Student Research Awards. *Equally contributed.

522. The Odd-Skipped-Related Transcription Factors Regulate Boundaries of Craniofacial Skeletal Development in Mice. Yu Lan, Qingru Wang, Kathleen M. Maltby, and Rulang Jiang. Center for Oral Biology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Osr1 and *Osr2* encode two Odd-skipped class C2H2-type zinc finger containing putative transcription factors in mammals. They exhibit distinct as well as overlapping expression patterns during mouse craniofacial development. We recently showed that Osr2 is an essential intrinsic regulator of palatal growth and patterning. Here, we report that the Osr1 gene is also essential for craniofacial morphogenesis. Mice homozygous for a targeted null mutation in *Osr1* exhibit micrognathia and cleft palate. Histological and skeletal preparations show that the *Osr1−/−* mutants have shortened mandible with ectopic cartilage growth from the rostral Meckel's cartilage into the developing tongue, which anchors the anterior tip of the tongue to the mandible. In addition, the *Osr1−/−* mutants have aberrant sternal bony fusions as well as fusion between the cranial base and the body of the first cervical vertebrae. Furthermore, mice homozygous for mutations in both *Osr1* and *Osr2* exhibit bony syngnathia and other aberrant skeletal fusions that are not seen in the single mutants. These data, together with the fact that *Osr1* and *Osr2* normally exhibit overlapping expression in tissues surrounding the craniofacial skeletal primordia, indicate that Osr1 and Osr2 normally regulate boundaries of craniofacial skeletal morphogenesis. This research is supported by the NIH/NIDCR grant R01DE13681.

523.Withdrawn.

524. Wnt9a is Required for Maintenance of Synovial Joint Integrity and Temporal Regulation of Chondrocyte Maturation. Daniela Spaeter and Christine Hartmann. Institute of Molecular Pathology, Vienna 1030, Austria.

The formation of skeletal elements of the limb starts with mesenchymal condensations, which form the cartilage templates that are finally replaced by bone via endochondral ossification and are separated by synovial joints. Wnt9a (formally Wnt14) a member of the Wnt gene family, encompassing 19 genes in vertebrates which encode secreted glycoproteins, expressed in the joint and perichondrium in chick and mouse. Gain-of-function studies in chick suggested that Wnt9a is sufficient to induce early steps of joint formation (Hartmann and Tabin, 2001). To address if Wnt9a is necessary for this process, we generated loss-of-function mutations in mouse. Mice lacking a functional Wnt9a gene die within the first 24 h after birth and show normal joint formation. However, in one particular joint, the humeral–radial joint, we observe that synovial cells differentiate into chondrocytes, a condition known in humans as synovial chondroid metaplasia, suggesting that Wnt9a is required for maintaining joint integrity. The joint also serves as an important signaling center involved in controlling chondrocyte maturation. Interestingly, this process is affected in Wnt9a mutants resulting in shortened long bones. Detailed analysis revealed that Wnt9a is temporarily required at E12.5–E13.5 to control the expression of the central regulator of chondrocyte differentiation, Indian hedgehog (Ihh), resulting in temporal down-regulation of Ihh-signaling. Such a temporal and maybe even regional regulation of chondrocyte maturation could serve as a mechanism to fine-tune the size of individual skeletal elements.

525. Canonical Wnt Signaling is Required for Pronephric Kidney Tubule Development. Jon P. Lyons,[1] Tom Deroo,[2] Hong Ji,[1] Elizabeth A. Jones,[3] Kris Vleminckx,[2] and Pierre D. McCrea[1]. [1]Univ. of Texas M.D. Anderson Cancer Center, GSBS Program in Genes & Development, Houston, TX 77030; [2]Ghent Univ., B-9052 Ghent, Belgium; [3]Univ. of Warwick, Coventry, UK.

During vertebrate kidney development, Wnt4 is essential for tubulogenesis; however, the downstream signaling mechanisms are unclear. Here, we elucidate the requirement for canonical Wnt signaling during organogenesis of the *Xenopus laevis* pronephric kidney. A morpholino directed against *xWnt4* has previously been shown to ablate pronephric kidney tubule development. To prove that β-catenin signaling plays a critical role downstream of the Wnt4 signal in the context of tubulogenesis, we have employed constructs that inhibit the canonical Wnt pathway. We find that inhibition of β-catenin signaling using two distinct inhibitory constructs ablates formation of pronephric kidney tubules in a dose-dependent manner. By utilizing an inducible construct to temporally inhibit β-catenin signaling just prior to *xWnt4* pronephric expression, we find that the undesired effect of axis bending is eliminated and somitogenesis is normal even when kidney tubules are absent. We are currently addressing the mechanism for loss of kidney tubulogenesis that

results from inhibition of β-catenin signaling. For example, we are testing if decreased cell proliferation or death is involved, and for possible effects upon cell specification and/or differentiation. Last, we are employing inducible constructs to activate β-catenin signaling to rescue the xWnt4 morpholino depletion phenotype.

526. Requirement of the Mesonephros for the Specification of the Indifferent Gonad. Jamil B. Scott[1] and Thomas M. Schultheiss[2]. [1]Harvard Medical School; [2]Beth Israel Deaconess Medical Center.

The indifferent gonad is a unique organ in that it has the potential to give rise to either an ovary or a testis, while in other organ systems, one specific tissue primordium gives rise to only one specific terminally differentiated organ. Much effort has gone towards understanding later time points in gonad development, such as sex determination and sexual differentiation, whereas relatively little attention has been given to understanding the events that cause the initial specification of the indifferent gonad. Using the avian embryo, we have determined the time and place of expression of various genes with a role in gonad development. These genes include WT-1, Lhx9, DMRT-1, AMH, and Sox-9. Expressed at HH stage 20, Sox-9 was determined to be the earliest gonad-specific marker. We have designed an in ovo microsurgery experiment that tests the requirement of the mesonephros in the specification of the indifferent gonad and have learned that in the absence of the mesonephros, gonad-specific genes are not expressed. We have set up an in vitro culture system to determine which tissues are specified to give rise to the gonad and when they gain this specificity. At stages 17 through 19, the urogenital ridge can be isolated and cultured for at least 3 days after the operation. These explants go on to express the gonad marker, Lhx9. We will further characterize the effects of the loss of the mesonephros on gonad specification and development by investigating cell death, cell replication, and the migration of primordial germ cells.

527. Growth and Development of the Müllerian Duct: Cellular Behavior and its Relation to the Wolffian Duct. Grant D. Orvis and Richard R. Behringer. Univ. of Texas M.D. Anderson Cancer Center, Houston.

Birds, reptiles, and mammals form two genital ducts, the Wolffian and Müllerian ducts, which are located lateral to the fetal gonads. Both ducts are simple tubes consisting of an epithelium with a surrounding mesenchyme. Initially, the Wolffian ducts form, giving rise to male-specific organs, followed laterally by the Müllerian ducts. The Müllerian ducts differentiate into female-specific organs, but little is known about the genetics and behavior of cells as they organize into the female reproductive tract. Prior studies suggest that the developing tip of the Müllerian duct grows using the Wolffian duct as a guide. Previously, experiments have shown that when Wolffian duct development is interrupted by the loss of *Lim1*, the Müllerian duct does not grow past the point of interruption. This suggests that the existence of the Wolffian duct is essential for Müllerian duct development. It is our goal to decipher the relationship of the Müllerian duct to the Wolffian duct and understand cellular behavior during uterine organogenesis. We will utilize transgenic and knock-in mice to visualize the epithelial and mesenchymal interactions of the two ducts during Müllerian duct development. *Lim1* is expressed in the epithelia of both the Wolffian and Müllerian ducts, *Wnt7a* is expressed in the Müllerian duct epithelium, while *Amhr2* is expressed in the Müllerian duct mesenchyme. We will exploit the expression patterns of these three genes to visualize the relationship between the two ducts using lacZ alleles as well as new and improved variants of GFP.

528. Hypoplasia, Duplex Ureters and Hydronephrosis in p53-deficient Mice. Zubaida R. Saifudeen, Jana Stefkova, Susana Dipp, and Samir S. El-Dahr. Department of Pediatrics, Tulane University Health Sciences Center, New Orleans, LA 70112.

Metanephric kidney development depends on reciprocal inductive interactions between the ureteric bud and the metanephrogenic mesenchyme (MM), a specified nephrogenic region at the caudal end of the intermediate mesoderm. Release of glial-derived neurotrophic factor (GDNF) by the MM stimulates the outgrowth of a single ureteric bud (UB) from a specific position on the nephric duct (ND). We show here that up to 50% of the progeny of p53 null mice examined at E13.5, postnatal days 1 and 23 exhibited one or more abnormality in metanephric development, including duplex ureters, aberrant UB branching, hypoplasia, and hydronephrosis. These defects were recapitulated by conditional kidney-specific inactivation of p53 in genetic crosses of Ksp-cadherin1.3-Cre+ to p53loxP/loxP mice. In situ hybridization at E13.5 revealed that p53 transcripts are expressed in the mesonephros, ND, and MM. In the MM, p53 mRNA expression overlaps with that of Eya1 and Pax2. Furthermore, in transient transfection assays, p53 strongly activates the transcription of a 4.3-kb mPax2 promoter–reporter construct in cultured UB cells. Given that Pax2 represses p53 but activates GDNF transcription, these findings suggest that p53 may be part of the transcriptional network in the MM which control UB budding and subsequent branching.

529. The Mouse *Fgf8* Gene is Required for Nephrogenesis but not for Somitogenesis. Mark Lewandoski,[1] Olga Timofeeva,[2] Florence Naillat,[3] Charmaine Richman,[1] Sangeeta Pajni-Underwood,[1] Catherine Wilson,[1] Seppo Vainio,[3] and Alan O. Perantoni[2]. [1]Cancer and Developmental Biology Laboratory, National Cancer Institute, NCI-Frederick, Frederick, MD 21702; [2]Laboratory of Comparative Carcinogenesis, National Cancer Institute, NCI-Frederick, Frederick, MD 21702; [3]Biocenter Oulu and Department of Biochemistry, Faculties of Science and Medicine, University of Oulu, PO Box 3000, FIN-90014 Oulu, Finland.

To bypass the essential gastrulation function of *Fgf8* and study its role in primitive streak derivatives, we have generated and characterized a new mouse line, T-Cre, in which Cre is driven by *Brachyury* regulatory sequences. Using T-Cre, we have generated mouse embryos with pan-mesodermal loss of *Fgf8* expression. Surprisingly, despite previous models assigning *Fgf8* a pivotal role in segmentation/somite differentiation, *Fgf8* is not required for these processes. However, mutant neonates display severe renal hypoplasia with a lack of nephronic epithelial differentiation. In mutant kidneys, aberrant cell death occurs within the metanephric mesenchyme (MM), particularly in the cortical nephrogenic zone, which provides progenitors for recurring rounds of nephron formation. Mutants fail to convert MM to the epithelia of the nephron. Prior to phenotypic changes in mutant morphology, *Wnt4* and *Lim1* expression, which is essential for nephrogenesis, is absent in developing MM. Furthermore, comparative analysis of Wnt4-null homozygotes reveals a similar renal phenotype with a concomitant downregulation of *Lim1*. Our data support a model whereby FGF8 and WNT4 function in concert to induce expression of *Lim1* for MM survival and tubulogenesis.

530. The Role of Gata3 During Kidney Development. David Grote,[1] Abdallah Souabni,[2] Meinrad Busslinger,[2] and Maxime Bouchard[1]. [1]McGill Univ., Montréal, Canada; [2]Research Institute of Molecular Pathology, Vienna, Austria.

Gata3 belongs to the conserved Gata-family of Zn-finger transcription factors, which perform essential functions during organogenesis. The significant role of Gata3 in human development was first revealed in a human inheritable disease, the HDR syndrome. This autosomal dominant disorder can be characterized by hyperparathyroidism, deafness, and renal dysplasia. Furthermore, there exists growing evidence that Gata3 might be involved in tumorigenesis. It was previously shown that Pax2/8 specify the onset of the nephric lineage. A cDNA-microarray screen for Pax2 target genes in the pro/mesonephros (1st and 2nd embryonic kidneys) identified

Gata3 as a promising candidate gene. Expression studies ranked Gata3 among the earliest lineage markers. In addition, Gata3 levels are down-regulated in the pronephros of Pax2−/−Pax8+/− embryos, confirming our micro-array results. To investigate the role of Gata3 in more detail, we generated Gata3-KO mice. The analysis of the Gata3-deficient embryos reveals a strong nephric phenotype. Strikingly, the induction of the metanephros (adult kidney) fails completely in the Gata3−/− embryos and the development of the mesonephros (nephric duct) is severely affected. Most notably, Gata3 appears to be involved in the maintenance of epithelial integrity.

531. Forming Organs from Tubes: Identification of a Common Transcriptionally Regulated Morphogenetic Step for Many Organs. Todd Evans and Audrey Holtzinger. Albert Einstein College of Medicine, Bronx, NY 10461.

Tube formation from an epithelial sheet is a well-studied first step of organogenesis. In contrast, genetic programs that orchestrate the subsequent growth of organs from tubes are less well understood. In particular, it has not been considered that organs as diverse as heart and liver might share common morphogenetic programs. We developed a loss-of-function model in zebrafish for a transcription factor of the GATA class. We find that primordial cardiac and gut tubes form normally in the deficient embryos. Initial morphogenetic movements also occur, including heart tube constriction and movement to the left, intestinal tube looping to the left, and initial pancreatic budding. However, subsequent organ growth fails for the heart, intestine, liver, pancreas, and swim bladder. Therefore, a single transcription factor regulates formation of many organs. Our results suggest the existence of a common morphogenetic step, shared by disparate organ systems derived from diverse tube primordia, regulated by a single transcription factor, and associated with organ growth following tube formation.

532. Novel Functions for GATA4 Revealed by the Zebrafish Model. Audrey Holtzinger and Todd Evans. Department of Developmental and Molecular Biology, Albert Einstein College of Medicine.

GATA4 is a member of a family of zinc finger transcription factors that are highly conserved in vertebrates. Mutations in the human GATA4 are implicated in causing congenital cardiomyopathies. Mouse embryos lacking GATA4 die by E9.5 due to a requirement in visceral endoderm for normal ventral morphogenesis, but the embryonic lethality of the mouse mutation has precluded a complete analysis of GATA4 function. Zebrafish embryos do not rely on extra-embryonic structures, and can survive well into larval stages without a normal heart. Therefore, we generated GATA4-deficient zebrafish embryos using morpholino oligomers. We used GATA4::GFP reporter fish to confirm the ability of the morpholino to target GATA4 mRNA. The heart tube forms in GATA4-deficient fish and expresses differentiated heart markers, such as Nkx2.5, amhc, vmhc, cmlc2, and tie2. However, the complete looping of the heart tube fails to occur, leading to a heartstring phenotype by 4dpf. RT-PCR as well as whole mount in situ hybridization analysis showed normal endoderm development. The use of a GATA6::GFP reporter fish allowed us to track the development of endoderm into different organs. In fish depleted for GATA4, the gut tube does not differentiate properly and fails to give rise to the intestine, liver, or pancreas. These data were confirmed by histology and analysis of markers by in situ hybridization. More surprisingly, fish deficient for GATA4 lack blood. While primitive hematopoiesis and the vasculature appear normal, the blood defect observed here appears to correlate with a block in definitive hematopoiesis.

533.The Role Of BMP Signaling in Patterning the Common Thymus–Parathyroid Primordium. Julie Gordon, Seema R. Patel, Farah Mahbub, and Nancy R. Manley. University of Georgia, Athens, GA 30602.

The thymus and parathyroids originate from the third pharyngeal pouches, endodermal outpocketings that form on embryonic day 9 (E9.0) of mouse development. The common thymus–parathyroid primordia separate from the pharynx around E12.0. We have previously shown that the Foxn1 and Gcm2 are expressed in the thymus and parathyroid domains within the primordium prior to formation of the organs proper. Using Bmp4-lacZ reporter mice, we demonstrate that Bmp4 expression is confined to the thymus domain, while Noggin, a Bmp4 antagonist, seems to be co-expressed with Gcm2. Bmp4-lacZ expression originates at the ventral tip of the third pharyngeal pouch at E10.5 then extends throughout thymus domain in a pattern similar to Foxn1. This pattern suggests two possible roles for Bmp signaling: in the initiation of thymus organogenesis and the induction of Foxn1 expression, and in the formation and/or maintenance of the organ boundary between the thymus and parathyroid domains. Preliminary results from conditional gene knockout studies in which Bmp signaling is ablated, via either the Bmp4 ligand or its receptor, BmpR1A, in the pharyngeal endoderm, the early thymic epithelial cells or the neural crest-derived mesenchyme suggest that Bmp signaling is involved in maintenance or establishment of the thymus–parathyroid organ boundary within the common primordium, and formation or maintenance of the outer capsule. Additional studies suggest that ectopic or over-expression of Bmp4 can induce cells in the parathyroid domain to change fate, suggesting a role in patterning the early primordium.

534. Genetic Analysis of the Role of *Gcm2* in Parathyroid Organogenesis and PTH Expression. Zhijie Liu and Nancy R. Manley. Genetics, University of Georgia, Athens, GA 30602.

In mice, the parathyroid gland develops with the thymus from bilateral, shared primordia that form from the third pharyngeal pouch, one of a serial transient outpockets of the pharyngeal endoderm. The parathyroid/thymus shared primordia form on E11, and physically separate into parathyroid and thymus organs only by E13.5. *Gcm2*, a murine homolog of the *Drosophila Gcm* gene, expresses in the presumptive parathyroid domain from E9.5 stage. Consistent with its expression pattern, *Gcm2* null mutation led to the complete absence of parathyroid. According to our data, the parathyroid failure in *Gcm2* null mutant is caused by the loss of the parathyroid domain in the parathyroid/thymus shared primordia during organogenesis, suggesting that *Gcm2* is required to specify or maintain the parathyroid domain in the parathyroid/thymus shared primordia. To our surprise, *Gcm2* also expresses at low level in the thymus, and in Gcm2 null mutants, thymic parathyroid hormone (PTH) but not calcium-sensing receptor (CasR) expression was greatly reduced, suggesting that *Gcm2* is also required to maintain PTH expression in these parathyroid-like cells in the thymus. In order to clarify the mechanism by which *Gcm2* functions in the parathyroid cells, we induced ubiquitous *Gcm2* ectopic expression. *Gcm2* ubiquitous expression caused neonatal lethality due to respiration problem. We found high concentrations of ionic calcium and inorganic phosphorus in the mutant mice serums, suggesting that ectopic or unregulated *Gcm2* expression results in a phenotype similar to hyperparathyroidism. We are continuing to check whether forced *Gcm2* expression induces ectopic parathyroid-like cells or causes unregulated PTH secretion.

535. Jagged2-Notch1 Signaling is Required for Proper Oral Epithelial Differentiation and Palate Development. Liam M. Casey,[1] Yu Lan,[1] Eui-Sic Cho,[2] Kathy M. Maltby,[1] Thomas Gridley,[3] and Rulang Jiang[1]. [1]Univ. of Rochester School of Med. and Dent., Rochester, NY 14642; [2]Chonbuk Nat. Univ. School of Dent., Chonju, Rep. of Korea; [3]The Jackson Lab, Bar Harbor, ME 04609.

Cleft palate is a frequent birth defect that occurs when any of a number of genes controlling different steps in the process of normal palate

development is disrupted. The Jagged 2 (Jag2) gene, encoding a ligand for the Notch signaling pathway, is expressed throughout the developing oral epithelium (OE). Targeted disruption of Jag2 causes the developing palatal shelves to fuse with the tongue [Jiang et al., *Genes Dev* 12:1046–57, 1998]. We report that ectopic adhesion is more widespread in the OE of Jag2 mutants and this phenotype is also observed in mice homozygous for the spontaneous syndactylism mutation, a hypomorphic missense point mutation in the Jag2 gene. We show that Notch1, the predominant Notch receptor expressed in the developing OE, is normally activated in the suprabasal and periderm layers of the OE and tongue. In Jag2 mutants, epithelial activation of Notch1 is significantly reduced, which correlates with defective organization of the tongue epithelium. Further evidence that Jag2-induced Notch signaling in the tongue is critical comes from recombinant palate–tongue explant culture experiments, which suggest that loss of Jag2 in the tongue alone can result in adhesion between the palate and the tongue. Analysis of other signaling pathways that play critical roles in regulating normal palate fusion shows that these signaling pathways are unaffected in Jag2 mutants. These data indicate that Jag2-Notch1 signaling plays a primary role in OE differentiation.

536. Mammalian Twisted Gastrulation Gene Loss of Function and Embryonic Salivary Gland Branching. Tina Jaskoll,[1] Anna Petryk,[2] George Abichaker,[1] Dan Witcher,[1] and Michael Melnick[1]. [1]Univ. of Southern California, Los Angeles, CA 90089-0641; [2]Univ. of Minnesota, Minneapolis, MN 55455-0356.

Mammalian twisted gastrulation gene (*Twsg1*) expression is found throughout embryonic development, including substantial levels in the first branchial arch that gives rise to the submandibular salivary gland (SMG). Immunolocalization studies demonstrate the distribution of Twsg1 protein throughout SMG branching epithelia. We addressed the proposition that normal *Twsg1* expression is critical to normal SMG ontogenesis by investigating SMG development in $Twsg1^{-/-}$ embryos in C57BL/6 background, as well as wild-type and heterozygote littermates. $Twsg1^{-/-}$ embryos with a primitive mandibular arch, but no evidence of several arch-derived structures (Meckel's cartilage, bone, teeth, tongue), exhibited an SMG that is abnormally positioned and ontogenically arrested. Salivary glands from phenotypically normal $Twsg1^{-/-}$ embryos exhibited age-appropriate SMG development, identical to that of their normal $Twsg1^{+/+}$ and $Twsg1^{+/-}$ littermates. Twsg1 genotype and external craniofacial phenotype were correlated to SMG phenotype. Our results indicate that the widely variable phenotypic expression noted previously in the craniofacies of $Twsg1^{-/-}$ mice was also found in the salivary glands. Supported by NIDCR (NIH) Grant number: RO1 DE014535 (TJ/MM); NICHD (NIH) Grant number: K08-HD043138 (AP).

537. The Making of the Liver. Catherine S. Lee, Joshua R. Friedman, James T. Fulmer, and Klaus H. Kaestner. University of Pennsylvania, Philadelphia, PA 19104.

The specification of the vertebrate liver is thought to occur in a two-step process, beginning with the establishment of the competence of the foregut endoderm to respond to organ-specific signals, followed by the induction of liver-specific genes. It has been proposed that the Foxa proteins establish competence by opening compacted chromatin structures within liver-specific target genes, based on the timing and location of their expression as well as in vitro studies. Here, we show that Foxa1 and Foxa2 are required in concert for hepatic specification. No liver bud is evident and expression of the hepatoblast marker alpha-fetoprotein (afp) is lost in embryos deficient for both genes in the foregut endoderm. Furthermore, Foxa1/Foxa2-deficient endoderm cultured in the presence of exogenous FGF2 fails to initiate albumin or transthyretin expression. Thus, Foxa1 and Foxa2 are required for the establishment of competence within the foregut endoderm and the onset of hepatogenesis.

538. Genetic and Genomic Approaches to Study Liver Development in Zebrafish. Jinrong Peng, Honghui Huang, Lin Guo, Chuan Gao, Wei Cheng, Mengyuan Aw, Jun Chen, and Evelyn Ng. Functional Genomics Laboratory, Institute of Molecular and Cell Biology, Singapore.

The main goal of this lab is to identify genes involved in liver initiation and development in zebrafish. We have screened ~600 F1 families for liver defect mutants based on prox1 signal as a screening marker and 21 putative mutants have been isolated. Three rounds of backcross have been performed to clean the genetic background of these mutant lines. One mutant gene has been cloned recently via positional cloning method and another mutation has been narrowed down to a contig containing three BAC clones. The rest of mutants have been characterized by using molecular markers for four major digestive organs, namely, gut tube (IFABP), liver (LFABP), exocrine pancreas (trypsin), and endocrine pancreas (insulin) and mutants are further classified into five categories based on the phenotype displayed by these organs. We aim to clone all mutant genes in the near future with hope to reveal how these genes are involved in controlling liver development. In addition, our lab has obtained ~15,000 unique cDNA clusters via our EST sequencing project and generated zebrafish cDNA microarrays using these unique clusters. We have used these homemade microarrays and also gene chips from Affymetrix to identify liver-enriched genes. ~150 genes obtained in our microarray hybridization were further confirmed as genuine liver-enriched genes via Northern blot hybridization. These genes will be used to compare the gene expression patterns between mutants versus the WT control to identify downstream genes and as candidates for functional study via gene knock-down methods in the future.

539. Vhnf1 Integrates BMP, RA, and FGF Signaling Pathways to Regulate Pancreas Development in Zebrafish. Jianbo Song, Hyon Kim, and Shuo Lin. Univ. of California, Los Angeles, CA 90095.

BMP, RA, and FGF signaling pathways have been implicated to play important roles in pancreas development. However, how these signaling pathways are integrated in vivo is not fully understood. Vhnf1 is an important transcription factor for liver, kidney, and pancreas development. Zebrafish vhnf1 mutants exhibit underdeveloped pancreas and liver. It is reported that Vhnf1 is required for Pdx-1 expression in the gut endoderm. We investigated the signaling pathways that regulate Vhnf1 expression. BMP signal establishes the dorsal–ventral axis of zebrafish. It has been shown that BMP signaling also regulates the anterior–posterior endoderm pattering in zebrafish. We show that BMP signaling is required for Vhnf1 expression in the endoderm. In Chordin Morphant, Vhnf1 expression in endoderm is expanded. Beta cells in Chordin Morphant also expanded as shown by in situ hybridization using insulin probe. On the other hand, in alk8 morphant, Vhnf1 expression in endoderm is reduced. We have also shown that FGF signaling participates the regulation of Vhnf1 expression pattern in endoderm. Embryos treated with RA inhibitor failed to develop pancreas. Vhnf1 mRNA injection in embryos treated with RA inhibitor rescues the development of pancreas. Taken together, our data suggest that Vhnf1 integrates the signaling pathways of RA, BMP/Chordin, and FGF to regulate pancreas development in zebrafish.

540. Cdx4 is Required to Correctly Position the Zebrafish Pancreas. Mary D. Kinkel, Martha Alonzo, David Stafford, and Victoria Prince. The Univ. of Chicago, Chicago, IL 60637.

Previous work in our lab has demonstrated that retinoic acid (RA) is required to specify the vertebrate pancreas. While anterior endoderm is competent to respond to exogenous RA by differentiating into β-cells, posterior endoderm is not. This suggests that additional mechanisms act to restrict β-cell differentiation to the proper AP location. Here, we examine

the role of the caudal homeobox gene *cdx4* in positioning the pancreas along the AP axis. Cdx genes function upstream of Hox genes and are thought to integrate RA and FGF signaling. In 48 hpf zebrafish embryos mutant for *cdx4*, whole mount in situ hybridization for *insulin* and *glucagon* shows that the endocrine pancreas is shifted posteriorly by two somite widths. Expression patterns of *trypsin* and *cebp4* indicate, respectively, that the exocrine pancreas and liver are also shifted posteriorly. This suggests that Cdx4 has a critical role in regionalizing the endoderm. Time-lapse recordings of live embryos expressing insulin-GFP demonstrate that pancreatic precursors initially appear in two clusters anterior to somite one on either side of the midline. Over the course of several hours, these cells converge and move posteriorly to become associated in a single cluster subjacent to somite four. Live imaging of insulin-GFP embryos lacking Cdx4 reveals that pancreatic precursors are initially spread farther along the AP axis, are delayed in converging, and ultimately move farther posteriorly than in wild-type embryos. This suggests that *cdx4* functions early in endodermal regionalization to position organs at the appropriate AP level.

541. Manic Fringe Induces Endocrine Differentiation Through Transcription Factor Myt1. Guoqiang Gu, Yanwen Xu, Sui Wang, Jia Zhang, and Aizhen Zhao. Vanderbilt Medical Center, Dept. of Cell and Dev. Biol., Nashville, TN 37232.

During vertebrate embryogenesis, a group of endodermal cells that locate in the foregut–midgut junction gives rise to the adult pancreas. Throughout later stages of gestation, these seemingly equivalent endodermal progenitors continuously differentiate into acinar, pancreatic duct, and four major endocrine cell types. Notch signaling plays an essential role in controlling both exocrine and endocrine differentiation. It is generally accepted that active Notch prevents pancreatic progenitors from differentiating, whereas inactive Notch allows pancreatic differentiation. Because the Notch receptors and their ligands seemed ubiquitously expressed in all pancreatic progenitor cells, it is not known what signal(s) initiates pancreatic differentiation only in a selective set of progenitors. Using a micro-array-based gene expression analysis, we have identified manic fringe (Mfng) as a gene that is exclusively expressed in the endocrine progenitor cells. Because Mfng encodes a glycosyltransferase and it modifies Notch signaling, we postulated that Mfng expression initiates endocrine differentiation. Using chicken embryonic endoderm as an assay, we have demonstrated that ectopic Mfng expression is sufficient to initiate islet formation by inducing the expression of a zinc finger transcription factor, MYT1. Further analysis indicates that Myt1 expression is not only sufficient, but also necessary for ectopic endocrine development. Our data suggest the expression of Mfng as the trigger for endocrine islet differentiation.

542. Role of Ptf1a in Exocrine and Endocrine Pancreas. Zeina H. Jarikji[1] and Marko E. Horb[2]. [1]Univ. de Montreal, Canada; [2]Institut de Recherches Cliniques de Montreal (IRCM), Canada.

In *Xenopus* tadpoles, endocrine and exocrine cells initially develop separately—endocrine cells come from the dorsal pancreas and exocrine cells from the ventral pancreas. The bHLH protein Ptf1a is known to be one of the key transcriptional regulators of exocrine pancreas development. Recent results have suggested that Ptf1a may also be involved in endocrine cell fate specification. To address this question, we have examined the role of Ptf1a in endocrine and exocrine cell development in *Xenopus* pancreas. We have cloned the full length of *Xenopus* Ptf1a cDNA and found that it is expressed in both the dorsal and ventral pancreas at the earliest stages. Two Ptf1a Morpholinos were designed to inhibit either translation initiation or splicing and were injected separately or together into *Xenopus* embryos. Ptf1a-MO-injected tadpoles do not develop any exocrine cells. In addition to the exocrine phenotype, morphants show reduced expression of the endocrine marker insulin. We have also performed gain-of-function studies by overexpressing Ptf1a in the liver in transgenic tadpoles and found that Ptf1a is able to convert liver to pancreas. These results suggest that the Ptf1a is a master switch gene that has a role in the specification of both exocrine and endocrine pancreas.

543. The Basic Leucine Zipper Transcription Factor MafB Regulates Glucagon Gene Transcription and is Critical for Alpha and Beta Cell Development. Isabella Artner,[1] John Le Lay,[1] Bruno Blanchi,[2] Michael Sieweke,[2] and Roland Stein[1]. [1]Vanderbilt University Medical Center, Nashville, TN 37232; [2]Centre d'Immunologie de Marseille-Luminy, Marseille, France.

Glucose homeostasis is maintained by counter-regulatory actions of the pancreatic hormones insulin and glucagon. Defects in the production of these hormones by islet alpha (glucagon+) and beta (insulin+) cells preclude diabetics from maintaining glycemic control. Identification and characterization of transcription factors regulating insulin and glucagon expression have not only revealed their significance in islet function, but also during pancreatogenesis. Here, we show that the MafB transcription factor is only expressed in alpha cells in the adult pancreas. MafB was shown to contribute to cell-type-specific expression of the glucagon gene. This factor is expressed in developing alpha and beta cells, as well as in proliferating hormone negative cells during pancreatogenesis. Initial analysis of mafB−/− embryos indicates an important role during endocrine cell differentiation, as mutant animals have reduced alpha and beta cell numbers. Moreover, the production of insulin+ cells was delayed in MafB mutant embryos until E13.5, a time point consistent with the onset of MafA expression, suggesting a critical role for MafB in both insulin and glucagon transcription during development. These results indicate that MafB not only regulates islet alpha cell function, but is also involved in endocrine cell differentiation.

544. Identification and Characterization of CTGF as a Factor Involved in Islet Development and Beta Cell Expansion. Laura A. Wilding,[1] Elizabeth Tweedie,[1] Braden Boone,[1] David R. Brigstock,[2] Karen M. Lyons,[3] Shawn Levy,[1] and Maureen A. Gannon.[1] [1]Vanderbilt University, Nashville, TN 37232; [2]The Ohio State University, Columbus, OH 43212; [3]University of California, Los Angeles, Los Angeles, CA 90095.

We have used a transgenic mouse model of HNF6 over-expression to identify genes involved in pancreatic islet morphogenesis. Since HNF6 transgenic (Tg) animals possess islets with a disrupted architecture and function, we used them as a tool to identify factors important for pancreatic islet formation and function. Factors underlying this process remain largely unidentified to date. Using the Affymetrix mouse genome array, we compared differences in gene expression profiles of pancreata from HNF6 Tg and wild-type (WT) animals at postnatal day 1, a time point critical in islet morphogenesis. Results from our microarray analysis demonstrate a 2-fold down-regulation of *Connective Tissue Growth Factor* (*ctgf*) in HNF6 Tg animals compared to WT. These results have been confirmed using qRT-PCR. Subsequent analysis has shown that Ctgf is expressed during development in insulin-expressing cells of the WT pancreas at stages critical for islet morphogenesis and β cell replication. Additionally, Ctgf expression is associated with proliferation in experimental models known to cause β cell replication and neogenesis (pregnancy and 60% partial pancreatectomy). Analysis of ctgf null mutant mice reveals that the effects of *ctgf* inactivation differ in the dorsal and ventral pancreas and appear to be non-cell autonomous. We report Ctgf as a new factor potentially important in islet development and β cell replication.

545. Nkx2.2 Functions as a Transcriptional Repressor in Islet Development. Michelle J. Doyle, Keith Anderson, and Lori Sussel. Univ. of Colorado HSC, Aurora, CO 80045.

Nkx2.2, a homeodomain transcription factor, is essential for the development and differentiation of pancreatic islet cells. Mice lacking Nkx2.2 have a complete loss of β cells and a significant loss of a cells and PP cells. Conversely, the islets of Nkx2.2 mutant mice have a striking increase in cells producing ghrelin. While it is clear that Nkx2.2 plays a critical role in the differentiation of islet cell types, it is unclear how Nkx2.2 functions at the molecular level. To determine the molecular function of Nkx2.2 in the islet, we have generated transgenic mice expressing dominant derivatives of Nkx2.2 in the islet. Transgenic mice carrying the dominant repressor derivative of Nkx2.2 (Nkx2.2-repressor) survive into adulthood, but are overtly diabetic. In Nkx2.2-repressor mice, islet architecture is disrupted due to mislocalization of α cells, while β cells appear to be unaffected. To determine whether the Nkx2.2-repressor can functionally substitute for wild-type Nkx2.2, we introduced the Nkx2.2-repressor transgene into the Nkx2.2 null mouse background. The Nkx2.2-repressor partially rescues the Nkx2.2 mutant pancreatic phenotype. There is both an increase in the number of insulin cells and a decrease in ghrelin cells compared to the Nkx2.2 null background. These studies are being complimented by analysis of the dominant active Nkx2.2 derivatives. In Nkx2.2-activator mice, there is a loss of mature hormone-producing islet cells; therefore, differentiation of endocrine cells appears to be blocked. Preliminary experiments suggest Nkx2.2 functions through NeuroD, an islet transcription factor that is essential for terminal endocrine cell differentiation.

546. Gene Expression Analysis of Embryonic Pancreas Differentiation. Kirstine Juhl,[1] Gerard Gradwohl,[2] Jan Jensen,[1] and John Hutton[1]. [1]BDC, UCHSC, Denver, CO 80262, USA; [2]INSERM, Unit 381, 67200, Strasbourg, France.

The mammalian pancreas is specified on the foregut endoderm and starts to bud at e9.5 and expands by branching morphogenesis. During this development, the expression of a number of genes, including those encoding transcription factors, growth factors, and signaling pathways, are enriched in the epithelium and/or mesenchyme of the distal buds which differentiates into the major endocrine, exocrine, and ductal cell types over the ensuing 10 days. We wanted to investigate in detail the transcriptome that are differentially expressed in the pancreas during these processes. To do this, we combined DNA microarray analysis (Affymetrix) with immunohistochemistry, in situ hybridization, and RT-PCR. We have focused on pancreatic tissue at day e11.5 to e18.5 where the majority of pancreatic proliferation and differentiation takes place. Epithelial–mesenchymal interactions which play an important role in e11.5 pancreatic anlage were associated with tissue-specific expression of lhx9, hoxa7, nkx2.5, Tgfbi in the mesenchyme and Nkx2.2, Ambp, Dsg2, Ces3, AA791885, Vanin1 in the epithelium. Interesting new candidates in the early pancreas development Sox11, Pitx2, Sfrp1, and Snai2 were highly expressed in both M&E at this stage. Studies with Ngn3 knock-out mice were used as a model to study endocrine specific expression as the hormone-producing cells fail to develop in these animals. The importance of Ngn3, NeuroD1, Pax4, Pax6, Brn4, Isl1, Nkx2.2, and Nkx6.1 in endocrine cell specification was confirmed. Other molecules of potential interest as regulators of this process were identified as Pet1, Insm1, and Wbscr14.

547. Sympathetic Innervation of the Murine Pancreas During Development. Regina E. Burris and Matthias Hebrok. UC San Francisco Diabetes Center, San Francisco, CA 94143-0540.

The developing pancreas of the mouse provides a dynamic system in which to study autonomic nervous development during pancreas organogenesis. Tools and techniques have been refined with which to assay sympathetic innervation of the pancreas from early organogenesis (e10.5) through organ maturation to the adult stage (p21). This analysis places neural development and pancreatic islet innervation in the context of both endocrine organization and pancreatic vascularization. Comparison of wild-type and Pax4-Shh transgenic animals reveals that a transient developmental delay in islet formation does not interfere with stages of sympathetic neural development in the pancreas. The RIP-cmycER mouse line, a model of β-cell regeneration, is being utilized to explore the effect of large-scale apoptosis and regeneration of beta cells on the maintenance of sympathetic islet innervation.

548. The Regulation of Sympathetic Nervous System Development by BMP and bHLH Factors. Yuka Morikawa, Baris Genc, Taneasha M. Washington, and Peter Cserjesi. Dept. Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

The sympathetic nervous system (SNS) is derived from the neural crest (NC) lineage that migrate to the dorsal aorta and other major blood vessels from which an inductive cue emanates to promote SNS-specific differentiation. BMP is expressed in blood vessels and has the ability to activate the SNS developmental program in vitro while BMP inhibition in vivo prevents NC cells from differentiating into neurons. BMP induction of SNS differentiation activates the expression of a number of transcription factors including two members of basic helix–loop-helix (bHLH) family, Mash1and Hand2. Loss of Mash1 results in a hypotrophic SNS while little is known about the role of Hand2 in SNS development due to early lethality in Hand2 null embryos. However, ectopic studies in vitro and in vivo have suggested that Hand2 promotes SNS development. We show that activation of *Hand2* and *Mash1* expression occurs concurrently and that Hand2 can activate the expression of *Mash1*. This suggests that these bHLH factors are regulated independently and that Hand2 activation of SNS developmental occurs in part through the activation of *Mash1*. We show that Mash1 is required for the amplification of SNS neuroblast precursor cells by promoting their proliferation and BMP induces NC differentiation and acts to promote their proliferation. This suggests that BMP has a dual role in SNS development, activation of the differentiation program, and maintenance of proliferation, possibly through the activation of *Mash1*. This work was supported by grants to P.C. from NSF (IBN-0345924) and NIH (2RO1NS015547-22).

549. Migratory Behaviors of Adrenomedulla-Fated Neural Crest Cells are Instructed by the Adrenocortical Cells. Daisuke Saito and Yoshiko Takahashi. Center for Developmental Biology, RIKEN, Kobe, Japan.

During organogenesis, distinct populations of cells originating from different places encounter each other to make functional organs. A subpopulation of neural crest cells (NCCs), fated to differentiate into the chromaffin cells of the adrenal medulla, migrate ventrally until they encounter the adrenal cortex, originating from the coelomic epithelium. Exploiting the simple composition of the adrenal gland, we asked which component, the medulla or cortex, directs the encounter of these two cell types. Electroporation with *Steroidogenic Factor-1* (*SF-1*), a gene encoding a nuclear receptor essential for the formation of adrenal gland, into the coelomic epithelium of a non-adrenal gland-forming region resulted in the formation of an ectopic cortex-like structure, expressing several cortical markers. In addition, tyrosine hydroxylase (TH)-positive NCCs cells, normally observed in the sympathetic ganglia and adrenal medulla, were located adjacent to the *SF-1*-electroporated cells. Thus, the *SF-1*-expressing cells appear to attract NCCs. In contrast, overexpression of *Noggin* in the adrenal-forming region prevented NCCs from participating in the medulla. Our findings suggest that during the formation of the adrenal gland, the nascent cortex instructs the migration of NCCs, which ultimately differentiate into the adrenal medullary chromaffin cells. And these interactions appear to be mediated by *SF-1* and *BMPs*.

550. Chick-quail Intestinal Transplants—A New Method for Studying Enteric Nervous System Development. Nandor Nagy and Goldstein M. Allan. Department of Pediatric Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA.

Neural crest cells migrate, proliferate, and differentiate within the wall of the gastrointestinal tract to give rise to the neurons and glial cells of the enteric nervous system (ENS). The intestinal microenvironment plays a critical role in this process and endothelin-3 (ET-3) is one of the factors known to have an essential role. While mutations of this gene are known to cause distal colonic aganglionosis, its mechanism of action is poorly understood. In order to determine the role of ET-3 in development of the hindgut ENS, we developed a novel embryologic assay using chick–quail chimeric intestinal transplants. Pre-ganglionic hindguts from 5-day-old quail embryos were treated with ET-3 or BQ788 (an ET-3 inhibitor), transplanted into the coelomic cavity of 3-day-old chick embryos, and allowed to develop for 7 days. In this assay, untreated control quail grafts develop normal submucosal and myenteric plexuses which are comprised entirely of chick neural crest cells, indicated by immunoreactivity to both the chick-specific antibody, 8F3, and Hu, a neuronal marker. In the presence of ET-3, ganglia are significantly increased in size and number while treatment of the grafts with BQ788 leads to a marked reduction in the size and density of enteric ganglia. These results suggest that ET-3 promotes the development of enteric ganglia in the hindgut. Chick-quail intestinal transplants offer a useful new method for studying the molecular regulation of ENS development.

551. The *C. elegans* VAB-1/Eph Receptor Tyrosine Kinase Functions with the SAX-3/Robo Receptor During Cell Moments and Axon Guidance. Ian D. Chin-Sang. Queen's University, Kingston ON, Canada K7L 3N6.

Mutations that affect the single *C. elegans* Eph receptor tyrosine kinase, VAB-1, cause defects in cell movements during embryogenesis. Genetic and molecular evidence suggests that the VAB-1 Eph receptor functions with another neuronal receptor, SAX-3/Robo, for proper embryogenesis. The VAB-1 tyrosine kinase domain can physically interact with the juxtamembrane and CC1 region of the SAX-3/Robo receptor. Gene dosage, non-allelic non-complementation, and colocalization data are also consistent with a model in which these two receptors may form a complex and function together during embryogenesis. Similarly, the SAX-3/VAB-1 receptor complex may also function together during axon morphogenesis. Loss-of-function mutations that affect the VAB-1 Eph receptor cause low penetrance axon guidance defects with the predominant phenotype resulting in an overextension of axons. Interestingly, constitutively active VAB-1 tyrosine kinase signaling causes a lack of axon outgrowth or an early termination phenotype, opposite to the loss of function phenotype. The combination of loss-of-function and gain-of-function analysis suggests that the VAB-1 Eph receptor is required for targeting or limiting axons and neuronal cells to a specific region, perhaps by transducing a repellent or stop cue.

552. A Screen for Epithelial Defects in the *Drosophila* Ovary Reveals a Role for Cytoplasmic Dynein in the Targeting of Apical Proteins. Sally Horne-Badovinac and David Bilder. Department of Molecular and Cell Biology, UC Berkeley.

The polarized architecture and dynamic movements of epithelial cells play critical roles in the sculpting of embryonic organs and body plans. To investigate the forces that underlie epithelial cell shape and cell movement, we have focused on the follicle cell epithelium (FCE), which is a somatic monolayer that surrounds the clusters of germ cells in the *Drosophila* ovary. We are currently employing a directed mosaic approach to screen for mutations specifically in the FCE. To date, we have identified ~50 mutations that affect various aspects of epithelial architecture and morphogenesis. One particularly interesting complementation group disrupts *Drosophila*'s lone cytoplasmic Dynein heavy chain gene, Dhc64C. We have isolated five alleles of Dhc64C that cause loss of apico-basal polarity and multilayering in the posterior of the egg chamber. Posterior multilayering is commonly associated with loss of function of apical proteins such as Crumbs. The phenotypic similarity between Dhc64C and crumbs, combined with the fact that the minus ends of FC microtubules are directed toward the apical surface, has prompted us to examine the role of Dynein in apical protein targeting. Indeed, in Dhc64C mutant clones, Crumbs is no longer restricted to the apical surface and protein levels are greatly reduced. We are presently investigating whether Dynein plays a direct or indirect role in the apical trafficking of Crumbs as well as whether it is responsible for the apical localization of other proteins.

553. A Two-Dimensional Mathematical Model for Dorsal Appendage Cell Patterning in *Drosophila*. Bruce S. Gardiner, David W. Smith, and Peter Pivonka. Univ. of Melbourne, Parkville VIC 3010, Australia.

Shvartsman et al. [1] have recently proposed a mathematical model of the EGF receptor pathway activation in the predevelopment of dorsal appendages (DA) in *Drosophila*. In particular, a one-dimensional model for the interaction of the ligands: Gurken, Rhomboid, Spitz, and Argos, was presented, with a predefined Gurken shape and evolution. With their model, they were able to reproduce many of the observed behaviors of these ligands about the midline of the anterior–dorsal region of the oocyte, and even predict mutations. A subsequent experimental study [2] showed that the number of DA varies between species of *Drosophila* and is related to differences in the expression of Argos and Rhomboid. This is a startling demonstration of the power of mathematics for predicting the range of states available to a biological system. Here, we present results of a 2-dimensional (2D) extension to the above model. With an axisymmetric concentration of Gurken, the 2D model predicts the possibility of concentric rings of DA. Indeed concentric rings have been observed in experiments in which the oocyte nucleus was (abnormally) localized in the posterior region [3]. By including an additional morphogen, Decapentaplegic (Dpp), which is known to display an anterior–posterior gradient in anterior–dorsal follicle cells, we demonstrate that Dpp can determine the anterior–posterior extent of the DA follicle cell patterning.

554. *In Toto* Imaging of Organogenesis in Zebrafish. Sean G. Megason and Scott E. Fraser. Caltech, Pasadena, CA 91125.

Organogenesis transforms a simple field of cells into a complex organ through the precise orchestration of cell proliferation, migration, and differentiation. To better understand these dynamic and complex cell behaviors, we have developed a technology called in toto imaging that allows us to digitally track every cell in a developing organ throughout its complex morphogenesis.

There are several technical hurdles to in toto imaging. First, embryos must be labeled so that all cells are individually distinguishable. We are using Histone2B-EGFP and membrane-localized mCherry followed by channel subtraction to cleanly separate nuclei. The next challenge is to image embryos at sufficient spatial and temporal resolution for tracking every cell without damaging the embryo. We have developed mounting, confocal imaging, and data storage techniques that allow us to continuously image a developing zebrafish embryo at very high spatial and temporal resolution for 48 h and to archive the gigabytes of images. The final and toughest challenge is image analysis. To this end, we have developed a software package called GoFigure that automatically segments cells in very large 4D image sets to form cell tracks and cell lineages.

We are first applying in toto imaging to determine the complete lineage of the inner ear from before otic placode formation to the differentiation of its basic cell types: hair cells, support cells, and neurons. This digital reconstruction provides a uniquely detailed view of how orchestrated cell behaviors form an organ. We believe that in toto imaging of transgenic and mutant zebrafish can form the basis for the construction of a digital fish.

555. Differentiation of a Sexually Dimorphic Spinal Nucleus in a Teleost Fish. Nydia L. Rivera-Rivera, Jose L. Serrano-Velez, and Eduardo Rosa-Molinar. Univ. of Puerto Rico-Rio Piedras, PR 00931-1809.

Convergent genitalic evolution is involved in the insertion of the male intromittent organ in the female sex-specific genitalia during coitus in vertebrates, thus altering early embryonic development and reproductive strategies. Despite obvious convergent evolution in terms of reproductive strategies in mammals and teleosts, sexually dimorphic areas in the central and peripheral nervous system associated with the control of coital reflexes have been studied in mammals but not in teleosts or other viviparous species with sexually dimorphic intromittent organs. Although the intromittent organs of one taxa of teleosts, the Atherinomorpha, have been the subject of extensive taxonomic study, no work has focused on the central and peripheral nervous system associated with the control of coital reflexes. The Western Mosquitofish, exhibits prominent sex differences in the anal (median), pectoral (paired) fin structure, and internal suspensorium, as do other cyprinodontoidei males. Descriptions of the sexually dimorphic anal fin, vascularization, peripheral nerve supply, functional morphology, and development abound, providing detailed knowledge of the morphological characters of this sexually dimorphic anal fin. However, nothing is known about differences in the nervous system in these species. We describe a novel sexually dimorphic spinal nucleus in male Western Mosquitofish that controls the circumgenital musculature of the intromittent organ. These data will increase understanding of convergent changes in the spinal cord among mammals and teleosts with respect to neural sexual dimorphism. Supported by NSF/IBN-0091120 and NIH/NS390405-06.

556. Role of Desmosomal Cadherins in Zebrafish Vascular Morphogenesis. Michael J. Harrington,[1] Michael Tsang,[2] Igor Dawid,[3] Marnie Halpern,[4] and Rachel Brewster[1]. [1]Univ. of MD Baltimore County, MD 21250; [2]Univ. of Pittsburgh School of Medicine, PA 15261; [3]NICHD, National Institutes of Health, MD; [4]Carnegie Institution of Washington, MD 21210.

Desmosomal cadherins, *desmogleins* (*dsg*) and *desmocollins* (*dsc*), are important for mediating intercellular adhesion. As the transmembrane components of desmosomes, desmosomal cadherins have traditionally been thought of as "biological glue," necessary for maintaining tissue integrity. However, recent evidence has revealed a role for these proteins in tissue morphogenesis. For example, Runswick et al. (2001) demonstrated that blocking specific desmosomal cadherin cell adhesion recognition sites inhibits mammary epithelial morphogenesis and cell-type-specific positioning. In the zebrafish deficiency line, *c1032*, a large deletion on chromosomes 20 removes three desmosomal cadherins, including the zebrafish homologue of *desmoglein2* (*zfdsg2*). Homozygous mutants show severe neural and cardiovascular defects. The latter include: decreased blood circulation, pericardial edema, and abnormal vessel morphology. Interestingly, knockdown of *zfdsg2* using morpholinos causes vascular defects comparable to those observed in *c1032* mutants, suggesting that this gene may be required for proper vascular development. This hypothesis is further being tested by expression analysis of zfdsg2 RNA and protein and by detailed characterization of the *c1032* mutant and *zfdsg2* knockdown phenotypes. In addition, rescue experiments involving the injection of full-length *zfdsg2* RNA into *c1032* mutants and *zfdsg2* morpholino-injected embryos will confirm whether zebrafish Desmoglein 2 is solely responsible for the vascular defects.

557. The Role of N-cadherin in Zebrafish Neurulation. Elim Hong,[1] Michael Tsang,[2] Marnie Halpern,[3] and Rachel Brewster[1]. [1]Univ. of Maryland Baltimore County, Baltimore, MD 21228; [2]Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261; [3]Carnegie Institution, Baltimore, MD 21210.

A critical step in central nervous system development is the shaping of the neural tube (or neurulation). Cell adhesion molecules are likely to mediate multiple aspects of this process, including the maintenance of neuroepithelial integrity, convergence extension movements that shape the neural tube as the embryo elongates and the sorting of neuroepithelial cells away from their non-neural epidermal neighbors. However, the role of adhesion proteins and in particular of cadherins in neurulation is still poorly understood. Interestingly, *N-cadherin* (*N-cad*), a calcium-dependent, homophilic-binding cell adhesion molecule, was recently shown to be required for neurulation in the zebrafish (Lele et al., 2002). Loss of *N-cad* function in *parachute* (*pac*) mutants results in a failure of lateral neural plate cells to converge towards the dorsal midline, resulting in an abnormally shaped neural tube, that is most striking in the midbrain–hindbrain region. In order to better understand the role of *N-cad*, we are carrying out a detailed analysis of cell behaviors at different stages of neurulation in both WT and mutant embryos. In addition, we are investigating the role of another gene, *bumpy brain* (*bpb*), that appears to function in the same or parallel pathway as *N-cad* to orchestrate neurulation.

558. Role of Non-Classical Cadherins in Shaping the Zebrafish Neural Tube. Foyin C. Fasanmi, Gebeyehu M. Ayalew, and Rachel Brewster. University of Maryland Baltimore County, Baltimore, MD 21250.

The neural tube, the precursor of the central nervous system (CNS), is formed by a series of morphogenetic movements called neurulation. Efforts to uncover the molecular mechanisms underlying neurulation have resulted in the identification of over 50 genes, directly or indirectly implicated in the process. Surprisingly, only a couple of these genes are implicated in the control of cell adhesion, which is thought to be essential for proper neurulation (1). It has recently been shown that N-Cadherin (N-Cad) mutants in the zebrafish have severe neural tube defects, suggesting that this gene plays a key role in mediating the morphogenetic movements that shape the CNS. Cadherins are a superfamily of genes encoding calcium-dependent cell adhesion molecules that facilitate cell–cell contact. Despite the widespread expression of N-Cad in the CNS, the neural tube defects in the N-Cad mutants are surprisingly restricted to dorsal regions. These findings suggest that other members of the cadherin family such as Ventral Neural-Cadherin (VN-Cad) and Flamingo may also be required for shaping the neural tube. Vn-Cad is a homologue of cadherin 11 that has previously been implicated in the convergent extension movement in the mesoderm. Flamingo is a seven-transmembrane pass cadherin and is a member of the non-canonical wnt-signaling pathway. Here, we use a reverse genetics approach to investigate the role of these two genes in CNS morphogenesis. (1) Colas, JF and Schoenwolf, GC (2001). Toward a cellular and molecular understanding of neurulation. Dev. Dyn, 221: 117–145.

559. Differentially Directed Cell Movements Drive Feart Tube Assembly in Zebrafish. Nathalia S. Glickman,[1] Huai-Jen Tsai,[2] and Deborah Yelon[1]. [1]Skirball Institute, NYU School of Medicine, New York, NY; [2]National Taiwan University, Taipei, Taiwan.

The embryonic heart tube begins as a simple cylinder, built through the cooperative efforts of bilateral populations of cardiomyocytes. By tracking the movements of individual GFP-expressing cardiomyocytes, we demonstrate that two differentially regulated phases of coordinated cell movements underlie tube assembly. First, coherent medial movement brings the cardiomyocytes toward the midline. Next, as the bilateral populations approach each other, we observe a striking transition in cardiomyocyte behavior. Rather than continuing to move medially, a regionally restricted subset of cardiomyocytes begin to move in an angular direction, toward the centrally located endocardial precursors. This transition to angular movement does not require proximity of the contralateral cardiomyocyte

populations, as indicated by the examination of cardia bifida mutants, such as *miles apart*, which demonstrate lack of medial movement but maintenance of angular movement. In contrast, angular movement does require interactions between the endocardium and myocardium, as demonstrated by examination of *cloche* mutants, which lack endocardium and exhibit only medial movement. Furthermore, loss of all cardiomyocyte movement in *miles apart;cloche* double mutants suggests that endocardial–myocardial interactions play a role in inducing cardiomyocyte movements. Together, our results indicate a new role for the endocardium in regulating cardiomyocyte movement and organization during a critical phase of heart tube assembly (Supported by the AHA).

560. Trilobite/van Gogh-Like 2 Regulates Polarized Cell Behaviors Underlying Directed Migration. Jason R. Jessen and Lila Solnica-Krezel. Vanderbilt University, Nashville, TN.

Our goal is to understand the molecular and cellular mechanisms underlying cell movement during zebrafish gastrulation. Convergence and extension movements narrow and elongate embryonic tissues and are driven by diverse cell behaviors including mediolateral intercalation and directed migration. We have shown that in the lateral gastrula domain, mesodermal cells undergo a transition from slow and ineffective movement to fast and effective dorsal migration. This transition is associated with mediolateral cell elongation and is dependent on the function of noncanonical Wnt pathway components Tri/Vangl2 and Knypek/Glypican 4. We are identifying cellular events leading to the transition in cell behaviors and testing the requirements for Tri/Vangl2 and noncanonical Wnt signaling. In these polarized cells, Tri/Vangl2 protein is uniformly distributed on cell membranes. We have identified when and where this transition occurs in the gastrula and demonstrated that prior to elongation, mesodermal cells become tightly packed with increased neighbor contacts. In contrast to wild type, *tri* null mutant cells exhibit delayed packing and formation of neighbor contacts. Whereas wild-type cells extend numerous membrane protrusions towards intercellular gaps and efficiently pack via a blebbing-type movement, tri cells extend protrusions but show ineffective packing. We hypothesize that Tri/Vangl2 interacts with components of noncanonical Wnt signaling to regulate the formation and/or polarization of membrane protrusions necessary for cell movement. We are currently using fluorescent labels and confocal microscopy to examine these cell behaviors in more detail.

561. Investigating Naked Function During Wnt and EGFR Signaling. Terence J. Van Raay, Lilianna Solnica-Krezel, and Robert J. Coffey. Vanderbilt University, Nashville, TN.

Cell polarity and migration are complex biological behaviors involved in normal development and disease. The Wnt and EGFR signaling pathways have been directly implicated in a significant number of invasive cancers and both can regulate cell polarity and migration behaviors. Recent studies shed light on how Wnt and EGFR signaling may cooperate; the proposed Wnt signaling antagonist Nkd2, but not Nkd1, is required for escorting the EGFR ligand, TGF-α, to the basolateral membrane in polarized cells in vitro. To better understand the role of Nkd1 and Nkd2 in Wnt and EGFR signaling in vivo, we have initiated functional studies of their zebrafish homologues. We are testing the hypothesis that Nkd1 and Nkd2 have different roles in canonical and non-canonical Wnt signaling. Expression of zebrafish *nkd2* is maternal and ubiquitous while expression of nkd1 starts only after the onset of zygotic transcription and appears to recapitulate sites of active canonical Wnt signaling. In mutants defective in Wnt signaling, Nkd1 and Nkd2 may affect both gene transcription or cell morphogenesis. Inhibition of Nkd2 with antisense morpholino oligonucleotides in homozygous null *headless/tcf3a* mutants rescues the eyeless phenotype while overexpression shortens the mutant embryos at early segmentation. In *silberblick(slb)/wnt11* mutants, overexpression of Nkd1 or Nkd2 exacerbates the moderate cyclopia observed in maternal zygotic slb mutants. Analysis of molecular markers at various stages of development in these mutants as well as analysis of EGFR, Nkd1, and Nkd2 function in other mutants is currently underway.

562. A Cell Cycle Regulatory Gene, *gadd45β* is Involved in Zebrafish Mesoderm Patterning. Katie S. Brown and Sharon L. Amacher. Department of Molecular and Cell Biology, University of California, Berkeley, CA.

The *gadd45* genes belong to a family of genes induced under growth arrest and DNA damaging conditions. Gadd45 proteins have been extensively studied in mammalian cell lines and interact with a variety of cell cycle regulatory proteins and signal pathways. A zebrafish homolog of one of the *gadd45* genes, *gadd45β*, is expressed in a bilateral stripe in the presomitic mesoderm that is positioned about two segments posterior to the most recently formed somite, suggesting a possible link between cell cycle regulation and segmentation [1]. Manipulation of *gadd45β* expression results in somite defects [2]. In zebrafish, *gadd45β* expression is initiated just anterior to the broad posterior FGF8 expression gradient. Because the expression of *fgf8* and *gadd45β* in the presomitic mesoderm is mutually exclusive, we hypothesize that one may antagonize the expression of the other. Consistent with negative regulation of *gadd45β* by FGF8, we find *gadd45β* expression is altered in *ace/fgf8* mutant embryos. We are investigating the possibility that *gadd45β*, in conjunction with FGF8, may refine positioning of somite boundaries. As a first step, we are manipulating FGF expression and observing the effects on *gadd45β* expression. We will present our most recent findings. 1. Durbin, L., et al., Anteroposterior patterning is required within segments for somite boundary formation in developing zebrafish. Development, 2000. 127(8): pp. 1703–1713. 2. Kawahara, A., et al., Zebrafish GADD45-beta genes are involved in somite segmentation. Proc. Natl. Acad. Sci. U. S. A., 2005. 102(2): pp. 361–366.

563. Role of Hedgehog Signaling in Anterior Neurocranium Development. J.K. Eberhart, M.E. Swartz, and C.B. Kimmel. Univ. of Oregon, Eugene, OR 97403.

The vertebrate neurocranium is essential for support of the brain and head sensory organs. It develops from an assembly of multiple cartilages that later fuse to form the brain vault. Studies in several species have shown that cells of different origins, mesoderm, and neural crest, contribute to distinct cartilages in the head. We are interested in the genes and tissue interactions that sculpt these cartilages. We show Hh directs patterning of the neurocranium by regulating development of the stomodeum, or oral ectoderm. Post-migratory crest cells fated to become the anterior neurocranium intimately associate with stomodeal ectoderm in a maxillary region (MX) of the first pharyngeal arch. Both MX and stomodeal pitx2 expression are lost in mutants for the Hh co-receptor *smoothened* (*smo*). Timed treatment with the Hh-pathway inhibitor cyclopamine suggests that signaling is required near the end of gastrulation for MX formation and the stomodeal expression of *pitx2*. Additionally, genetic mosaics between *smo* mutants and wild-type embryos show that the critical target tissue receiving the Hh signal is the stomodeum, not the postmigratory neural crest itself. Two Hhs, *shh* and *twhh*, are expressed by prechordal plate mesoderm and ventral brain at the time Hh signaling is crucial for MX development. However, mosaic analyses with embryos injected with *shh* and *twhh* morpholinos show that only the ventral brain is an important source of Hh. These combined results show that Hh signaling from the ventral brain at the end of gastrulation specifies a role, perhaps an adhesive function, of the stomodeum in development of skeletogenic crest cells.

564. New Functions for a Vertebrate Rho Guanine Nucleotide Exchange Factor in Cilia-Dependent Processes. J.R. Panizzi, J.R. Jessen, and L. Solnica-Krezel. Vanderbilt University, Nashville, TN.

We have identified a zebrafish homolog of a human PDZ-domain-containing guanine nucleotide exchange factor for Rho (PDZ-RhoGEF). Rho can influence many cellular processes ranging from transcriptional regulation to cytoskeletal modulation. In cell culture studies, human PDZ-RhoGEF was shown to associate with actin, modulate neurite outgrowth, and alter cell morphology. A similar RhoGEF in *Drosophila*, DRhoGEF2, is able to induce cell shape changes during gastrulation and associates with microtubules. Here, we investigate the functions of the zebrafish homolog of human PDZ-RhoGEF in the developing embryo through loss-of-function studies using morpholino oligonucleotides and a dominant-negative construct. RNA encoding PDZ-RhoGEF is maternally-contributed and expressed ubiquitously through early somitogenesis, while later becoming restricted to the head, neural tube, and pronephric ducts. Similar to the human homolog, zPDZ-RhoGEF can induce actin stress fiber formation via Rho activation in cell culture. Overexpression in the embryo leads to pronounced gastrulation defects. Loss-of-function data implicate PDZ-RhoGEF in several processes mediated by primary cilia, such as establishment of left/right asymmetry, otolith formation, and kidney function. Antibody staining reveals PDZ-RhoGEF expression in ciliated regions, further supporting this notion. In addition, data from in situ hybridization using asymmetrically-expressed probes show left–right patterning is randomized after morpholino injection. Our current experiments aim to investigate the role of PDZ-RhoGEF in cilia and the protein interactions that are required for its function.

565. Coordinating the Left–Right (LR) Asymmetry Along Zebrafish Posterior–Anterior Axis by *Southpaw* (*Spw*) Wave. Xinghao Wang, Jeffrey D. Amack, Brent W. Bisgrove, Jeffrey J. Essner, and Joseph H. Yost. Univ. of Utah, Salt Lake City, UT 84108.

Left Lateral Plate Mesoderm (LPM) expression of *nodal* family members is a conserved mechanism of establishing LR asymmetries along the AP axis during vertebrate development. *Spw*, a *nodal* family member, is expressed in the left LPM and regulates LR development in zebrafish. To further understand how expression of *spw* is established, we carefully studied the temporal and spatial expression of *spw* by using *myoD* as a somite stage marker. In consistence with a previous report, the expression of *spw* starts around the tail bud at 11–12 s. We further show that the anterior expression boundary of *spw* moves anteriorly in a speed of 3–4 somite lengths per somite generation time between 12 and 21 s. At 24 s, the expression of *spw* in LPM is greatly diminished. Thus, the expression of *spw* can be described as a PA "wave". Preliminary fate mapping results suggest that the likely mechanism for *spw* LPM expression is autoregulation rather than the migration of the posterior cells. We further demonstrate that the expression of *pitx2* shows a similar wave pattern in LPM. Interestingly, *lefty-1* expression in the midline also exhibits a PA wave coordinated with the *spw* wave. In contrast to *spw* and *pitx2*, the midline *lefty-1* expression starts posteriorly before the initiation of *spw* in LPM. Based on morphant analysis, it is likely that the bilateral expression domain of *spw* in the tail bud is responsible for the initiation of *lefty-1* expression. Our future goal is to understand how this wave contributes to the LR asymmetries in organs. We are generating GFP transgenic zebrafish driven by *spw* or *pitx2* promoter.

566. Analysis of the Tight Junction Component *claudin d* During Zebrafish Development. Ashley E. Bruce,[1] Zina S. Jeyapalan,[1] Monica Dixon Fox,[1] and Isaac Skromne[2]. [1]Univ. of Toronto, Toronto, ON M5S3G5; [2]Univ. of Chicago, Chicago, IL 60637.

We are interested in how cell movements are coordinated during embryonic development to generate the adult body plan. One mechanism for regulating cell motility is by modulating the strength and specificity of cell adhesions. This has led us to investigate the potential role of the tight junction component *claudin d* in zebrafish morphogenesis. We have shown by in situ hybridization that *claudin d* is a maternal transcript that is ubiquitously expressed from the 1-cell stage to the sphere stage (4 h post-fertilization, hpf). Subsequently, *claudin d* transcript levels rapidly decline and are undetectable by 30% epiboly (4.7 hpf). Intriguingly, the down-regulation of the transcript correlates with the initiation of the first coordinated cell movement during zebrafish development, epiboly. Epiboly begins at the dome stage (4.3 hpf), when the yolk bulges up into the blastoderm, which sits on top of the yolk. We speculate that the sharp decline in *claudin d* transcripts during doming may reflect a requirement for modulating tight junctions during this process. We are currently examining protein localization using a green fluorescent protein–*claudin d* fusion construct and we are investigating the function of *claudin d* in over-expression studies.

567. Integrin Alpha5 and Delta/Notch Signaling Have Complementary Spatiotemporal Requirements During Zebrafish Somitogenesis. Scott A. Holley,[1] Dörthe Jülich,[1] Robert Geisler,[2] and Tübingen 2000 Screen Consortium[2]. [1]Yale University, New Haven, CT 06520; [2]Max Planck-Institut für Entwicklungsbiologie, Tübingen, D-72076.

Somitogenesis is the process by which the segmented precursors of the skeletal muscle and vertebral column are generated during vertebrate embryogenesis. While somitogenesis appears to be a serially homologous, reiterative process, we find that there are differences between the genetic control of early/anterior and late/posterior somitogenesis. We have found that point mutations can cause segmentation defects in either the anterior, middle, or posterior somite in the zebrafish. We demonstrate that mutations in zebrafish integrin a5 disrupt anterior somite formation, giving a phenotype complementary to the posterior defects seen in the notch pathway mutants after eight/deltaD and deadly seven/notch1a. Using fluorescence time-lapse microscopy, we show that Integrin a5-GFP clusters along the basal surface of prospective somite border cells at the onset of somitogenesis and that this Integrin clustering can be observed along the irregular borders that form in the posterior of the notch pathway mutants. In fact, integrin a5 is necessary for the formation of these irregular borders as double mutants between the notch pathway and integrin a5 display somite defects along the entire body axis with a complete loss of the mesenchymal to epithelial transition and Fibronectin matrix assembly in the posterior trunk. Together, our data suggest that notch- and integrin a5-dependent cell polarization and Fibronectin matrix assembly occur concomitantly and interdependently during somite border morphogenesis.

568. Interactions Between Muscle Fibers and Segment Boundaries in Zebrafish. Clarissa A. Henry,[1] Wendy A. Durst,[2] Sarah E. Munchel,[2] and Sharon L. Amacher[2]. [1]University of Maine, Orono, ME; [2]University of California, Berkeley, CA.

Somites give rise to vertebrae and much of the musculature. We currently define three stages of somite boundary formation. Stage one is initial somite boundary formation. Stage two is a transitional stage prior to stage three: myotome boundary formation, where muscle precursor cells have formed long muscle fibers that attach to the extracellular matrix-rich myotome boundary. Formation of the initial somite boundary requires Notch signaling; Notch pathway mutants in both zebrafish and mouse show initial defects in somitogenesis. However, many Notch pathway zebrafish mutants are homozygous viable, suggesting that segmentation of their body plan at least partially recovers. We addressed this segmental recovery using a mutation for the Notch ligand, *aei/deltaD*, and show that stage three myotome boundaries form in *aei/deltaD* mutant embryos. Inhibition of Hedgehog-induced slow muscle in *aei/deltaD* mutant embryos suggests that slow muscle is necessary for myotome boundary formation in these embryos. As we have previously demonstrated that slow muscle migration triggers fast

muscle cell elongation in zebrafish, we hypothesize here that migrating slow muscle facilitates myotome boundary formation in *aei/deltaD* mutant embryos by patterning coordinated fast muscle cell elongation. In addition, we utilized genetic mosaic analysis to show that somite boundaries can also function to limit the extent to which fast muscle cells can elongate. Combined, our results indicate that multiple interactions between somite boundaries and muscle fibers mediate segmentation of the zebrafish body plan.

569. The Effects of the Widely Used Herbicide Atrazine on Early Amphibian Development. Jenny R. Lenkowski and Kelly A. McLaughlin. Department of Biology, Tufts University, Medford, MA 02155.

In recent years, use of the herbicide atrazine has been the topic of much debate because of its implicated, but poorly described, effects on non-target organisms. Atrazine levels persist long after application to crops and have been found in the aquatic environments that amphibians and other species depend on for reproduction and early development. Amphibians have been found to be particularly sensitive to agents classified as endocrine disruptors and as such are frequently used as bio-indicators of environmental health. As a consequence, many of the previous studies examined atrazine exposure only at later stages of amphibian development, largely focusing on metamorphosis and sexual differentiation. It is important to remember atrazine is typically applied early in the growing season, and due to normal agricultural run-off, can be found at surprisingly high concentrations in vernal breeding ponds during time periods overlapping with early amphibian development. In order to identify windows of susceptibility to the effects of atrazine, our research focuses on investigating the potentially teratogenic effects of atrazine in *Xenopus laevis* at significantly earlier stages of amphibian development preceding metamorphosis. Our preliminary results suggest that atrazine can dramatically affect amphibian development at non-lethal, environmentally relevant doses during earlier stages of development than previously described in the literature while demonstrating the importance of adopting an interdisciplinary approach in the investigation of possible environmentally hazardous chemicals.

570. Cell Behaviors Associated with Somite Formation in *Xenopus laevis*. Minh Ho,[1] Bonnie Glosier,[1] Caroline Meloty,[2] Kathleen Wunderlich,[3] Jean Gustin,[4] and Carmen Domingo[1]. [1]Dept. of Biology, SFSU, San Francisco, CA 94132; [2]Dept. of Cell Biology, UC Davis, Davis, CA; [3]Biomolecular Resource Center, UCSF, San Francisco, CA 94143; [4]Oregon Health Sciences Univ., Portland, OR 97201.

Somites give rise to vertebrae, muscle, and the dermis of the dorsal skin. In *Xenopus laevis*, somitogenesis consists of segmentation of the presomitic mesoderm and then a 90° rotation of somitic cells to form muscle fibers that are elongated along the anterior–posterior axis. We used a combination of cell and molecular tools to examine the cell behaviors underlying somite formation. Using live time-lapse digital microscopy of explants, we show that somite boundary formation is dynamic in that fissures initiate from lateral, medial, or central regions of the presomitic mesoderm. Using a membrane-tagged GFP to outline cell morphologies, we find that somite boundaries form prior to cellular shape changes. Moreover, somite formation is not associated with an epithelialization event characteristic of most vertebrate embryos examined thus far. Soon after, boundary formation cells exhibit changes in their shape and protrusive activity that appear to be associated with the reorientation of cells along the anterior–posterior axis to generate muscle fibers. Finally, we show that boundary formation and somite rotation appear to be two separate mechanisms. Interestingly, we observe a lag time between segmentation and rotation in anterior somites, whereas in posterior trunk somites, rotation is almost immediate after segmentation. Together, our results show that somitogenesis is a dynamic process driven by specific cell behaviors.

571. Syndecan-4 Regulates Convergent Extension Movements in *Xenopus* Embryos. Juan Larrain, Mauricio Moreno, Carlos Oliva, and Rosana Muñoz. P. Universidad Catolica de Chile, Fondap-Biomedicine.

Convergent Extension (CE) movements, in which polarized dorsal mesodermal cells intercalate mediolaterally, narrowing the embryo and elongating the anteroposterior (A/P) axis, play a key role in convergent extension. We have cloned Syndecan-4 (xSyn-4) from gastrulae *Xenopus* embryos. In situ hybridization assays showed maternal expression; at gastrula stage, it is expressed in the mesoderm; and later, it is expressed in branchial arches, brain, eyes, pronephric duct, somitic mesoderm, and tailbud. Either gain- or loss-of-function assays in *Xenopus* embryos resulted in open neural tubes, shortened A/P axis, and dorsal flexure. These phenotypes resemble abnormal CE movements. To confirm the function of xSyn-4 in CE, we realized activin assays in animal caps overexpressing xSyn-4 mRNA or Morpholino xSyndecan-4 (MoSyn-4). We observed inhibition of explant elongation with normal levels of dorsal mesoderm markers, indicating that xSyn-4 must regulate cell behavior and not cell fate. To characterize the CE defects in xSyn-4 or MoSyn-4 injected embryos, we examined the expression of different molecular markers. In injected embryos, the expression domain of Xbra was more irregular; in fact, dorsal midline domain was shorter and wider than control stage 12 embryos. Also, the midline expression domain of Chordin was broader and shorter in the injected compared with uninjected stage 16 embryos. Finally, the neural plate domain of Sox-2 at stage 16 injected embryos was clearly broader. Altogether, these results allow us to conclude that xSyn-4 plays an important role in CE movements. We are currently studying the role of syndecan 4 in non-canonical Wnt-signaling.

572. The KIAA0888 Homolog of *Xenopus tropicalis* is Essential for Early Embryogenesis. Marcela E. Torrejon, Rakhi Gupta, Kay Walter, Sonja Gennuso, and Sigrid Reinsch. NASA-Ames Research Center, Moffett Field, CA.

We are using a gene-trap in *Xenopus tropicalis* to identify genes involved in neural and vestibular development. Green fluorescent protein (GFP) is the transgene. Using the GFP as a molecular tag, we can ultimately identify and clone the mutated gene using RACE- or inverse-PCR. A founder animal with an insert in the KIAA0888 gene was identified using 5′RACE. This is a developmentally normal female with an apparent maternal effect lethal phenotype. All offspring die, both transgenic and non-transgenic. The majority die prior to or during gastrulation. The remainder die with severe axial, endoderm, cardiac, and craniofacial defects. A transcript was cloned from St. 44 wild-type tadpoles and predicted to encode a 79-kDa protein. Several transcripts were cloned by 5′ RACE from transgenic animals. These indicate that a duplication of at least 1 exon has occurred that may account for the maternal effect lethal phenotype. In situ analysis shows expression in the developing CNS, neural crest, and muscle in neurulating and tailbud embryos. Morpholino injection into early embryos gives a dose-dependent response. High concentrations cause cleavage failure, developmental delay, exogastrulation, and severe axial defects. Intermediate concentrations allow development to later stages and phenocopy the F1 tadpole phenotype. A low concentration gives phenotypically normal tadpoles that show later defects in development of the gut and growth reduction. This growth reduction can be rescued by co-injection of RNA encoding the full-length predicted protein fused to GFP. However, the injected RNA does not rescue early defects.

573. Analysis of *Xenopus laevis* Claudins (*Xcla*) Tight Junction Genes in Development. Jianzhen Xie and Brenda Brizuela. North Carolina State University, Raleigh, NC 27695.

Claudins are the integral membrane proteins in tight junction. Traditionally, work done in cell culture system defines tight junction proteins as

structural proteins which control the paracellular permeability and cell polarity. New evidence has shown that claudins are adhesion molecules which play an important role in regulating the developmental process. Using the *Xenopus laevis* embryonic model system, we can analyze claudins from the developmental aspect. In this study, eight *X. laevis* claudin genes: *Xcla1*, *Xcla4B*, *Xcla5*, *Xcla6*, *Xcla12*, *Xcla16*, *Xcla18*, and *Xcla19* were cloned and sequenced. Their normal mRNA expression patterns were shown from cleavage stage to tadpole stage by whole mount in situ hybridization. The protein expression of *Xcla5* was detected at neural stage using mouse antibody. To study the estrogenic regulation activity on claudins, the ectopic mRNA expression patterns of *Xcla4A*, *Xcla4B*, *Xcla5*, and *Xcla7* after exposure to bisphenol A (BPA) were shown and compared. To study its function, *Xcla5* was overexpressed by injecting synthetic mRNA into 8 cell-stage embryos. The results presented here showed the dynamic and complementary expression patterns (including in neural crest cells, brachial arches, sensory placodes, surface ectoderm, brain, eyes, gut, heart, and vascular endoderm) of different claudin species, which suggest that claudin may play an important role in neural crest cell migration, epithelial–mesenchymal transition and ultimately organogenesis during the embryonic development. The patterns of the gene expression can be drastically altered in the presence of BPA. The morphology can be altered when *Xcla5* is overexpressed.

574. A Nodal Ligand and its Inhibitor Define a Left–Right Organizer in *Xenopus* Neurula. Alin Vonica and Ali H. Brivanlou. Rockefeller University, New York, NY 10021.

Members of the DAN domain family of secreted proteins are inhibitors of TGFb and Wnt pathways implicated in developmental processes like head formation and left–right axis. We present here a role in left–right axis determination for *Xenopus* coco. In early neurula (stage 13), coco was expressed in a limited, bilateral, posterior paraxial territory. At stage 16, a nodal ligand, Xnr1, which is later expressed asymmetrically in the left lateral mesoderm, is coexpressed with coco. Expression of both factors is maintained until stages 22–24. Morpholino depletion of coco protein on the right side of the embryo leads to bilateral expression in lateral plate mesoderm of Xnr1 and other left-sided genes, like Xlefty1, 2, and Pitx2c, and to left–right axis randomization. These can be rescued by morpholinos against Xnr1, confirming that coco inhibits Xnr1 function on the right side. The Xnr1 morpholinos alone randomize the axis only when injected on the left side. We also found that sectioning the left, but not the right, paraxial mesoderm anterior to where coco and Xnr1 are expressed produced the same randomized left–right axis phenotype as the loss of coco on the right side and of Xnr1 on the left side. The effect of the section can be rescued with left side injection of an inducible nodal pathway activator. We conclude that bilateral Xnr1 expression is turned into a left-sided signal by coco inhibition. This signal is transmitted from the left–right organizer in the posterior paraxial mesoderm to the anterior lateral plate through the left side paraxial mesoderm to induce asymmetric gene expression.

575. Shroom is a Multifunctional Protein that Initiates a Suite of Different Cell Behaviors During Neural Tube Closure. Chanjae Lee and John B. Wallingford. Univ. of Texas, Austin, TX 78712.

Neural tube closure is an essential event during early development in vertebrates. This morphogenetic event involves several cell behaviors, including apicobasal cell heightening, apical constriction, and apical actin accumulation. The molecular and cellular mechanisms that control this event are still unclear. In previous studies, Shroom, an actin-binding protein, has been shown to be necessary for neural tube closure in mice and frogs. Here, we describe the normal pattern of cell-shape changes during *Xenopus* neurulation using 4D confocal microscopy. Moreover, we show that Shroom is required for apical constriction, and that defective apical constriction results in a failure of medial movement of neural cells, but not anterior–posterior movement. To test the effects of Shroom on cell behaviors in *Xenopus*, we cloned full-length Shroom from *Xenopus*. Using a gain of function assay, we confirmed that the Xshroom protein functions in a manner similar to that described for mouse Shroom. In addition, we show that Xshroom (*Xenopus* Shroom) induces apicobasal cell heightening and basally-directed nuclear migration. Finally, we provide evidence that the distinct cellular events initiated by Shroom require different complements of small GTPases. Together, these data demonstrate that Shroom is a multi-functional protein that plays several important roles in neural tube closure.

576. XNF-ATc3 is Necessary for Morphogenesis of the *Xenopus* Neural Tube. Annette G. Borchers and Julie C. Baker. Department of Genetics, Stanford University Medical School, Stanford, CA 94062, USA.

Convergent extension is the main force elongating the anterior–posterior body axis. In *Xenopus*, convergent extension occurs in the gastrulating mesoderm and the neural ectoderm. Molecular evidence suggests that neural and mesodermal convergent extensions are mediated by conserved signaling pathways. However, differences in cell behavior and polarity in these two processes suggest that divergent molecular mechanisms exist. To date, the differences in these molecular mechanisms are not understood. Here, we demonstrate that XNF-ATc3 is only necessary for *Xenopus* neural tube convergent extension. To tease apart the effect of XNF-ATc3 on dorsal mesoderm from that of neural ectoderm, we have enlisted a variety of temporal and spatial inhibitory approaches. Blocking XNF-AT signaling in explants or whole embryos using the chemical inhibitor Cyclosporin, applied over time or dominant-negative XNF-ATc3 expressed in particular tissues demonstrate that, regardless of reagent or method, neural convergent extension, but not mesodermal, is affected. Consistent with a function in neural convergent extension, we show that XNF-ATc3 is expressed and transcriptionally active within the neural tube. This work presents XNF-ATc3 as the first known transcriptional regulator specific for morphogenetic movements within the *Xenopus* neural tube.

577. Time-Lapse Analysis of Epidermal Cell Behavior During Vertebrate Neurulation. Julie M. Hayes, Esther K. Kieserman, Chanjae Lee, and John B. Wallingford. University of Texas at Austin.

Successful neurulation is dependent on a suite of cell shape changes. The neural plate is known to undergo apical constriction and convergent extension as its lateral edges move medially towards one another. Epidermis will eventually cover the neural tube, and this tissue is required for neural tube closure. The cell shape changes of the epidermis have yet to be analyzed in *Xenopus*. It has been suggested that the epidermis may actively move medially to assist neural tube closure; however, others argue that the movements of the epidermis are a passive result of being pulled by the moving neural tissue. In order to morphometrically analyze cell behaviors in the epidermal layer during neurulation, we used in vivo confocal time-lapse imaging. Using fluorescent-tagged proteins (RFP and GFP), we have visualized cell membrane, actin filaments, and microtubules. Data from these studies will set a foundation for future experiments aimed at elucidating the mechanisms of cell shape change in the epidermis during neurulation.

578. The Role of Calcium in Somite Morphogenesis. Andrea Harstock,[1] Caroline Meloty-Kapella,[2] Minh T. Ho,[1] and Carmen R. Domingo[1]. [1]Dept. of Biology, SFSU, San Francisco, CA 94132; [2]Dept. of Cell Biology, UCD, Davis CA.

The vertebrate embryo is comprised of three embryonic germ layers, referred to as the ectoderm, mesoderm, and endoderm. The adult musculature system arises from the mesodermal germ layer through a complex combination of signaling events and cell behaviors. Previous studies have

shown that calcium signaling may be involved in this process (Walingford et al., 2001; Ferrari and Spitzer, 1999). To further investigate the role of calcium in myotome formation, we treated *Xenopus laevis* embryos with several calcium channel inhibitors (Benzohydroquinone [BHQ], Thapsigargin, Ryanodine, and 2-Aminoethoxydiphenylborate [2-APB]) for varying time intervals. The embryos were scored for defects in myotome morphology, somite number, and tail length. Each calcium channel inhibitor presented different morphologies ranging from no defect in the number of somites in BHQ-treated embryos to cessation of somite formation and disorganized myotome in Thapsigargin-treated embryos. Using a membrane-tagged GFP and confocal microscopy, we further characterized the effects of these calcium channel inhibitors on the cell behaviors associated with somite formation. We show that calcium plays a role in the formation and differentiation of somites. Furthermore, since these inhibitors target different intracellular calcium stores, multiple calcium-dependent signaling mechanisms are likely to be involved in somitogenesis in *X. laevis*.

579. Vertebrate orthologues of the *Drosophila* PCP genes Fuzzy and Inturned play unexpected roles craniofacial morphogenesis. John B. Wallingford,[1] Tae Joo Park,[1] Saori L. Haigo,[2] and Sara M. Peyrot[2]. [1]University of Texas, Austin; [2]University of California, Berkeley.

Previously, vertebrate orthologues of *Drosophila* planar cell polarity (PCP) genes have been found to be uniformly involved in regulating convergent extension cell movements. Here we show that vertebrate orthologues of the *Drosophila* PCP genes inturned and fuzzy display radically different loss-of-function phenotypes as compared to other vertebrate PCP genes. Disruption of either Xinturned (Xin) or Xfuzzy (Xfy) with morpholino-oligos has only modest effects on convergent extension, but results in severe defects in the timing of both anterior and posterior neural tube closure. Far more striking is that loss of either gene results in a complex spectrum of patterning defects in the skull and jaw. At early stages, these embryos display disruptions of normal patterns of craniofacial gene expression. At later stages, we observe disruptions of specific cartilages in the skull and jaw, while other cartilages form normally. Affected cartilages include both neural crest- and mesoderm-derived craniofacial elements. For example, the Meckels' cartilages of the lower jaw are hypomorphic and displaced medially, while the ceratohyal and branchial arch cartilages are hypomorphic, but patterned normally. The cartilages that form the base of the braincase fail to develop, though the adjacent cartilages of the upper jaw remain unaffected. In *Drosophila*, Inturned is a peripheral membrane protein, but we observed expression of functional Xin-GFP fusion proteins at the membrane and also in the nucleus, perhaps explaining the unexpected and novel function for these genes in vertebrate animals.

580. Correct Subcellular Localization of Dishevelled is Essential for Planar Cell Polarity and Rho/Rac Activation but not for Canonical Wnt Signaling in Vertebrate Embryos. Tae Joo Park,[1] Ryan S. Gray,[1] Raymond Habas,[2] and John B. Wallingford[1]. [1]Univ. of Texas-Austin; [2]Robert Wood Johnson School of Medicine.

Wnt signaling plays a critical role in many cellular processes including cell fate specification, cell movement, and oncogenesis. Dishevelled mediates two distinct Wnt signaling pathways, the canonical Wnt signaling and the planar cell polarity (PCP) pathway. Canonical Wnt signaling mainly controls cell fate specification and PCP regulates polarity and a morphogenetic process called convergent extension in vertebrate embryos. Dishevelled is in the center of both signaling pathways. Moreover, Dishevelled-dependent convergent extension is critical for neural tube closure in vertebrates. It has been suggested that pathway specificity for Dvl in two distinct signaling pathways is achieved by subcellular localization. However, the function of subcellular localization of Dishevelled in vertebrate embryos has not been tested. Here, we show that sequestration of Dishevelled away from the cell membrane inhibits convergent extension movement and mediolateral polarization of dorsal marginal zone (DMZ) cells of *Xenopus* embryo. RhoA and Rac1 are critical regulators for convergent extension. Sequestration of Dishevelled away from the cell membrane inhibits RhoA and Rac1 activation in DMZ. We also demonstrate that either cytoplasmic or membrane-associated Dishevelled can activate the canonical Wnt signaling more potently than does wild-type Dishevelled. From these data, we suggest that proper membrane localization of Dishevelled is critical for PCP signaling during convergent extension but not necessary for canonical Wnt signaling in vertebrate embryos.

581. Tumorhead Function in *Xenopus* and Fission Yeast Possibly Involves PAK Signaling. Chuan Fen Wu,[1] Peirong Yang,[1] Edwin E. Traverso,[1] Nathalie Morin,[2] Stevan Marcus,[1] and Laurence D. Etkin[1]. [1]Univ. of Texas M.D. Anderson Cancer Center, Houston, TX 77030; [2]Centre de Recherche de Biochimie macromoléculaire, CNRS, Montpellier, France.

Tumorhead (TH) is a maternal gene product in *Xenopus laevis*. We previously demonstrated that TH is involved in cell proliferation and differentiation of ectodermal derivatives in the *Xenopus* embryo. TH is also involved in cortical F-actin organization and cell polarity of neural plate cells. To find the mechanism and molecular pathway of TH function, we recently used genetically tractable fission yeast, *Schizosaccharomyces pombe*, as an experimental model. TH expression in *S. pombe* resulted in severe morphological defects, characterized by effects on cell growth and polarity similar to those observed in *Xenopus*. TH expression also caused cytoskeletal defects, including depolarization of the cortical F-actin cytoskeleton and increased microtubule formation. As in *Xenopus*, TH protein localizes to the cell cortex of the yeast. Interestingly, TH cortical localization is dependent on the *S. pombe* PAK homolog, Shk1. Moreover, TH expression inhibited the growth of a mutant defective in Shk1 function, suggesting that TH may interact with a component of a PAK-mediated morphogenetic regulatory pathway in *S. pombe*. In *Xenopus*, our preliminary results show that expression of kinase-deficient *Xenopus* PAK1 protein (Xpak1KR) produced a phenotype similar to that produced by TH overexpression. Loss of TH function, however, attenuated the Xpak1KR phenotype. Taken together, our findings demonstrate that TH functions through a mechanism that is conserved from the yeast to vertebrates and probably involves Pak signaling.

582. New Tools for Quantitative Analysis of Morphogenesis in Spherical Embryos. Julian M. Tyszka,[1] Andrew E. Ewald,[2] John B. Wallingford,[3] and Scott E. Fraser[1]. [1]Biological Imaging Center, Division of Biology, California Institute of Technology, Pasadena, California, USA; [2]Department of Anatomy, University of California, San Francisco, California, USA; [3]Institute for Cellular and Molecular Biology, University of Texas, Austin, Texas, USA.

Many embryos are essentially spherical during their early development, with cell motion and organization often influenced by their spherical geometry. We demonstrate a potentially generalizable coordinate system and associated geometric methods for quantitative imaging studies in developing spherical embryos using 2D, 3D, and 4D data acquired using conventional optical, surface imaging, and magnetic resonance microscopy. Spherical geometric corrections of surface area measurements are shown to significantly improve quantitative accuracy and sensitivity of morphometric analyses. Extractions of anatomically relevant spherical shells and planes from 3D and 4D imaging data are also demonstrated to be a valuable visualization and analysis tool in early development. Specific application areas include morphometric analysis and surface gene expression domain quantitation in the early development of *Xenopus laevis*. These new tools are generalizable to other embryos and can be adapted to a wide variety of

developmental problems which previously have been investigated only semi-quantitatively. Software implementations of the methods are made freely available to the general developmental biology community.

583. Tension Affects Apoptosis in Chick Chorioamniotic Membrane. Rebecca E. Pulver and Beatrice Holton. Univ. of Wisconsin Oshkosh.

Physical forces, such as shear force and tension, have been shown to influence cell proliferation and cell survival through cell–matrix interactions. Tension in the avian chorioamniotic (ChorAm) membrane is maintained, throughout its development, along the leading edge, sweeping over the embryo and enclosing it in the amniotic sac and chorion. Here, we show that an inverse relationship between tension and cell death exists in the developing chick ChorAm membrane. We propose that apoptosis acts as a mechanism to maintain tension during ChorAm development. Previously, our lab has shown that during normal ChorAm development, apoptotic cells are found within the leading edge and are asymmetrically distributed to the right of the midline in stage 1416 (HH) embryos. We hypothesized that the increase in the density of apoptotic cells in this region may be caused by loss of tension to the right of the midline, after the membrane stretches over the heart and moves down the relatively narrow trunk. Experiments in which tension was increased to the right of the midline resulted in a significant decrease in the density of apoptotic cells on that side (F = 16.481, P = 0.02). Furthermore, an increase in the density of apoptotic cells resulted when tension was decreased to the left of the midline (F = 5.433, P = 0.037). These results demonstrate that changes in tension can influence cell survival in epithelial sheets, and suggest a possible role for mechanotransduction in the development of the avian ChorAm membrane.

584. Indian Hedgehog Regulates Skeletal Angiogenesis in Association with Cartilage and Bone Development. Céline Colnot,[1] Luis de la Fuente,[1] Steve Huang,[1] Diane Hu,[1] Chuanyong Lu,[1] Benoit St-Jacques,[3] and Jill A. Helms[2]. [1]Departments of Orthopaedic Surgery, University of California, San Francisco, California, 94143-0514, USA; [2]Department of Plastic and Reconstructive Surgery, Stanford University, Stanford, CA 94305, USA; [3]Genetics Unit, Shriners Hospital, Montreal, Quebec, Canada H3G 1A6.

A null mutation in Indian hedgehog (Ihh) results in the absence of bone in the limbs. Here, we showed that this defect was not attributable to a permanent arrest in cartilage differentiation since Ihh−/− chondrocytes underwent hypertrophy and terminal differentiation and expressed angiogenic markers like Vegf. Though initial vascular invasion of cartilage took place, vessel expansion and persistence were impaired. The vascular defect was not because the Ihh−/− skeleton is anti-angiogenic; in an ex vivo environment, wild-type vessels invaded the Ihh−/− cartilage and expanded into a marrow cavity. This was in sharp contrast to the Ihh−/− endothelial cells, which invaded but failed to persist. In addition, Ihh−/− cells, likely of perichondrial origin, differentiated into osteoblasts and deposited a bony matrix without benefit of exogenous Hh in the ex vivo environment. The earliest Ihh-dependent skeletal defect occurred during the formation of chondrogenic condensations when cells surrounding the mutant condensations failed to aggregate, elongate, and flatten. In conclusion, Ihh is involved in multiple steps and acts through multiple mechanisms in the program of skeletogenesis, ranging from a direct role in cartilage and perichondrial differentiation to a role in determining osteoblast and endothelial cell fate.

585. The Cytoplasmic C-Terminal Tail of Claudin-1 is Essential for Randomizing the Direction of Heart Looping in Chick Embryos. Erminia Di Pietro, Annie Simard, and Aimee K. Ryan. Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada.

In vertebrates, the asymmetric arrangement of internal organs relative to the midline is evolutionarily conserved. We performed a subtractive screen using cDNA prepared from the left and right sides of chick embryos to identify novel molecules involved in asymmetric morphogenesis. One of the molecules identified was Claudin-1, a component of epithelial tight junctions. Claudins contain 4 transmembrane domains and a cytoplasmic C-terminus that interacts with proteins in the cytoplasmic plaques associated with tight junctions. In addition, the cytoplasmic C-termini regulate the stability of claudin molecules and phosphorylation of the C-termini have been shown to affect the permeability of tight junctions. Retroviral overexpression of chick Claudin-1 on the right side of HH stage 4–8 chick embryos randomizes the direction of heart looping. The Claudin-1 cytoplasmic C-terminus contains a putative mitogen-activated protein kinase (MAPK) or a protein kinase C (PKC) site at amino acid 206. A series of constructs were prepared where the C-terminus of Claudin-1 was either deleted or T206 was mutated to a neutral residue (alanine), a negatively charged residue (glutamic acid), or a positively charged residue (arginine). HH stage 4 embryos were infected with retroviral particles expressing these Claudin-1 protein mutants. Our results suggest that the MAPK/PKC consensus site in the cytoplasmic C-terminus of Claudin-1 is important for its left–right patterning function.

586. SDF1 and its Receptor CXCR4 Control Cell Migration and Patterning During Somitogenesis. Emi Ohata,[1] Toshiharu Kasai,[2] Takashi Nagasawa,[3] and Yoshiko Takahashi[2]. [1]NAIST, Nara, Japan; [2]Center for Developmental Biology, RIKEN, Kobe, Japan; [3]Kyoto Univ., Kyoto, Japan.

During vertebrate somitogenesis, numerous intercellular signaling events must occur. By using a signal sequence trap method (Tonegawa et al., Developmental Biology, 2003), we identified genes encoding extracellularly acting proteins synthesized in the chicken presomite/somite tissue. Among those genes, CXCR4, a receptor for the chemokine SDF1, exhibits a specific and dynamic pattern of expression. We here report that the signaling mediated by CXCR4/SDF1 plays important roles in tissue interactions between somite and neighboring tissues. When CXCR4 is electroporated into presomitic mesoderm (PSM) cells, the electroporated cells become preferentially localized in the dorsal region of the PSM, adjacent to the overlying ectoderm that is expressing high levels of SDF1. This behavior of cells is mediated by SDF1 since CXCR4-expressing cells are also attracted to implanted COS cells producing the SDF1 protein. In contrast, cells electroporated with CXCR4-RNAi become confined ventrally in the PSM. At a later stage when the SDF1-expressing area shifts from the dorsal ectoderm to the ventral region around the dorsal aorta, CXCR4-electroporated cells migrate toward the dorsal aorta. Disrupted function of CXCR4 causes augmented apoptosis in dermis, as observed in CXCR4-KO mice. Thus, chemotactic behaviors necessary for the patterning of PSM/somite cells are dynamically mediated by somitic CXCR4-expressing cells responding to SDF1 produced by the surrounding tissues.

587. Activation of Notch in the Posterior Compartment of a Somite Determines the Differentiation, Directed Migration, and Patterning of the Dorsal Aorta Precursors. Yuki Sato,[1] Tadayoshi Watanabe,[2] Jun Kouyama,[3] Hideyuki Okano,[3] and Yoshiko Takahashi[1]. [1]Center for Developmental Biology, RIKEN, Kobe, Japan; [2]NAIST, Nara, Japan; [3]Keio Univ. School of Medicine, Tokyo, Japan.

Most of the blood vessel endothelial cells in early vertebrate embryos arise de novo from mesenchymal cells of the lateral plate mesoderm or somites. It remains unexplored about how the angioblastic mesenchyme achieves the formation of stereotypic patterning of blood vessels in the body. We here report a novel mechanism underlying the formation and

patterning of the dorsal aorta from cells of the posterior somitic compartments. By electroporating a Notch–reporter construct into the chicken somitic mesoderm, we have detected cells exhibiting endogenous Notch activation. These cells are confined to the posterior half of individual somites. Consistent with this localization, experimentally Notch-activated and -inactivated cells are located to the posterior and anterior halves of a somite, respectively. Somitic cells whose Notch activity is either endogenous or experimentally induced ultimately participate in the formation of the dorsal aorta and intersomitic vessels. In addition, we show that the Notch-active cells respond to a long-range attractant signal emanating from the dorsal aorta. We present a model in which the three-dimensional patterning of blood vessels is achieved by sorting out and directed migration of angioblastic precursors along both the A–P and D–V axes of the somite, and where Notch and extracellular attractant signals play central roles in these coordinated behaviors of cells.

588. Novel Localization of Claudin-16 Tight Junction Protein in the Goblet Cells of Chick Intestine. Ozkan Ozden, Betty Black, and Brenda Brizuela. North Carolina State University, Raleigh, NC 27695.

Tight junction (TJ) proteins form major barriers in epithelial and endothelial tissues and are responsible for regulating paracellular transport. Claudin protein family members are important structural and functional components of the TJ complex. Mutations in Cla16 have been linked to impaired reabsorption of magnesium and calcium leading to kidney disease, and Cla16 is reported to play a role in forming aqueous pores in the paracellular pathway within Henle's loop. This work investigates, for the first time, the localization of Cla16 in the intestine of chick embryos during the week before hatching and in 1-day-old chicks. The localization of Cla16 was determined in sections of frozen and paraffin-embedded tissue from duodenum, jejunum, and ileum using an immunostaining protocol. Interestingly, the expression of Cla16 was primarily detected in two regions of chick intestine: (1) goblet cells within the intestinal epithelium and (2) smooth muscle layers. Cla16 was not detectable in embryonic epithelium until day 19 (2 days before hatching) even though goblet cells containing mucin appear at 14 days and are abundant by day 18. Cla16 was present in embryonic circular muscle from 14 days (the earliest stage examined) through posthatch. Light microscopy of the goblet cell associated Cla16 suggests that it resides within the membranes of mucus granules. Thus, it may play a role in the mucus secretion process, possibly by creating calcium channels or junctional complexes during exocytosis. Cla16 in the smooth muscle fibers might function in calcium movement between cells or across organelle membranes.

589. Optic Cup Formation Requires Downregulation of Sox3 Expression in Chick Embryonic Eye. Yasuo Ishii and Takashi Mikawa. Weill Med. Col. Cornell Univ., New York, NY 10021.

The optic cup forms through invagination of the distal part of the optic vesicle, giving rise to the photosensitive neural retina and the underlying pigmented epithelium of the eye. Little is known about the molecular and cellular mechanisms responsible for this morphogenetic process. We have found that an HMG-box transcription factor, *Sox3*, is expressed throughout the optic vesicle but downregulated as the vesicle starts to invaginate to form the optic cup. In the present study, we examined the potential role of the downregulation of *Sox3* in optic cup formation. Constitutive overexpression of *Sox3* in the optic vesicle via electroporation induced apical constriction in transfected optic vesicle cells, resulting in ectopic sites of invagination. In contrast, the diencephalon, where endogenous expression of *Sox3* is maintained, was not affected by overexpression of *Sox3*. These results suggest that downregulation of *Sox3* expression is required for normal optic cup formation perhaps by allowing expansion of the apical surface in the retinal primordium. Supported in part by NIH and the Charles H. Revson foundation.

590. TIMP-2 Regulates Cardiac Neural Crest Morphogenesis in Avian Embryos. Mark V. Reedy,[1] Veronica Cantemir,[2] Marilou Holmberg,[2] Jennifer Sanmann,[1] Maureen Stroscheim,[1] D.H. Cai,[2] and Philip R. Brauer[2]. [1]Department of Biology, Creighton University, Omaha NE; [2]Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE.

Tissue inhibitor of metalloprotease-2 (TIMP-2) regulates morphogenesis in two ways: by controlling the activity of extracellular matrix–degrading matrix metalloproteases (MMPs), and by binding to integrins and triggering a signal transduction cascade in cells. Here, we report that TIMP-2 is an important regulator of cardiac neural crest (NC) morphogenesis in chicken embryos. Beginning at stage 11, TIMP-2 mRNA is expressed in the neural tube and in migrating cardiac neural crest cells. Addition of small amounts of exogenous TIMP-2 protein increased cardiac NC cell motility in vitro, whereas addition of the related protein TIMP-1 had no effect. Antisense morpholinos (AMOs) directed against TIMP-2 mRNA significantly inhibited proMMP-2 activation and cardiac NC migration in vitro. In ovo electroporation of anti-TIMP-2 AMOs drastically reduced both the number of migrating cardiac NC cells and the distance that these cells migrated. Conversely, over-expression of TIMP-2 in the neural tube and NC cells in ovo resulted in a dramatic increase in the number of migrating cardiac NC cells. Together, these data demonstrate that TIMP-2 is an important regulator of cardiac NC development in avian embryos. Supported by a grant from the American Heart Association Heartland Affiliate #0051638Z and #0355397Z to PRB and NIH INBRE award P20 RR016469 to MVR.

591. Control of Dorsal–Ventral Innervation Choice in the Vertebrate Limb. Victor Luria and Ed Laufer. Columbia University, New York, NY 10032.

We study how target tissue signals influence the trajectories of nerves that innervate the vertebrate limb. Neurons in the lateral division of the lateral motor column, LMC(l), project axons to the dorsal limb; medial, LMC(m), neurons project axons to the ventral limb. To elucidate how LMC(m) axons are targeted, we asked whether axonal trajectories change in mutants (chick limbless, mouse conditional BmprIa−/−) with bi-dorsal limbs that lack ventral limb tissue. We labeled motor axons genetically and embryologically and analyzed the correlation between axon identity and target tissue. In FGF-rescued bi-dorsal limbless limbs: (i) a DV axon branching occurs and the angle between branches is more obtuse in the mutant than in wild-type; (ii) the dorsal branch originates from LMC(l), the ventral branch from LMC(m). In BmprIa−/− hindlimbs: (i) DV axon branching occurs; (ii) LMC(l) axons extend in both dorsal and ventral branches. Accurate interpretation of the projection patterns requires determining whether the bi-dorsal limb transformation is complete in the mutants. Using functional molecular markers of limb DV identity in both mouse and chick at the time when motor axons make their projection choice, we find that the bi-dorsal limb transformation in limbless, although extensive, is incomplete, while that of BmprIa−/− hindlimbs is complete. Our limbless results are consistent with LMC(m) axons having an absolute preference for the ventral limb, in contrast to the known relative LMC(l) preference for the dorsal limb. A definitive statement awaits resolution of the LMC(m) projection patterns in BmprIa−/− hindlimbs, currently underway.

592. The Adapter Protein SH2-B? Regulates Nerve Growth Factor-Induced Gene Expression. Linyi Chen and Christin Carter-Su. Univ. of Michigan Medical School.

The adapter protein SH2-B binds to activated nerve growth factor (NGF) receptor and enhances NGF-induced differentiation in pre-neuronal PC12 cells, while a dominant-negative mutant, SH2-B(R555E), blocks it.

SH2-B undergoes nucleocytoplasmic shuttling, implying its role in regulating expression of genes that determine neuronal differentiation. We used Affymetrix gene array to compare gene expression profiles of NGF-treated and control PC12 cells expressing GFP, GFP-SH2-B, or GFP-SH2-B(R555E). NGF-induced expression of multiple genes was enhanced by expressing SH2-B but not SH2-B(R555E), including genes encoding urokinase plasminogen activator receptor (uPAR), and matrix metalloproteinase 3 and 10 (MMP3 and 10). QT-PCR confirmed Affymetrix results. NGF-induced activation of MMP3/10 was enhanced in cells expressing GFP-SH2-B but reduced in cells expressing SH2-B(R555E) compared to cells expressing GFP. uPAR, and MMP3 and 10 regulate interaction between the cell and extracellular matrix (ECM) that is continuously regulated during differentiation. uPAR has been shown to prime PC12 cells for NGF-induced differentiation. MMP3-mediated growth cone invasiveness has been shown to contribute to neurite outgrowth. Consistent with SH2-B increasing the expression and activity of MMP3 and 10, SH2-B overexpression enhanced NGF-induced invasiveness of PC12 cells. This increased invasiveness was reduced by TIMP2, suggesting that increased ECM degradation by MMP3/10 allows more differentiating cells to penetrate Matrigel. These results suggest that SH2-B enhances NGF-induced expression of uPAR, and MMP3 and 10. Furthermore, SH2-B enhances neurite outgrowth at least partially through activating MMP3 and 10.

593. Identification and Developmental Expression Analysis of a Novel Homeobox Gene Located in the Proximal Region of Mouse Chromosome 18. Han Liu,[1] Wenjin Liu,[2] Kathy M. Maltby,[2] Yu Lan,[2] and Rulang Jiang[2]. [1]University of Rochester, Rochester, NY 14627; [2]University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Cleft lip and/or cleft palate are common birth defects affecting 1 in 500 to 1000 children worldwide. Both genetic and environmental factors contribute to the etiology of facial clefting. One good animal model for studying the genetic basis and molecular mechanisms of cleft palate pathogenesis is the *Twirler* mutant mouse. The *Twirler* mutation arose spontaneously and causes inner ear defects in heterozygous and cleft lip or cleft palate in homozygous mutant mice. This mutation was previously localized to proximal Chromosome 18. Here, we report the identification of a novel homeobox gene, *Irxl1*, from the vicinity of the *Twirler* locus. *Irxl1* encodes a TALE-family homeodomain protein, with its homeodomain exhibiting the highest (54%) amino acid sequence identity to that of the Irx subfamily members. However, the *Irxl1* protein lacks the Iro-box, a specific amino acid sequence conserved in all known members of the Irx subfamily. Searching the databases showed that *Irxl1* is evolutionarily conserved in *Xenopus*, chick, and mammals. In situ hybridization analyses of different stage mouse embryos showed that *Irxl1* mRNA is highly expressed in the frontonasal process, in the palatal mesenchyme during palate outgrowth, in the myotome prior to the onset of migration and persists in a subset of muscular structures. The developmental expression pattern and its close linkage to the *Twirler* mutation suggest that this new homeobox gene may play important roles in regulating craniofacial development.

594. Wnt5a Regulates SHH and FGF10 Signaling During Lung Development. Changgong Li,[1] Lingyan Hu,[1] Jing Xiao,[2] Hongyan Chen,[1] John Li,[1] Saverio Bellusci,[3] Stijn Delanghe,[3] and Parviz Minoo[1]. [1]Univ. of Southern California, Los Angeles, CA 90033; [2]Dalian Medical University, Dalian 116027, China; [3]Saban Research Institute of Children's Hospital Los Angeles, Los Angeles, CA 90027.

We have previously shown that targeted disruption of Wnt5a results in over-branching of the epithelium and thickening of the interstitium in embryonic lungs. To further determine the role of Wnt5a, we generated and characterized transgenic mice with lung-specific over-expression of Wnt5a from the SpC promoter. Over-expression of Wnt5a interferes with normal epithelial–mesenchymal interactions resulting in reduced epithelial branching and dilated distal airways. During early stage of lung development, over-expression of Wnt5a in the epithelium resulted in increased Fgf10 in the mesenchyme and decreased Shh in the epithelium. Both levels and distribution of SHH receptor, Ptc, were reduced in SpC-Wnt5a transgenic lungs and were reciprocally correlated to the changes of Fgf10 in the mesenchyme, suggesting the SHH signaling is decreased by over-expression of Wnt5a. The increased expression of Fgf10 in the mesenchyme could be modulated by recombinant SHH, suggesting that the SHH pathway is still functional in regulating mesenchymal Fgf10 gene expression and distribution. Cultured mesenchyme-free epithelial explants from Wnt5a transgenic lungs responded abnormally to FGF10 with dilated branch tips that mimic the in vivo phenotype, suggesting that over-expression of Wnt5a disrupts the response of epithelium to FGF10 signaling. In conclusion, Wnt5a regulates SHH and FGF10 signaling during lung development.

595. The Transcription Factor HNF-6 Controls Differentiation of Ductal Pancreatic Cells. Christophe E. Pierreux,[1] Sabine Cordi,[1] Frédéric Clotman,[1] Miguel Maestro,[2] Jorge Ferrer,[2] Guy G. Rousseau,[1] and Frédéric P. Lemaigre[1]. [1]Université catholique de Louvain and Institute of Cellular Pathology, Brussels, Belgium; [2]Institut d'Investigacions Biomédiques August Pi i Sunyer, Barcelona, Spain.

During embryogenesis, Hepatocyte Nuclear factor (HNF)-6 is expressed in the pancreatic epithelium. We have previously shown that HNF-6 controls the expression of Pdx-1 in the foregut endoderm, and of HNF-1β and ngn3 in precursors of the endocrine lineage. We have now investigated the role of HNF-6 in the morphogenesis of pancreatic ducts by analyzing the phenotype of Hnf6−/− mice. Whole mount immunochemistry to detect mucin as a marker of the ductal lumen revealed that the pancreatic epithelium of Hnf6 knockout is disturbed as early as e11.5. Later on, branching defects were observed and epithelial cysts appeared at e15.5. These cysts were located in intra- and interlobular ducts. The cells lining these cysts were not overproliferating, but failed to display the differentiation characteristics of ductal cells. They did not express HNF-1β and failed to form primary cilia on their apical surface. Since dysfunction or absence of cilia in kidneys is associated with cystic diseases, we analyzed in Hnf6−/− pancreas the expression of genes mutated in these diseases. We found that the expression of cystin and of fibrocystin was down-regulated in Hnf6−/− pancreas. In conclusion, HNF-6 is required for ductal morphogenesis and differentiation in the pancreas. It also controls the formation of primary cilia and the expression of a network of ciliogenic genes.

596. Identification of a Novel Gene Expressed Exclusively in the Zone of Polarizing Activity in the Vertebrate Limb. Jason R. Rock and Brian D. Harfe. Univ. of Florida, Gainesville, FL 32610.

Classical experiments identified the zone of polarizing activity (ZPA) as being responsible for patterning the anteroposterior limb axis. Subsequent experiments determined that Sonic hedgehog (Shh) is responsible for the polarizing activity associated with this region of the developing limb. To date, Shh is the only gene known to be expressed exclusively in the ZPA in the limb. To identify other genes expressed in the ZPA, we took advantage of a transgenic mouse allele in which we had inserted GFP into the Shh locus. Embryos with this transgene expressed GFP in all cells that normally express Shh. Cells expressing Shh were cell sorted from E10.5 limbs and labeled complementary RNAs from GFP-positive (the ZPA) and GFP-negative (rest of the limb

bud) cell populations were hybridized to Affymetrix GeneChips. Analysis of the data revealed that Shh and ~15 other genes were exxpressed at higher levels in the ZPA than in the rest of the limb. One of these genes, an uncharacterized EST with 8 transmembrane domains, was analyzed further. We have provisionally named this gene TM1. Whole mount RNA in situ hybridization revealed that TM1 is expressed in the limb in a pattern temporally and spatially identical to Shh. TM1 is a member of a family of transmembrane proteins that share no similarity to any of the known transmembrane proteins functioning in the Shh-signaling pathway. In the chick limb, we have found that the TM1 homolog is also expressed exclusively in the ZPA. RNA in situ hybridization analysis of TM1 expression in mutant mouse embryos suggests that Fgf-signaling is necessary for normal TM1 expression, but Shh is not.

597. MicroRNA Regulation of Murine Limb Development. Danielle M. Maatouk,[1] Michael T. McManus,[2] and Brian D. Harfe[1]. [1]Univ. of Florida, Gainesville, FL 32610; [2]Univ. of California, San Francisco, San Francisco, CA 94143.

MicroRNAs (miRNAs) encode short untranslated mRNAs that have been shown to be essential for the normal development of several organisms. MiRNA transcripts are initially processed to produce a precursor hairpin transcript of ~70–80 nt in length (pre-miRNA). The hairpin pre-miRNA is then further processed by the Dicer enzyme, producing a mature ~21 nt miRNA. Mature miRNAs regulate gene expression by binding to target sequences resulting in mRNA degradation or translational inhibition. There are over 250 miRNAs encoded in the mammalian genome; however, only a few have been assigned functions. We are interested in uncovering the roles of miRNAs during murine limb development. Mice null for Dicer die by 7.5 dpc, well before limbs form. To investigate the roles of miRNAs at later developmental stages, we have generated a conditional null allele of Dicer. Previously, we found that removal of Dicer specifically from the limb mesoderm results in loss of miRNA processing, increased cell death, and abnormal gene expression. To further investigate the role of miRNAs in limb development, we are using microarray technology to uncover target genes that are misregulated upon loss of mature miRNAs. Loss of miRNAs may directly or indirectly influence gene expression. To determine which genes obtained in our screen are direct targets of miRNAs, genes will be screened for known miRNA binding sites. By identifying genes which are direct targets of miRNAs, our studies will help elucidate the roles of miRNAs during vertebrate limb development.

598. Molecular Cascade Leading to the Morphological Segmentation During Somitogenesis: Ephrin-Eph Signaling Downstream of Notch and cMeso1. Tadayoshi Watanabe,[1] Yuki Sato,[2] Jun Kouyama,[3] Hideyuki Okano,[3] and Yoshiko Takahashi[2]. [1]NAIST, Nara, Japan; [2]Center for Developmental Biology RIKEN, Kobe, Japan; [3]Keio Univ. School of Medicine, Tokyo, Japan.

During early development in vertebrates, the presomitic mesoderm (PSM) undergoes segmentation to form somites, the basis of the reiterated structures along the antero-posterior axis of the body. We previously found, using electroporation, that the posterior border cells at the prospective boundary of PSM exhibit a fissure-inducing activity (called the "segmenter"). We also showed that this inductive action involves Notch signaling (Sato et al., *Development*, 2002). By extending this study, we here report a signaling cascade that leads to the production of the segmenter. The bHLH transcription factor *cMeso1*, a homolog of mouse *MesP2*, exhibits the segmenter activity when an ectopic boundary of *cMeso1* expression was created in a non-segmenting region. Overexpression of *cMeso1* results both in activation of a Notch reporter construct, and also in ectopic expression of *EphA4*. Since Notch is sufficient and also required for EphA4, the molecular cascade, cMeso1→Notch→EphA4, can be drawn. Furthermore, we have found that the ephrin-reverse signal in the anterior border cells is sufficient to make a fissure, and Eph-forward signal seems to be dispensable. We present a model in which a molecular cascade originating from cMeso1 at the prospective boundary site is mediated by Notch signal, and this cascade is ultimately resolved into the ephrin-reverse signal, which plays a critical role during morphological segmentation.

599. Failure of TGF-β Signaling Via Alk5 in Neural Crest Cells Leads to Facial Clefting. Marek Dudas,[1] Jieun Kim,[1] Andre Nagy,[1] Jonas Larsson,[2] Stefan Karlsson,[2] and Vesa Kaartinen[1]. [1]Developmental Biology Program, Children's Hospital Los Angeles, Los Angeles, CA 90027, USA; [2]Lund University Hospital, Sweden.

Cranial neural crest contributes significantly to formation of facial bones and cartilage. TGF-β signaling is involved in specification and patterning of many cell types including neural crest cells. However, detailed signaling mechanisms, particularly in vivo, are poorly known. We have deleted the Tgf-β type-I receptor Alk5 specifically in the neural crest cell lineage. Mutant mice display severe facial and calvaria defects. While the external calvaria phenotype is very similar to that seen in mutants lacking the TGF-β type-II receptor (Tgfbr2) in the neural crest, the facial phenotypes including nasal clefting and dramatic mandibular hypoplasia are uniquely different. These defects can be at least partly attributed to the increased rate of apoptosis of cells derived from the neural crest in the mesenchyme of the first pharyngeal arch, and failure to appropriately form the maxillary processes of the first pharyngeal arch. Moreover, expression of Msx1 and Tgif, both genes with a well-established role in facial morphogenesis, was notably attenuated in the maxillary primordia of mutants at E10–E11. The current results demonstrate that Alk5 plays a unique non-redundant cell-autonomous role in the neural crest during facial development. The remarkable divergence between Tgfbr2 and Alk5 phenotypes raises the possibility that Alk5 is activated not only by TGFβRII, but also by other type-II receptors in vivo during facial development.

600. Craniofacial Dysmorphogenesis in qkk2 Quaking Null Mutation Embryos. Olga V. Lakiza,[1] Jennifer Northrop,[3] Monica Justice,[3] David Walterhouse,[1] Elizabeth B. Goodwin,[2] and Phil Iannaccone[1]. [1]Children's Memorial Research Center, Northwestern Univ. Feinberg School of Medicine, Chicago, IL; [2]Univ. of Wisconsin, Madison, WI; [3]Baylor College of Medicine, Houston, TX.

Quaking belongs to the STAR family and has been shown to bind RNA and repress translation via an element in the 3′UTR, called the TGE. Mouse quaking transcripts are expressed beginning at E7.5. During development, quaking expression is found in the head folds, nascent brain, and neural tube. The ENU-induced embryonic lethal allele of quaking, qkk2, contains a T to A transversion in the KH (RNA binding) domain. Quaking plays a critical role in blood vessel development and blood vessel defects are the cause of embryonic death of qkk2 mutant embryos. Some qkk2 mutant embryos have shown evidence of severe neural tube defects, open head folds, abnormal somites and abnormal axial development. Here, we evaluate the craniofacial defects in detail. We find that qkk2 cranial defects vary from pure midbrain exencephaly (9 of 19 abnormal embryos) to severe craniofacial dysmorphology (5 of 19 embryos) associated with hemorrhage in the head mesenchyme and hyperplasia of the neuroectoderm and/or mesenchyme. In 2 of 19 mutant embryos, there were caudal neural tube defects. Remarkably, 9 of 19 qkk2/qkk2 embryos exhibited embryonic axis defects. In these embryos, the trunk region or middle part of the embryonic axis rotates in a clockwise direction relative to the direction of the tail. The mechanism responsible for craniofacial dysmorphogenesis in the qkk2 mutants is still not understood. Supported in part by ES010549 from the NIH, DHHS.

601. *Fgfr1* and *Fgfr2* are Necessary for Craniofacial Development in the Mouse. Graham Smyth,[1] Kai Yu,[2] David M. Ornitz,[2] Juha Partanen,[3] and Erik N. Meyers[1]. [1]Duke University Medical Center, Durham, NC 27710; [2]Washington University Medical School, St. Louis, MO 63110; [3]Viikki Biocenter, Developmental Biology Program, PO Box 56, Viikinkaari 9, 00014-University of Helsinki, Finland.

The development of the vertebrate face and heart are critically dependent upon neural crest cells (NCCs). NCCs are specified along the dorsal neural tube and migrate away to contribute to cardiovascular structures and facial primordia. Signaling interactions between ectoderm, endoderm, and underlying NCC coordinate the outgrowth of the pharyngeal arches, which, together with muscle and other tissues, form the adult face, as well as components of the cardiovascular system. Previous studies have suggested a role for fibroblast growth factor (Fgf) signaling in NCC patterning and survival. *Fgf8* in particular is expressed in the ventral ectoderm of the face as well as in the pharyngeal ectoderm and endoderm, thus implicating it as an important signaling ligand for the development of the these structures. To further examine the role of Fgfs in neural crest development, we characterized the expression pattern of Fgf receptors (Fgfrs) within developing NCC and surrounding tissues between e8.5 and 11.5. In addition, we used a genetic approach to delete Fgf receptors (Fgfrs) from migratory neural crest using the Cre-loxP system. Comparison and contrasts of the results from deleting *Fgfr1* and/or *Fgfr2* will be presented. These results support a model where *Fgfr1* and *Fgfr2* are functionally redundant in some tissues and required by only a subpopulation of NCC.

602. Regulation of Craniofacial Morphogenesis by Dlx Family Members. Peter G. Farlie, Sonja J. McKeown, and Donald F. Newgreen. Murdoch Children's Research Institute, Melbourne, Victoria 3052, Australia.

Craniofacial morphogenesis involves the coordination of complex signalling events and the conversion of these cell surface signals into tightly regulated changes in cellular behaviour. The Dlx family of homeodomain transcription factors are expressed in a nested fashion throughout the branchial arches and targeted deletion in mice has revealed roles in patterning and development of the craniofacial skeleton. This indicates that the Dlx genes may be intermediaries involved in the conversion of cell surface signals into specific morphogenetic events. We have investigated the function of the Dlx genes using in ovo electroporation and cell culture. Ectopic expression of Dlx2 within the neural tube beginning prior to emigration of neural crest cells at E1.25 drastically inhibits the migration of transfected cells and induces aggregation of transfected neuroepithelial cells within the neural tube. Electroporation of the Dlx2 expression construct into branchial arch mesenchyme induced N-cadherin and NCAM, resulted in a dramatic increase in cell–cell adhesion relative to controls, and promoted mesenchymal condensation. Interestingly, while similar results were obtained for Dlx5, no change in cell adhesion or condensation is observed following overexpression of Dlx1. We are currently investigating antagonistic relationships between Dlx family members. These findings suggest that the Dlx genes are involved in regulating craniofacial morphogenesis by controlling mesenchymal condensation through changes to the cell adhesion properties of cranial neural crest cells.

603. Perioptic Mesenchymal Target for Retinoic Acid Synthesized in Eye Field Ectoderm. Andrei Molotkov, Natalia Molotkova, and Gregg Duester. The Burnham Institute, La Jolla, CA 92037.

Retinoic acid (RA) is a cell–cell signaling molecule derived from vitamin A (retinol) that is required for vertebrate eye development. RA deficiency leads to microphthalmia, but the mechanism of RA action in the eye field is unclear. To address this question, we are analyzing three retinaldehyde dehydrogenase genes (Raldh1, Raldh2, and Raldh3) expressed in the mouse eye that control synthesis of endogenous RA from retinol. Raldh2 is expressed transiently in the optic vesicle, Raldh3 is expressed in the eye field surface ectoderm, and both Raldh1 and Raldh3 are expressed in the retina from the late optic vesicle stage throughout gestation. We have found that optic cup formation still proceeds relatively normally in Raldh1−/− and Raldh3−/− embryos. Raldh2−/− and Raldh1−/−:Raldh2−/− double mutants also exhibit normal optic cup formation as well as normal RA activity associated with Raldh3 expression in the eye, suggesting that both Raldh1 and Raldh2 are dispensable for optic cup formation when Raldh3 is present. In support of this hypothesis, Raldh2−/−:Raldh3−/− double mutants fail to invaginate the retina ventrally, and Raldh1−/−:Raldh3−/− double mutants exhibit excess perioptic neural crest migration into the anterior eye resulting in a recessed optic cup. Our findings suggest that ectodermal tissues of the eye field synthesize RA that is required to limit perioptic neural crest mesenchyme migration to allow proper invagination of the optic cup and proper placement of the optic cup relative to the surface. We find that Raldh3 alone is sufficient for eye RA synthesis, but that Raldh1 and Raldh2 together can replace this function in Raldh3−/− embryos.

604. A Novel Gene Required During Neural Tube and Eye Development. Tae-Hee Kim,[1] Kathryn V. Anderson,[1] and Lee Niswander[2]. [1]Cornell University and Sloan-Kettering Institute, New York, NY; [2]University of Colorado Health Science Center, Denver, CO.

Neural tube closure defects (NTDs) are one of the most common birth defects in humans. In mouse, mutations in genes involved in many different processes can lead to NTDs such as genes which regulate patterning of embryo, cell cytoskeleton, or cell proliferation. To better understand the genetics of NTDs, we have used an ethylnitrosourea (ENU) mutagenesis approach to identify recessive mutations causing NTDs in mice. One line: G2E has an open neural tube in the brain (exencephaly), and eye defects including coloboma. Characterization of the neural tube and retina phenotypes suggests that these defects are caused by abnormal proliferation. A missense mutation in a conserved domain of a novel protein phosphatase and actin interacting protein was identified in G2E line. Our data suggest that the G2E protein regulates cell proliferation by regulation of protein phosphatase and retinoblastoma (Rb) during neural tube and eye development.

605. The Actin Binding Protein Shroom Facilitates Neural Tube Closure by Regulating the Apical Positioning of Actomyosin. Jeffrey D. Hildebrand. Department of Biological Sciences, University of Pittsburgh, Pittsburgh PA, 15260.

Aberrant neural tube closure is one of the most common human birth defects. Mouse and *Xenopus* embryos deficient for the actin binding protein Shroom (Shrm) fail to complete neural tube closure, resulting in spina bifida and exencephaly. These defects stem from the inability of Shrm-deficient neuroepithelial cells to undergo apical constriction, a change in cell morphology required for the formation of hinge points and juxtaposition of the lateral edges of the neural folds at the dorsal midline. Shrm co-localizes with F-actin and Nectin at the apical-most aspect of adherens junctions of neural epithelium and to the Apical Junctional Complex (AJC) in MDCK cells. Expression of Shrm in MDCK cells causes them to undergo apical constriction and adopt a wedge-shaped morphology, likely mimicking the cell shape changes necessary for hinge point formation. Shrm induces apical constriction by orchestrating the assembly of an ectopic actomyosin network in the AJC. In vivo, Shroom-deficient neural epithelia show a marked reduction in apically positioned actomyosin. Genetic analysis suggests that Shrm-mediated actomyosin distribution may also play a role in the steps of neural tube closure that are regulated by

planar cell polarity pathways. Thus, Shroom regulates neural tube morphogenesis by facilitating the apical positioning of a contractile actomyosin network in neural epithelial cells and provides a novel mechanism for controlling epithelial cell shape and morphogenesis during elaboration of the vertebrate body plan. Supported by NIH grant GM41570.

606. Spontaneous Mutation in Mice Causes Branching of the Notochord and Fusions of Caudal Vertebrae. Deborah L. Chapman and Deborah R. Farkas. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

We have a spontaneous mutation that results in a kinky tail phenotype in heterozygotes and early embryonic lethality in homozygotes. The kinky tail phenotype varies within litters, with some individuals displaying a shortened kinked tail, others having looped tails, and still others having nearly normal sized tails with only a few kinks. Skeletal preparations showed that the kinky tail phenotype results from fusions and malformations of tail vertebrae. These vertebral malformations are restricted to the tail region. Alcian blue staining of e13.5 embryos revealed that the notochord branches dorsally in the tail region prior to noticeable vertebral abnormalities. *shh* expression in the kinky tail embryos supports the histological findings, namely, that in multiple regions of the tail, the notochord branches. In addition, *shh* is expressed in a broadened region suggesting a thickened notochord. Intercrossing of kinky tail mice resulted in a group of embryos with embryonic lethal phenotypes by e8.5, presumably representing the homozygous mutant embryos. Mesoderm production is severely impaired in these homozygous embryos. Phenotypic analysis of the heterozygous and homozygous mutant phenotype will be described. This spontaneous mutant is currently being mapped to identify the affected gene. Sponsored by NIH NICHD Grant: RO1-HD38786.

607. *Nap1*-Dependent Cell Migration is Required for Early Organogenesis and Axis Specification in the Mouse. Andrew S. Rakeman[1] and Kathryn V. Anderson[2]. [1]WGSMS, Cornell University, 445 East 69th Street New York, NY 10021; [2]Sloan Kettering Institute, 1275 York Ave., New York, NY 10021.

Cell migration in the early mouse embryo is important for morphogenetic events that shape the embryo as well as movement of organizer structures into juxtaposition with their target tissues. Despite their importance, little is known about the regulation these migration events. We identified an ENU-induced mouse mutant, *khlo*, with morphogenetic defects in the heart and foregut without defects in their patterning or specification. *khlo* mutants arrest at e8.5 having failed to form a single heart tube (cardia bifida) or close the foregut.

We identified a Leu to Pro missense mutation in the *Nap1* transcript in *khlo* mutants. Complementation tests with a gene-trap allele of *Nap1* proved that it is the gene responsible for the *khlo* phenotype. *Nap1* regulates actin remodeling by controlling the activity of WAVE proteins. Using a combination of embryological and explant analyses, we determined that *Nap1* is required for the efficient migration of the foregut endoderm during gastrulation. We demonstrate that coordinate morphogenesis of the foregut endoderm and cardiac mesoderm is critical for heart fusion. In addition to morphogenetic defects, a fraction (~15%) of *khlo* homozygotes have a duplication of the anterior–posterior axis. A–P axis specification requires movement of the anterior visceral endoderm (AVE) from the distal tip to the future anterior side of the pre-gastrula. We show that AVE migration is blocked or delayed in ~50% of *khlo* mutants, suggesting that defective AVE migration is responsible for axis duplication in *khlo* mutants.

608. The *chato* Mutation Disrupts Antero–Posterior Axis Elongation of the Mouse Embryo. Maria J. Garcia-Garcia and Kathryn V. Anderson. Sloan-Kettering Institute, New York, NY 10021.

During early embryogenesis, morphogenetic events responsible for patterning of the vertebrate body plan require the coordinated movement of cells. In *Xenopus* and zebrafish embryos, elongation of the body anterior–posterior (A–P) axis is achieved through cell growth and polarized cell movements of convergence and extension, a process regulated by non-canonical Wnt signaling. In the mouse, it is believed that directional mediolateral cell movements also promote the elongation of the A–P axis, but non-canonical Wnt signaling mutants disrupt neurulation and only show modest elongation defects. An ENU screen identified the recessive mutation *chato*, which disrupts elongation of the A–P body axis. *chato* mutants survive until e10.5, showing a characteristic shortening of the embryo. Analysis of markers of epithelial and mesenchymal cell types (such as *Krox20* and *Mox1*) revealed that, in *chato* mutants, these markers are expressed in domains laterally broader and apart from the midline of the embryo, a phenotype reminiscent of frog and fish embryos with defective convergent extension. However, the *chato* mutation does not genetically interact with mouse mutants in the non-canonical Wnt signaling pathway such as *Looptail* (*Strabismus*), suggesting that, in the mouse, other signaling pathways regulate A–P axis elongation. The positional cloning of *chato* revealed that it affects a zinc finger protein. Interestingly, zinc finger proteins of this family regulate elongation movements of imaginal discs in *Drosophila*. Our studies on *chato* provide evidence for the involvement of a novel signaling pathway controlling elongation of the body axis in vertebrates.

609. *limulus* and *wsnp*: Cell Adhesion During Embryonic Morphogenesis. Jeffrey D. Lee and Kathryn V. Anderson. Sloan Kettering Institute, New York, NY 10021.

The genes that regulate cell adhesion during mammalian embryonic morphogenesis are largely unknown. In the mouse embryo, mesoderm and endoderm form as cells delaminate from the primitive streak and then migrate to the positions appropriate for each cell fate. Gastrulation thus requires dynamic regulation of cell–cell and cell–substrate adhesion. To investigate this process, we are performing a phenotypic and molecular characterization of the recessive mutants *limulus* (*lulu*) and *wing-shaped neural plate* (*wsnp*) that were isolated in an ENU screen for mutations affecting embryogenesis. Both mutants die at 9.5 dpc with similar sets of defects: they are shortened along the AP axis, with deficits of posterior tissue and an apparent expansion of anterior neural tissue. At 7.5 dpc, mesodermal patterning appears normal, but by 8.5 dpc, numerous mesodermal defects are apparent: both mutants have a dramatic reduction of paraxial mesoderm, a discontinuous notochord, cardia bifida, and an abnormal node which blocks the establishment of left–right asymmetry. Similar phenotypes have been described for mutations that affect signaling through the Fibronectin–Integrin a5 pathway. Thus, *lulu* and *wsnp* may alter the motility or the adhesive properties of nascent mesodermal tissue, or of epiblast cells moving posteriorly toward the primitive streak. Genetic analysis of *lulu/wsnp* double mutants, including weaker allelic combinations, suggests that the genes encoding *lulu* and *wsnp* act in the same pathway. *lulu* encodes a FERM domain protein; together with data from the zebrafish *lulu* homolog, this implicates *lulu* and *wsnp* in the regulation of cell adhesion during gastrulation.

610. Middle Primitive Streak Assembly and Function During Mouse Gastrulation. Elizabeth Lacy, Sharon Strope, Roberta Rivi, Claudia Munoz, Floria Lupu, and Loydie Jerome-Majewska. Sloan-Kettering Institute, NY.

Fate mapping studies in the mouse divide the primitive streak into three functional domains: the proximal region, generating germ cells and yolk sac mesoderm; the distal region, producing gut endoderm and node derived axial mesoderm; and middle streak, giving rise to paraxial, intermediate, and lateral plate trunk mesoderm. To gain insight into mechanisms

mediating the assembly of the primitive streak into these functional regions, we are characterizing recessive mutations, either transgene or ENU induced, that specifically disrupt the formation and/or function of the middle streak. Studies on one of these mutations led to the discovery of Amn, a type I transmembrane protein that is expressed exclusively on the apical surface of the visceral endoderm, an extraembryonic layer surrounding the epiblast. Our recent studies implicate Amn as an essential component of the Cubilin receptor complex (Dev. 131:4787, 2004). In intestine and kidney proximal tubules, Cubilin functions as a scavenger receptor that mediates internalization and transcytosis of a variety of ligands. Ongoing experiments seek to understand how the endocytic, transcytotic, and potentially signaling activities of Cubilin/Amn on visceral endoderm impact epiblast cell behaviors involved in middle streak formation and function. Two ENU mutants, mermaid (merm) and 99/J, also impair middle streak derivatives, notably posterior paraxial mesoderm. Both exhibit somites of abnormal size and morphology, reduced amounts of presomitic mesoderm, and an irregular tail bud. Gene identification studies are in progress for 99/J but have revealed that merm encodes a nucleporin.

611. A Polarity Gene, Pals1 (Stardust), is Essential for Normal Gastrulation in Mice. Seonhee Kim,[1] N. Ray Dunn,[2] Jarema Malicki,[3] and Chris A. Walsh[1]. [1]HHMI/BIDMC, Harvard Med. School, Boston, MA 02115; [2]Harvard Univ. Cambridge, MA 02138 and ES Cell International, Singapore 138667; [3]Harvard Med. School/MEEI, Boston, MA 02114.

The establishment and maintenance of cell polarity is required for essential developmental processes such as cell proliferation, shape change, and fate decisions as well as directed cell migration. Cell polarity complexes are well characterized for their roles in invertebrate development, and evolutionarily conserved homologs of these genes have been identified in mammals. However, their function in early mammalian development has not been extensively explored by genetic approaches, and redundancy among polarity complex genes has been anticipated. Here, we describe the generation of a loss-of-function mutation in the mouse ortholog of a polarity gene, *Stardust* (*Drosophila*) and *Nagie oko* (*Zebrafish*), termed Pals1, and present data describing its crucial role during early embryonic development. Loss of Pals1 results in lethality by day 9 of gestation. Pals1-deficient embryos show striking morphological abnormalities due to abnormal cell shape changes and disrupted proliferation control within the early ectoderm. These defects significantly impair gastrulation, leading to a paucity of embryonic mesoderm and axis elongation defects, including loss of notochord and abnormal anterior neuroepithelium. Furthermore, the adherens junctions are not properly maintained in the neural tube of Pals1 mutant embryos. These results suggest that Pals1 plays an indispensable role in the maintenance of the cellular architecture within the early ectodermal and neuroectodermal epithelia. Supported by the NINDS and HHMI.

612. GON-1 and Fibulin have Antagonistic Roles in Control of Organ Shape. Daniel Hesselson,[1] Craig Newman,[2] Kyung Won Kim,[2] and Judith Kimble[3]. [1]Dept. Genetics; [2]Dept. Biochemistry; [3]HHMI and Depts. Biochemistry and Genetics, Univ. of Wisconsin-Madison, Madison, WI 53706.

The GON-1 ADAMTS metalloprotease is required for migration of the distal tip cell (DTC), which controls both size and shape of the developing gonad in the nematode *C. elegans*. We find that either of two human homologs, ADAMTS-4/aggrecanase-1 or ADAMTS-9, can rescue a *gon-1* null mutant, suggesting that human and nematode metalloproteases control related biological events. To investigate the role of the extracellular matrix (ECM) in DTC migration, we genetically removed individual ECM components from *gon-1* mutants and discovered one *gon-1* suppressor, called fibulin (*fbl-1*). Whereas the DTC fails to migrate in *gon-1* null mutants, it migrates extensively in *fbl-1 gon-1* double mutants. Therefore, wild-type fibulin must block DTC migration when GON-1 is absent. The *gon-1* suppression by depletion of fibulin is specific, since removal of other ECM components (e.g., collagen, perlecan, nidogen) did not suppress the *gon-1* DTC defect. In *fbl-1* single mutants, DTC migration is essentially normal, but the gonad is thicker than usual. Therefore, wild-type fibulin constrains gonadal width. Removal of GON-1 from *fbl-1* mutants restores the gonad to its normal width, suggesting that GON-1 and fibulin have antagonistic, but balanced, effects on gonadal width. GON-1 is expressed in the migrating DTC and non-gonadal tissues, while fibulin is expressed only in non-gonadal tissues. A rescuing fibulin–YFP fusion protein localizes to several basal laminas, including that around the gonad. We propose that the localized expression of GON-1 in the DTC overcomes a migration block imposed by fibulin.

613. Planarian Robo RNAi Knockdowns Result in CNS Patterning Defects and Disruption of Polarity During Regeneration. Francesc Cebrià and Phillip A. Newmark. Department of Cell and Structural Biology, Neuroscience Program, Univ. of Illinois at Urbana-Champaign, IL 61801, USA.

Freshwater planarians are able to regenerate a complete and functional central nervous system (CNS) from almost any part of their bodies in less than 2 weeks. During anterior regeneration, the newly differentiated cephalic ganglia must re-establish the proper connections with the regenerated ventral nerve cords and regain control over the pre-existing nervous system. In an attempt to unravel how the planarian CNS is rewired during regeneration, we are characterizing axon guidance cues from *Schmidtea mediterranea*. Roundabout (Robo) proteins are transmembrane receptors of the Ig superfamily and, together with their ligand Slit, are necessary for the proper wiring of the developing CNS in *Drosophila*, *C. elegans*, and vertebrates. Here, we have identified two robo homologues in *S. mediterranea*, which are expressed in the intact and regenerating CNS. RNAi analyses clearly show that *Smed robo1* is required for the proper regeneration of the CNS as well as for the stereotypical pattern of the axonal projections of the photosensitive cells. Surprisingly, *Smed robo1* RNAi knockdowns also result in polarity defects as revealed by ectopic pharynx formation and cephalic outgrowths.

614. Cell Motility and Steroid Hormone Signaling in *Drosophila*. Michelle A. Starz-Gaiano, Xuejiao Wang, Stacey Bridges, Mariana Melani, and Denise Montell. Johns Hopkins School of Medicine, Baltimore, MD.

Uncovering the mechanisms controlling cell migration is essential to further our understanding of embryonic development and tumor metastasis. We are studying a group of motile cells in the *Drosophila* ovary, the border cells, as a model system. These cells delaminate from a layer of follicle cells and migrate through a cluster of germ-line cells, stopping next to the developing oocyte. The movement of the border cells is regulated in part by signaling through ecdysone, the single steroid hormone in flies. Like steroid hormone signaling in mammals, ecdysone binds a receptor and the complex activates transcription. Ecdysone Receptor (EcR) in the border cells functions in cooperation with a p160-type coactivator, Taiman (Tai). The human homolog of Tai is AIB1, a breast cancer oncogene. Border cells mutant for *EcR* or *tai* are unable to migrate properly, but the transcriptional targets that contribute to this phenotype are unknown. Using microarray analysis, we have identified 159 potential EcR and Tai target genes in the border cells. These include potential adhesion molecules, post-translational regulators, and metabolic enzymes as well as some genes previously identified as ecdysone targets in other cells and tissues. Expression patterns and mutant phenotypes are

being examined. In addition to this analysis, we have screened 2950 new P-element insertion mutants for abnormal migration phenotypes. This has uncovered additional genes that contribute to border cell movement. We are working to determine in which pathways these genes function, and whether they act autonomously or non-autonomously in migration.

615. Dissecting the Role of VEGFR in Hemocyte Migration in *Drosophila*. M. Haesemeyer, D. Siekhaus, and R. Lehmann. HHMI, Developmental Genetics Program, NYU School of Medicine, New York, NY 10016.

Drosophila hemocytes are an attractive system to study cell migration in vivo. They are formed in the head mesoderm during stage 8 of embryogenesis and follow a fixed migratory pattern resulting in an even dispersal throughout the embryo by stage 15. The *Drosophila* VEGFR homolog (PVR) plays a key role in hemocytes. It controls their survival as well as their migration from the dorsal head region into the juxtaposed tail. In VEGFR mutants, hemocytes undergo apoptosis forming clumps in the head [Brueckner et al. Dev. Cell 7(1)73–84]. This phenotype can be rescued by suppressing apoptosis in hemocytes in a VEGFR mutant background thereby revealing the tail migration defect. To further elucidate the mechanism by which VEGFR controls this migratory step and to find further genes involved in hemocyte migration, we screened 1200 GAL4 P element insert lines. We identified 14 lines showing expression in hemocytes. 5 of these displayed defects in hemocyte migration. Currently, our focus is on an insertion in a signal transduction molecule that shows a clear defect in the same tail migration step that is also defective in VEGFR mutants. In situ hybridization as well as real-time RT-PCR revealed that VEGFR RNA levels are reduced dramatically in this mutant background. We therefore hypothesize that basal VEGFR levels are sufficient for hemocyte survival whereas efficient migration requires elevated VEGFR levels achieved by the action of the signal transduction molecule. We are investigating whether this change in VEGFR level is controlled transcriptionally and which transcriptional elements are responsible for this regulation.

616. At the Next Stop Sign, Turn Right: The Metalloprotease Tolloid-Related1 Controls the Defasciculation of Motoaxons in *Drosophila*. Hermann Aberle and Frauke Meyer. MPI for Developmental Biology, Spemannstr.35, 72076 Tuebingen, Germany.

The navigation of motoneuronal growth cones towards their muscle targets serves as a model system to study the molecular mechanisms that control axon guidance and synaptic target selection. In a large-scale mutagenesis screen, we identified piranha, a motoaxon guidance mutant that shows strong defects in the innervation pattern of somatic muscles. Neuromuscular junctions are often missing or located at wrong places. Transgenic visualization of motoaxons in embryos and larvae demonstrated that defasciculation errors occur at specific choice points in all motoneuronal pathways. Positional cloning of piranha revealed point mutations in the conserved metalloprotease gene tolloid-related-1 (tlr-1), a sequence homolog and genomic neighbor of tolloid. In piranha mutant backgrounds, ectopic expression of Tlr-1 in various tissues including muscles, neurons, fat body cells, or hemocytes completely restores the wild-type innervation pattern indicating that secretion of Tlr-1 into the hemolymph is sufficient for rescue. In contrast, rescue experiments using Tolloid or the related metalloprotease Kuzbanian failed to rescue the tlr-1 mutant phenotype. Similarly, loss-of-function mutations in tolloid or kuzbanian do not show motoneuronal innervation defects, suggesting that Tlr-1 is the only metalloprotease involved in steering motoaxonal growth cones to their targets. The data support the model that Tlr-1 is secreted into the extracellular space to cleave specific substrates at choice points, which enables motoaxons to defasciculate from their original nerve bundle and migrate into their target fields.

617. The Role of Eph/Ephrin Signaling During Neuronal Migration. Thomas M. Coate and Philip F. Copenhaver. Oregon Health and Sciences University, Portland, OR 97239.

During the formation of the enteric nervous system (ENS) in *Manduca sexta*, cells of the enteric plexus (named "EP cells") migrate in a stereotyped manner along eight visceral muscle band pathways. We have determined that Eph/ephrin signaling may be one of the key regulatory mechanisms that restrict the EP cells to their appropriate pathways. Although Eph/ephrin signaling has been well characterized in the context of axon guidance, its role in migration is poorly understood. In our simplified in vivo model system, only one Eph receptor (MsEph) and one A-class ephrin ligand (Msephrin) are expressed. Using methods to detect both mRNA and protein in whole-mount preparations, we have found that the migratory EP cells express Msephrin, while MsEph is distributed within adjacent interband regions that are normally inhibitory to migration. Manipulations in vivo using Fc-fusion proteins showed that treatment with Eph-Fc led to an overall repulsion response while treatment with ephrin-Fc caused EP cells to migrate off of their pathways. We are currently investigating other factors that may be associated with Eph/ephrin signaling in the enteric plexus of *M. sexta* in order to achieve a greater understanding of its role during neuronal migration.

618. Slit Gradient in the Brain: Neurobiologists' Imagination? Marie-Pierre Furrer, Irina Vasenkova, Daichi Kamiyama, Ozlem Unluer, Yaira Rosado, and Akira Chiba. Cell and Dev. Biol., Univ. of Illinois, Urbana, IL.

Slit is secreted at the midline of embryonic CNS. Genetic studies suggest that Slit coordinates the CNS development by signaling through neuronally expressed Robo receptors. Because of its influence over short and long ranges, and by analogy to morphogens, Slit has been hypothesized to establish a concentration gradient that descends steadily on each side of the midline. To date, however, direct evidence is missing for an in vivo Slit gradient or how it mediates long-range signaling. We examined the in vivo Slit distribution and its regulation through biotin-extraction of extracellular proteins, detergent-free quantitative immunostaining, transgenic probes, and in vivo trapping. We show an increasingly complex extracellular distribution of Slit during neurogenesis. During neuroblast determination and axogenesis, Slit maintains a steep but linear gradient. During subsequent dendrogenesis, a prominent local Slit accumulation occurs on the surface of the emerging lateral neuropil. Slit is transferred from its source to the neuropil through both direct filopodial delivery and diffusion-followed-by-capture by Slit receptors. There, long-range Slit induces dendrogenesis of pioneer neurons and its secondary Slit peak determines dendritic field position and size. Thus, Slit-Robo signaling repulses growth cones at the midline, while it promotes dendrogenesis at the lateral neuropil. Altogether, our data reveal a surprisingly complex spatiotemporal presentation of Slit in the embryonic CNS. They further suggest that neural differentiation is controlled by dynamic redistribution of diffusible signaling molecules such as Slit.

619. Role of Slits in Longitudinal Axon Guidance. Hikmet F. Nural, Amy L. Altick, William T. Farmer, and Grant S. Mastick. Univ. of Nevada, Reno, NV 89557.

Longitudinal axons travel lengthwise to connect different regions of the central nervous system. However, very little is known about the mechanisms of longitudinal axon guidance. The secreted Slit proteins are well-characterized guidance molecules for commissural axons and primarily act as repellents. Slits have been implicated in longitudinal axon guidance, and might play a role in dorsal–ventral positioning of longitudinal tracts. We have started analysis of Slit guidance using a variety of genetic and in vitro approaches. In Slit mutant mouse embryos,

dorsal hindbrain longitudinal axons diverge from their normal trajectory suggesting that Slit signals set the dorsal–ventral position of longitudinal tracts. To investigate Slit function in pioneer longitudinal axon guidance, we analyzed the expression pattern of Slits and their receptors (Robos). Slits are expressed in distinct patches in forebrain and in longitudinal stripes at the dorsal and ventral midlines, and longitudinal axons travel parallel to these stripes. Slit receptors are differentially expressed in clusters correlating with the neurons that pioneer longitudinal tracts. Longitudinal tracts, projecting at different dorsal–ventral positions, express distinct combinations of Robos. Thus, Slit and Robo patterns suggest a general mechanism guidance of longitudinal axons. To understand the mechanisms of Slit action, further experiments, including mis-expression in chick embryos and explant assays are underway to reveal the role of Slits in longitudinal axon guidance.

620. Sonic Hedgehog Sets Dorsal–Ventral Positioning of Early Longitudinal Axon Tracts. William T. Farmer, Amy L. Altick, Travis Hailstone, and Grant S. Mastick. University of Nevada, Reno.

The first longitudinal tracts of the vertebrate brain traverse the neural tube in specific dorsal–ventral positions paralleling the ventral midline (floorplate), yet little is known about the guidance mechanisms of dorsal–ventral positioning. Sonic hedgehog (Shh), a morphogen expressed by floorplate cells, is known to establish dorsal–ventral patterning by regulating cell fate. Recently, Shh has been shown to directly repel and attract spinal cord commissural axons. We postulated that Shh may act in dorsal–ventral positioning of longitudinal axons. Shh may either act indirectly by patterning the underlying neural epithelium, or directly by influencing longitudinal axon growth. To investigate Shh function, we ectopically expressed Shh in embryonic day 2 (E2, 10–20 somites) chick neural tube and found strong effects on longitudinal axons. Each of the three primary longitudinal tracts in the forebrain, midbrain, and hindbrain shifted their trajectory dorsally to avoid growing directly over Shh-expressing cells. Shh appears to affect axons at a distance. Ectopic Shh also induced floorplate-associated genes, including secreted axon guidance cues. It is not known if Shh is acting as a guidance cue. Experiments are in progress with the goal to separate direct from indirect mechanisms of Shh axon guidance. Our data indicate that Shh functions in early longitudinal axon guidance by establishing cues emanating from the floorplate.

621. C-Ephexin Regulates Motor Axon Stalling at the Limb Base. Catherine E. Krull,[1] Mustafa Sahin,[2] Michael Z. Lin,[2] Johann Eberhart,[3] and Michael E. Greenberg[2]. [1]Univ. of Michigan, Ann Arbor, MI 48109; [2]Harvard Univ., Boston, MA 02115; [3]Univ. of Missouri-Columbia, Columbia, MO 65202.

Motor axons from LMC neurons extend precisely from the neural tube to their targets in the limb. We are interested in the molecules and mechanisms that guide motor axon pathfinding and have focused on the function of Eph family members. Motor axons grow to the base of the limb where they stall and subsequently sort into dorsal/ventral nerve trunks. Ectopic expression of ephrin-A5 in limb mesoderm prevents EphA4+ axons from limb entry but sorting into dorsal/ventral trunks is normal. In contrast, ephrin-A5 acts as a positive factor for EphA4+ MMC(m) axons. How can EphA4+ motor neurons respond so differently to ephrin-A5? One possibility is that signaling cascades downstream of EphA4 are distinct in these two neuronal subsets. We cloned c-ephexin, an intracellular signaling molecule that interacts with EphA4, and found that LMC but not MMC(m) neurons express it. LMC neurons were then transfected with two c-ephexin shRNAs that reduced c-ephexin protein specifically in vitro and in vivo. When c-ephexin was reduced, LMC axons grew prematurely into the limb whereas control axons remained at the limb base. In combination, these results indicate that normal levels of c-ephexin protein are required for motor axon stalling at the base of the limb. Experiments are in progress to determine whether motor axons select the correct muscle targets after entering the limb prematurely.

622. Evidence that Motor Growth Cones Use Physical Force to Exit from the Spinal Cord. Kathryn W. Tosney, Denise Dehnbostel, Amy Wagnitz, and Kenneth Balazovich. The University of Michigan, Ann Arbor, MI.

Growth cones of motor axons exit from the spinal cord, despite the presence of a basal lamina barrier. During many other invasive events, cells escape an epithelium when its basal lamina disperses. Here, however, the basal lamina failed to widely disperse. Instead, we found evidence the basal lamina is penetrated by growth cones that use physical force to exit. During their initial interactions with the basal lamina, nascent motor growth cones displaced the resident neuroepithelial endfeet, spread on the basal lamina, and maintained its physical integrity. Then, small filopodia emerged at the interface. These protrusions deformed the basal lamina locally and tore small holes in it. These holes were avidly colonized and enlarged by exiting growth cones. In effect, the growth cones stabilized the basal lamina while their filopodia forced exit points. During the time when filopodia were distorting the basal lamina locally, the margin of the exit zone was also deformed on a larger scale, as though the entire margin was pulled inward. As documented by time-lapse recording, these large deformations were dynamic. These results support the idea that motor growth cones employ physical force to exit from the developing spinal cord, and supply a cogent example in which cellular force is employed in the embryo for a biological purpose.

623. Rac1 Regulates Integrin-Dependent Adhesion in Neuronal Growth Cones. Stephanie Woo and Timothy M. Gomez. Univ. of Wisconsin-Madison.

Axon outgrowth depends on the coordinated formation of actin-based protrusions and adhesion of these protrusions to the extracellular matrix (ECM). The Rho GTPase Rac1 is a regulator of cytoskeletal dynamics and integrin-dependent adhesion. We found that expression of dominant-negative (DN) or constitutively active (CA) Rac1 in *Xenopus* spinal neurons attenuated axon outgrowth and altered leading edge protrusion dynamics. Of the characteristics examined, only protrusion persistence was significantly reduced for both DN and CA Rac1, suggesting that unbalanced Rac1 activity slows axon outgrowth primarily by destabilizing protrusions. Therefore, we determined the effects of perturbing Rac1 on adhesion to the ECM. Immunocytochemical staining and live imaging of paxillin-GFP showed that DN Rac1 blocked the formation of adhesion sites. CA Rac1, however, produced unstable adhesions that were smaller in size and had shorter lifetimes. Studies have shown that stabilization and maturation of focal adhesions depends on myosin-II-based contraction downstream of RhoA. As Rac1 is known to antagonize RhoA signaling, we tested the effects of CA Rac1 on phosphorylation of the regulatory myosin light chain (MLC), a RhoA/ROCK target. We found that growth cones expressing CA Rac1 have reduced levels of phosphorylated, i.e., active, MLC. Moreover, we found that application the ROCK inhibitor Y-27632 perturbs growth cone adhesion sites similar to expression of CA Rac1. Together, our data suggest that Rac1 activity in growth cones promotes leading edge protrusion and formation of nascent adhesions, but gives way to RhoA activity which stabilizes adhesions through ROCK-dependent activation of myosin II.

624. Molecular Control of Axon Arborization of Trigeminal Sensory Neurons. Margaret Choy, Alvaro Sagasti, and Alexander F. Schier. Skirball Institute, New York, NY 10016.

In vertebrates, the trigeminal sensory ganglia (tgg) is responsible for sensing thermal, mechanical, and chemical stimuli in the face. Formed

from subpopulations of neural crest and placodal cells, tgg neurons undergo a phase of extensive axon outgrowth and arborization starting at midsomitogenesis. Tgg peripheral axons remain ipsilateral and cover the head region in a non-overlapping fashion. Live imaging and transplantation experiments suggest that the partitioning of the target field may be regulated by mutual repulsion of tgg axons; however, the molecules that mediate this behavior are unknown. Axon guidance molecules belonging to the semaphorin family might be involved in this process because of their known function in axon and dendrite repulsion as well as, in some cases, trigeminal phenotypes in knockout murine studies. We will describe loss- and gain-of-function studies to test the role of semaphorins in controlling the axon arborization pattern of trigeminal sensory neurons.

625. Nevermind/CYFIP2 is Required for Dorsoventral Optic Tract Sorting and Topographic Mapping in the Zebrafish Retinotectal System. Andrew J. Pittman and Chi-Bin Chien. Univ. of Utah Medical Center, Salt Lake City, UT 84132.

We are taking a genetic approach to understand the cellular and molecular mechanisms that regulate dorsoventral (D–V) topography in the zebrafish retinotectal system. In the nevermind (nev) mutant, a subset of axons from dorsal retina are missorted with ventral axons in the optic tract and project incorrectly to both the dorsal and ventral optic tectum rather than just the ventral optic tectum. While nev is larval lethal, the overall morphology of the body, brain, and the eye is grossly normal, as is the D–V polarity of the eye. Therefore, this mutation causes specific defects in D–V retinotectal topography. To determine the gene mutated in nev, we have genetically mapped nev to a 0.47-cM interval on linkage group 14, which contains the zebrafish homolog of cyfip2 (Cytoplasmic FMRP Interacting Protein 2). Allele sequencing has identified premature stop codons 80 amino acids (allele tr230b) and 330 amino acids (allele ta229f) into the protein CYFIP2 and injection of a cyfip2 splice-blocking morpholino phenocopies nev, demonstrating that nev is cyfip2. cyfip2 is broadly expressed in the nervous system from 24 hpf to 72 hpf and is expressed in the eye and brain during the development of the retinotectal system. In zebrafish, as in mouse and humans, there are two known CYFIP proteins, CYFIP1 and CYFIP2, which are 85% identical to each other. CYFIP proteins are known to interact with the Rac pathway as well as with the FMRP family, suggesting intriguing links to cytoskeletal dynamics as well as with mRNA regulation.

626. Expression of Metalloproteinase *adam10* in the Developing Visual System of *Xenopus laevis*. Yuanyuan Chen, Jennifer C. Hocking, Carrie L. Hehr, and Sarah McFarlane. [1]Genes and Development Research Group, University of Calgary, Health Sciences Centre, Calgary, Alberta T2N 4N1, Canada.

Zn-dependent metalloproteinases are implicated in key developmental processes such as neurogenesis, myelination, and neuronal migration. These include the matrix metalloproteinase (MMP) and the A disintegrin and metalloproteinase (ADAM) families. *Drosophila* mutant for the ADAM10 homologue, *kuzbanian*, exhibits axon extension and guidance defects. In this study, we investigate the spatial and temporal expression patterns of *adam10* mRNA in *Xenopus laevis* brain and retina by in situ hybridization during the period when retinal ganglion cell (RGC) axons navigate from the retina (stage 30) to their target (stage 40), the optic tectum. We found that *adam10* is expressed in the dorsal midbrain and diencephalon over this entire period. Initially, *adam10* is expressed throughout the retina. Subsequently, at stage 33/34, when RGC axons enter the contralateral brain, *adam10* is highly expressed in the peripheral retina but at lower levels in the mature central retina. At stage 37/38, when the first RGC axons reach their target, *adam10* is weakly expressed only in the most peripheral proliferative region of the retina, and by stage 40, *adam10* mRNA is undetectable in the retina. This expression pattern suggests that, initially, ADAM10 may act cell-autonomously in RGCs to modulate axonal behavior, and/or that during later development, ADAM10 processes the molecular substrate over which RGC axons extend. We will perform functional studies to examine whether ADAM10 function is important in the extension and guidance of vertebrate axons in vivo.

627. Beta-Catenin Independently Specifies Pathfinding and Targeting of Optic Axons in the Tectum. Katie Mirro, Michelle Lee, Heather Muhr, and Tamira Elul. Touro University College of Osteopathic Medicine, Vallejo, CA 94592.

Topographic maps are precisely organized neuronal circuits that mediate sensory information processing. Topographic maps form by the specific pathfinding and targeting of afferent axons in their brain targets. Cadherin function is required for development of topographic neuronal circuitry, but the role of β-catenin, an adaptor protein that links Cadherin to actin and thereby strengthens Cadherin-based adhesion, is unclear. We hypothesized that β-catenin acts in the Cadherin pathway to control specific pathfinding and targeting of optic axons in the retinotopic projection of *Xenopus laevis* tadpoles. We overexpressed a deletion mutant of β-catenin (NTERM) that uncouples Cadherin from actin in single ventral optic neurons, and examined the resulting changes in their terminal axonal projections in tectal midbrains of live tadpoles. Expression of NTERM in ventral optic axons perturbed both their entry into and their targeting in the tectum. In NTERM optic axons, the average position of entry was shifted posteriorly, and the average target position was shifted anteriorly and laterally. Significantly, in NTERM-expressing optic axons, position of entry and of targeting in the tectum were not correlated. These data suggest that β-catenin acts in the Cadherin pathway to independently specify pathfinding and targeting of optic axons. We propose that the β-catenin/Cadherin adhesion pathway functionally interacts with the Ephrin pathway to selectively specify targeting (but not pathfinding) of ventral optic axons in the tectum.

628. A Diffusible Guidance Cue, Wnt3, is a Candidate Opposing Signal for Medial–Lateral Retinotectal Mapping. Yimin Zou, Adam Schmitt, Jun Shi, Alex Wolf, Chin-Chun Lu, and Leslie King. Department of Neurobiology, Pharmacology and Physiology, Committees on Developmental Biology and Neurobiology, the University of Chicago.

Axonal connections are frequently organized in a topographic manner. We tested whether the Wnt family axon guidance cues play a role in map formation. We found that both chick and mouse retinal ganglion cell (RGC) axons from different dorsal–ventral positions showed graded response to Wnt3 at different concentrations, repelling both dorsal and ventral RGC axons at higher concentrations but attracting dorsal RGC axons at lower concentrations. Wnt3 is expressed in a medial–lateral decreasing gradient in the tectum. Wnt3 repulsion of ventral RGC axons at all concentrations and dorsal axons at higher concentrations can be blocked by antibodies against the ectodomain of Ryk, the mammalian homologue of a *Drosophila* Wnt receptor, Derailed. The attraction of dorsal axons at lower concentration can be blocked by sFPR2 protein but not by the Ryk antibodies. Expression of a dominant-negative Ryk in dorsal RGC axons caused a dramatic shift of termination zone medially. Ectopic expression of Wnt3 in medial tectum led to a lateral shift of termination zone. Therefore, a diffusible molecule Wnt3 plays a role in retinotectal mapping along the medial–lateral axis in collaboration with the opposing medial-directed EphrinB1-EphB activity.

629. Inhibition of Semaphorin Signaling by Dlx Homeobox Genes Promotes Tangential Migration of Interneurons from the Basal Forebrain to the Neocortex. S. Cheng, T.N. Le, J.T. Wigle, and D.D. Eisenstat. Depts. of Pediatrics, Biochemistry, and Anatomy, University of Manitoba, Winnipeg, Canada.

Dlx1/Dlx2 null mice die at birth with loss of tangential migration of GABAergic inhibitory interneurons to the neocortex. We have applied ChIP (chromatin immunoaffinity precipitation) to identify transcriptional targets of DLX homeoproteins derived in vivo from E13.5 ganglionic eminences (GE). Following enrichment for protein–DNA complexes, nucleoproteins were incubated with DLX antibodies and genomic DNA (gDNA) fragments, including putative DLX transcriptional targets, were characterized. PCR for the Neuropilin-2 promoter (NRP-2) showed that both DLX1 and DLX2 bind to this regulatory region in situ but neither binds to the Neuropilin-1 promoter. Gel shift assays confirm direct binding of DLX1 and DLX2 to the NRP-2 promoter in vitro. Both DLX1 and DLX2 repress NRP-2 expression, confirming the functional significance of DLX binding and consistent with increased and aberrant expression of NRP-2 in the Dlx1/Dlx2 null mouse. We have generated a Dlx1/Dlx2/Npn-2 triple knockout mouse. Analysis of the neocortex of the triple knockout compared to the Dlx1/Dlx2 null demonstrates a partial restoration of GABA and calbindin-expressing interneurons in the neocortex by P0 ($P < 0.05$). NRP-2 is a receptor for semaphorin axonal guidance ligands in the developing forebrain. We have provided evidence for NRP-2 as a direct Dlx homeodomain target from embryonic forebrain in situ. These findings improve our understanding of Dlx function in cortical development in vivo, especially regulation of tangential interneuron migration to the neocortex.

630. *Pax6*$^{-/-}$ Mice Have Defects in Non-Radial Cell Migration and Interneuron Specification. Pallavi P. Gopal[1] and Jeffrey A. Golden[2]. [1]Department of Neuroscience, University of Pennsylvania; [2]Department of Pathology, Children's Hospital of Philadelphia, PA 19104.

Neuronal migration is essential for normal nervous system development. Two main pathways of neuronal migration have been described: radial and non-radial. While the mechanisms underlying radial cell migration are well described, the molecular and cellular mechanisms underlying non-radial cell migration (NRCM) are just beginning to be defined. Mutant mice displaying abnormal cell migration have been important tools for elucidating the mechanisms of radial cell migration; we expect this approach will also facilitate defining the molecular and cellular interactions necessary for NRCM. Although several mouse mutants have been described with NRCM deficiencies, ***Pax6***$^{-/-}$ (***Small eye***, ***Sey***$^{-/-}$) is the only mutant reported to show increased migration and numbers of cortical interneurons. Based on these data, we hypothesized Pax6 is required to suppress permissive substrates or guidance cues, and/or induce restrictive molecules; we sought to use the ***Sey***$^{-/-}$ mouse to identify such candidates. Unexpectedly, we find that ***Sey***$^{-/-}$ mice have a cell non-autonomous reduction in the rate of NRCM. Also, in contrast to the conclusions drawn by others, we find no difference between the number of non-radially migrating cells in ***Sey***$^{-/-}$ and wild-type mice. Therefore, the increased number of cortical inhibitory interneurons observed in ***Sey***$^{-/-}$ cannot be explained by an increase in rate or number of non-radially migrating cells; instead, our data support the hypothesis that loss of Pax6 causes a patterning defect which results in ectopic specification of interneurons in the cortical ventricular zone.

631. The α5 Subunit of Laminin is Required for Proper Murine Neural Crest Cell Migration. Edward G. Coles,[1] Laura S. Gammill,[1] Jeffery H. Miner,[2] and Marianne Bronner-Fraser[1]. [1]Division of Biology, California Institute of Technology, Pasadena, CA 91125; [2]Washington University School of Medicine, St Louis, MO 63110.

Neural crest cells migrate away from the dorsal-most portion of the forming central nervous system and ultimately differentiate into many different cell types throughout the embryo. Laminins are a major extracellular matrix component and have been shown to influence many aspects of neural crest migration. Previously, in a screen designed to identify genes up-regulated during chick neural crest induction, laminin α5 was identified as an early response gene. Largely conserved between chick and mouse, the α5 subunit of laminin is localized in regions of the newly formed neural folds, in cranial and trunk regions and along migratory neural crest cell pathways. Here, we analyze the neural crest phenotype of laminin α5 mutant mice and show neural crest cells migrate in abnormal streams. Indeed, in vitro studies show that the migratory ability of neural crest cells in the presence of the laminin α5 subunit is decreased by a half when compared to cells migrating in the absence of the α5 subunit. During neurogenesis, laminin α5 mutant embryos exhibit defects in the forming cranial ganglia and condensing trunk ganglia. In particular, sympathetic ganglion formation is significantly delayed. The ganglia, however, are not totally missing and at later stages of development apparently recover. These data demonstrate that the function of the laminin α5 subunit, as a component in the extracellular matrix, acts as a non-permissive cue and is essential for proper migration and timely differentiation of some neural crest populations.

632. Identification of Genes Involved in the Induction and Migration of Neural Crest Cells. Meghan S. Adams, Laura S. Gammill, and Marianne Bronner-Fraser. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Neural crest cells are a transient and migratory population of cells that delaminate from the neural tube, migrate along precise pathways, and localize in characteristic and diverse derivatives. We have performed a comprehensive screen using a macroarrayed cDNA library from early chick embryos to identify genes up-regulated as a consequence of neural crest induction. Here, we used a secondary in situ hybridization screen to identify those genes expressed in migratory neural crest populations. The initial examination of over 550 up-regulated clones resulted in the identification of ~225 different transcripts. More than 85% of these genes are expressed by migrating neural crest cells. Collectively, we have identified many new markers of neural crest cells as they delaminate and migrate along precise pathways, as well as new candidate regulatory molecules. Future experiments will explore the function of some of these genes during neural crest migration.

633. Chain Migration of Cranial Neural Crest Cells, a Dynamic In Vivo Analysis. Paul A. Rupp and Paul M. Kulesa. Stowers Institute for Medical Research, Kansas City, MO.

The cranial neural crest is a migratory, pluripotent population of cells required for normal craniofacial and cardiovascular development. These cells differentiate into the melanocytes, cranial neurons, glia, cartilage, bone, and connective tissues of the head, as well as contribute to the enteric nervous system and the aorticopulmonary and conotruncal regions of the heart. Extensive information from transplants, fixed tissues, and static imaging has shed light on how cells sort into and maintain the observed stereotypical patterns of cranial neural crest migration. Unlike the neural crest that migrates from rhombomeres (r) 1 + 2, r4, and r6 in large wide streams, cells migrating from r7 do so as extended narrow chains. Small Rho GTPases have been shown to be involved in the migration of a variety of cell types. Using in vivo labeling techniques, embryonic culturing systems and 4D-confocal time-lapse microscopy, we are now able to analyze, at high resolution, the dynamic behavior of individual neural crest cells migrating in r7 chains. The establishment of these chains and the involvement of small Rho GTPases are studied.

634. Cranial Neural Crest Migration in the Avian Embryo and the Role of Ephrin-A5. Carole C. Lu and Scott E. Fraser. California Institute of Technology, Pasadena, CA 91125.

Cranial neural crest cells are multipotent cells that arise in the dorsal neural tube and migrate within discrete streams in the avian embryo. By labeling the premigratory neural crest cells with a GFP construct and conducting time-lapse microscopy, we are able to follow their cell behaviors and migratory patterns in the intact embryo. Similar to previous findings, the neural crest cells at the level of rhombomere 4 (r4) migrate in a tight lateral stream directly from the neural tube to branchial arch 2 (ba2), separated from other neural crest cells by crest free regions adjacent to r3 and r5. In the posterior hindbrain, the neural crest cells first form a field of cells that then segregate among ba3 and ba4. The underlying mechanism of this sorting is unclear. However, this is similar to what occurs in ***Xenopus***, where cranial crest cells first migrate as a wave and then segregate to different arches. Differential expression of Eph receptors and ephrins have been shown to be involved in segregating these streams. Differences in the avian r4 and r6 streams of neural crest cells suggest the mechanisms that guide their migration are different. To this effect, we are studying the role of ephrin-A5 in the migration of neural crest cells that populate ba3 and ba4. We find that when we misexpress ephrin-A5, the neural crest cells are less likely to populate ba3 but the other branchial arches are not affected. The combination of time-lapse microscopy with molecular perturbations allows us to characterize the migration and sorting of the neural crest cells into ba3 and ba4, and to dissect underlying changes in migration due to molecular perturbations.

635. Segmental Migration of Neural Crest Cells Through the Somites Requires Neuropilin-2/Semaphorin3F Signaling. Laura S. Gammill,[1] Constanza Gonzalez,[1] Chenghua Gu,[2] David Ginty,[2] and Marianne Bronner-Fraser[1]. [1]Division of Biology 139-74, California Institute of Technology, Pasadena, CA 91125; [2]Department of Neuroscience and Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In vertebrates, trunk neural crest cells migrate segmentally through the rostral half of each somitic sclerotome. This in turn is thought to generate the segmental organization of the neural-crest-derived dorsal root ganglia (DRG) of the peripheral nervous system. However, the molecular mechanisms that pattern trunk neural crest migration remain unclear. Here, we show that migrating neural crest cells express the receptor neuropilin-2 (npn-2). Moreover, npn-2 null mice exhibit a variety of defects during neural crest migration, including loosely condensed trigeminal ganglia and "bridges" of migrating cells crossing between cranial neural crest streams. In the trunk, npn-2 mutant neural crest cells no longer migrate exclusively through the rostral sclerotome; instead, they are evenly distributed throughout the sclerotome. The repulsive npn-2 ligand semaphorin3F (sema3F) is selectively expressed in the caudal sclerotome, and sema3F null mice exhibit an identical phenotype to that of npn-2 mutants. The results clearly show that npn-2/sema3F signaling is required to direct rostral-only migration of neural crest through the somite. Interestingly, DRGs still condense in a segmental pattern in npn-2 mutant mice, apparently sorting out from the sheet of migrating neural crest. This suggests that segmental neural crest migration may not be essential for the subsequent formation of individualized DRGs.

636. Fetal Alcohol Syndrome: The Effects of Ethanol on Murine Forelimb and Hindlimb Development. Yir G. Yueh, Catherine Cheng, Jennessa Janitell, Tanya Thal, and David P. Gardner. Midwestern University, Glendale, AZ 85308.

Fetal Alcohol Syndrome (FAS) is a condition that describes an abnormal pattern of developmental changes due to maternal ingestion of alcohol during gestation. Our previous data showed that ethanol exposure of pregnant mice at 9 days 8 h of gestation induces postaxial digit loss in forelimbs in a high percentage of embryos. Current data from both beta-gal staining and in situ hybridization experiments suggest that the teratogenic activity of alcohol may affect Fgf-8 gene expression as observed through ethanol-embryo limb bud blebbing of the AER in both forelimb and hindlimb if the ethanol exposure is delayed till 10 days 6 h. It is hypothesized that during limb outgrowth, signaling by bone morphogenetic proteins (BMPs) is moderated to maintain the signaling loop between the zone of polarizing activity where Shh is expressed and the apical ectodermal ridge (AER) where Fgf-8 is expressed. Gremlin, an extracellular Bmp antagonist, has been proposed to fulfill this function and therefore be important in limb patterning. Preliminary in situ experiments have been started with a Gremlin probe to determine the differences between Gremlin expression on ethanol-affected and control embryos. Interestingly, Gremlin expression appears to be weaker in the ethanol-affected embryos than the control embryos, presenting the possibility that ethanol affects the expression of Gremlin. Future work in the area of characterizing ethanol effects on limb bud gene expression will be concentrated with the Gremlin in addition to the FGF-8.

637. *OFD1*, a Human Disease Gene, is Required for the Assembly of Cilia and Centrosomes. Veena I. Singla, Andrew R. Norman, and Jeremy F. Reiter. University of California, San Francisco.

Orofaciodigital syndrome type I (OFD1) is a human congenital syndrome characterized by malformations of the face, oral cavity, and digits, as well as polycystic kidney disease. Polycystic kidney disease can be caused by defects in primary cilia function, suggesting that OFD1 may be due to ciliary dysfunction as well. Additionally, the product of the gene responsible for OFD1 localizes to basal bodies at the origin of primary cilia. To test the hypothesis that OFD1 is required for ciliary function, we generated a murine embryonic stem cell line with a loss-of-function mutation in the ***OFD1*** gene. OFD1-deficient cells completely and specifically lack primary cilia. The lack of cilia is due to the loss of OFD1, as revealed by the restoration of ciliogenesis upon reversion of the ***OFD1*** mutation. Immunoflourescence analysis of OFD1-deficient cells demonstrates that OFD1 is required for normal centrosomal assembly. Together, these data suggest that OFD1 is an essential component of the centrosome, and that in the absence of OFD1, the centrosome cannot support ciliogenesis. We are currently using live cell imaging, proliferation analysis, and transmission electron microscopy to understand how OFD1 controls cilia formation and other aspects of centrosomal function.

638. Dosage-Sensitive Interaction of TBX1 and CRKL Underlies the Etiology of Del22q11 Syndrome. Deborah L. Guris,[1] Virginia E. Papaioannou,[2] and Akira Imamoto[1]. [1]Committee on Developmental Biology, The Ben May Institute for Cancer Research, University of Chicago, Chicago, IL 60637; [2]Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

22q11 deletion (del22q11) syndrome, characterized by large heterozygous deletions within chromosome 22q11, is the most frequent deletion syndrome in humans (occurring 1 in 4000 live births). The hallmarks of this syndrome are congenital malformations of the aortic arch, heart, thymus, and parathyroid glands—a constellation of defects first described as DiGeorge syndrome (DGS). A critical, unsolved question is whether these developmental defects are caused by haploinsufficiency of a single gene, or instead multiple 22q11 genes. Here we report that compound heterozygosity of mouse homologues of two 22q11 genes, CRKL and TBX1, results in a striking increase in the penetrance and expressivity of a DGS-

like phenotype compared to heterozygosity at either locus. The developmental basis of the cardiovascular malformations seen in compound heterozygous mice was traced to loss of the fourth pharyngeal arch and its associated artery. Our results strongly suggest that DGS is a contiguous gene syndrome in which CRKL and TBX1 both play a major role. Furthermore, we show that these two genes have critical dose-dependent functions in pharyngeal pouch formation and subsequent patterning of the pharyngeal apparatus along the antero-posterior axis.

639. Protease-Activated Receptors (PARs) in Mouse Embryonic Development. Abby M. Cheng and Shaun R. Coughlin. Univ. of California, San Francisco, CA 94143.

Cellular responses to thrombin are mediated by PARs. In adults, the coagulation cascade, which generates thrombin, mediates hemostatic and inflammatory responses to injury. In the embryo, lack of the thrombin receptor PAR1, prothrombin (PT), or tissue factor (TF; the integral membrane protein that triggers coagulation) is associated with death at midgestation with failed vascular integrity and impaired remodeling of yolk sac vessels, but the penetrance of these phenotypes varies: ~85–100% for Tf −/−; ~50% for Pt−/−; and ~50% for Par1−/−. The partial penetrance of the PT phenotype can be ascribed to some maternal PTs reaching the embryo, but the greater penetrance of the Tf−/− vs. Par1−/− phenotype suggests that TF has actions that are independent of PAR1 and important for embryonic development. Whether such actions involve other thrombin targets or functions of TF outside of coagulation is unknown. We report that Pt−/−:Par1−/− embryos at least grossly recapitulated the TF phenotype, consistent with TF's functioning to trigger coagulation during development and implying that thrombin must have effectors other than PAR1. Like PAR1, the thrombin receptor PAR4 was detected in endothelial cells at midgestation, and combined deficiency of PAR1 and PAR4 also grossly recapitulated the TF phenotype. A detailed comparison Tf−/− vs. Par1−/−:Par4−/− phenotypes is in progress. Taken together, our data are consistent with the hypothesis that the coagulation cascade and PARs regulate the behavior of endothelial cells in a manner important for proper vascular development in the mouse embryo.

640. Hoxb13 and Nkx3.1 Have Overlapping and Distinct Functions in the Mouse Prostate. Kyriakos D. Economides,[1] Mario R. Capecchi,[2] and Cory Abate-Shen[1]. [1]CABM-UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854; [2]Univ. of Utah, Salt Lake City, UT 84112.

Among the transcription factors expressed during prostate development are the homeobox genes Hoxa13, Hoxb13, Hoxd13, and Nkx3.1. Of these, Hoxb13 and Nkx3.1 continue to be expressed in the adult prostate epithelium in both mice and humans. Mice lacking either Hoxb13 or Nkx3.1 display various defects of prostate differentiation, including loss of secretory function and perturbations in branching morphogenesis; however, the consequences of loss of these genes differ in the various prostatic lobes. In addition, mice lacking Nkx3.1 develop hyperplasia and dysplasia, as well as prostatic intraepithelial neoplasias (PINs). We now find that compound mutants lacking Hoxb13 and Nkx3.1 display hyperproliferative defects in the prostate epithelium, which are accelerated by increasing dosage of mutant alleles; however, development of PIN lesions appears to require mutations in Nkx3.1. Additionally, these defects are lobe specific and consistent with the differential expression patterns of Hoxb13 and Nkx3.1. Interestingly, loss of function of Hoxb13 and Nkx3.1 also results in defects in prostatic epithelial regeneration in response to testosterone. Together, our data suggest Hoxb13 and Nkx3.1 have distinct roles in different contexts, such as development versus regeneration, or anterior prostate versus ventral prostate. Importantly, the observation that Hoxb13 mutants display proliferative defects but not PIN suggests that these are potentially exclusive processes, which has implications for differences between benign prostate hyperplasia and prostate cancer in the clinical setting.

641. Corneal Disease in Mice Expressing a Dominant-Negative Clim/Ldb Transcriptional Co-Factor. Xiaoman Xu, Joel Spencer, and Bogi Andersen. Departments of Biological Chemistry and Medicine, Univ. of California, Irvine, CA 92697.

Co-factors of LIM domains (Clim/Ldb), which were originally identified as co-factors of LIM-homeodomain and LIM-only transcription factors, are thought to function as adaptors that facilitate the assembly of transcriptional complexes. Because Clim2 is expressed extensively in various epithelial tissues, we investigated its role in the regulation of epithelial homeostasis by generating transgenic mice expressing a dominant-negative Clim (DN-Clim) under control of the keratin (K) 14 promoter. The K14-DN-Clim mice developed progressive hair loss in addition to striking corneal abnormalities. Newborn K14-DN-Clim mice developed corneal stromal edema and epithelial blisters. Later, corneal wounds with leukocyte infiltration and neovascularization developed, presumably due to rupture of the blisters. As the mice aged, thinning of the corneal epithelium was observed, suggesting the possibility of a stem cell deficiency; DN-Clim is expressed in the corneal epithelial stem cell compartment of the limbus region. Finally, the corneal epithelium in transgenic mice acquired epidermal properties. Our study suggests that Clim co-factors are important for the homeostasis of cornea; specifically, we propose that Clims are involved in the regulation of genes required for adhesion of corneal epithelial cells as well as stem cell maintenance. The K14-DN-Clim mice provide a new mouse model that exhibits several features that characterize corneal diseases, and may be useful for testing therapeutic approaches to corneal diseases.

642. Cation Channel TRPM7 is Necessary for Survival of Embryonic Melanophores in Zebrafish and is Expressed in Human Melanoma Cell Lines. Robert A. Cornell,[1] Jennifer Paulsen,[1] Michael Elizondo,[2] Brigitte Arduini,[3] Erin Burninght,[1] Paul D. Henion,[3] David Parichy,[2] and Mei-Yu Hsu[1]. [1]Univ. of Iowa, Iowa City, IA 52242; [2]Univ. of Texas, Austin, TX 7812; [3]Ohio State Univ., Columbus, OH 43210.

To identify neural crest regulatory genes, we conducted a mutagenesis screen in zebrafish and isolated the mutant *touchtone*, in which embryonic melanophores differentiate poorly and a fraction undergoes cell death. We performed meiotic mapping and identified the disrupted gene as transient receptor potential melastatin 7 (*trpm7*), encoding a transmembrane channel for divalent cations. Previous studies have shown that expression levels of TRPM1, the founding member of the TRPM sub-group of the TRP channel superfamily, are down-regulated within cultured metastatic melanoma cells in comparison to within to normal melanocytes. In contrast, we show that expression levels of TRPM7 mRNA are comparable in cultured human melanocytes and in several metastatic and non-metastatic melanoma tumor cell lines, and preliminary siRNA-mediated knock-down experiments indicate TRPM7 is required for melanoma cell viability. Thus, analysis of a zebrafish mutant has revealed a pathway critical for cell survival of melanocytes and perhaps melanoma cells.