oocyte's apical/basal polarity complex, they pattern later embryonic development.

doi:10.1016/j.ydbio.2010.05.170

Program/Abstract # 131
Transcriptional Integration of the Wnt and Nodal Pathways during Organizer Formation
Christine D. Reid, Daniel S. Kessler.
Dept. of Cell & Dev. Biol., University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

During *Xenopus* embryogenesis, the Wnt and Nodal pathways overlap in the Spemann Organizer, a region of tissue on the dorsal side of the embryo essential for embryonic patterning and axis formation. Both the Wnt and Nodal pathways are essential for organizer formation, yet little is known about how these pathways interact at the level of target gene promoters to affect organizer gene expression. Gsc is an organizer specific gene with a well-defined promoter containing both a Wnt responsive element and a Nodal responsive element. Expression of Wnt effectors Sia and Twn with Nodal leads to synergistic activation of the Gsc promoter. Chromatin immunoprecipitation (ChIP) has revealed that Wnt effectors Sia/Twn and Nodal effectors Fast-1/Smad2/3 occupy the endogenous Gsc promoter. Occupancy of Smad2/3 at the Gsc promoter is increased upon addition of Nodal, as expected. However, a combination of Nodal and Sia/Twn, significantly increases Smad2/3 occupancy at the Gsc promoter. Sia/Twn occupancy at the Gsc promoter is enhanced with addition of Nodal. Taken together, these results suggest that the transcriptional synergy observed at the Gsc promoter maybe due in part to formation of a transcriptional complex at the promoter, consisting of effectors from both Wnt and Nodal pathways. This complex also likely contains p300, as p300 occupies the Gsc promoter in a Sia/Twn dependent manner. Defining the mechanism by which Wnt and Nodal pathway effectors function to promote formation of the organizer is critical to understanding how two distinct signaling pathways cooperate to establish the organizer.

doi:10.1016/j.ydbio.2010.05.171

Program/Abstract # 132
Atoh1 regulates cellular patterning through cell fate specification and cell-cycle control in the mammalian cochlea
Michael C. Kelly, Ping H. Chen
Dept of Cell Bio, Emory Univ, Atlanta, GA, USA

The mammalian auditory sensory organ, the organ of Corti, consists of precisely patterned sensory hair cells interdigitated with nonsensory supporting cells. The bHLH transcription factor Atoh1 has been shown to play a necessary role in the differentiation of sensory hair cells during the development of the organ of Corti and ectopic delivery of Atoh1 results in the generation of ectopic hair cells in neonatal animals. To further test the competence of Atoh1 as a hair cell differentiation factor in various cell types of the developing and mature cochlea, we generated a transgenic mouse line with Dox-inducible Atoh1 expression throughout the cochlear epithelium. When Atoh1 expression is induced early in development, cells in most regions of the cochlea appear capable of initiating a hair cell differentiation program. Atoh1 induction in neonatal tissue results in hair cell differentiation in a more regionally restricted manner. Induced ectopic hair cells express MyoVI and display well-formed hair bundles. Although Atoh1 is presumably expressed throughout the cochlear epithelium, the ectopic sensory regions exhibit a mosaic cellular pattern similar to that of the organ of Corti, with alternating sensory and nonsensory cell. Furthermore, we observed significant cell proliferation within the normally post-mitotic organ of Corti in transgenic cochleae. These results suggest that Atoh1 plays a key role in the regulatory network that coordinates cell fate specification and cell cycle control for the precise patterning of the auditory sensory organ, and identify cochlear regions competent for hair cell differentiation.

doi:10.1016/j.ydbio.2010.05.172

Program/Abstract # 133
Genetic interaction of Lmx1a and Lmo4 in the mouse inner ear
Jennifer K. Hill[a], Lin Gan[b], Doris K. Wu[a]
[a]*National Institute on Deafness and Other Communication Disorders; Rockville MD USA*
[b]*University of Rochester, Rochester NY USA*

LIM homeodomain (LIM-hd) proteins participate in heteromeric transcriptional complexes critical to many cell fate decisions and patterning organs. *Dreher* (dr^J/dr^J) mice, which are deaf and have abnormal brain development, are functional nulls of the LIM-hd protein Lmx1a. Inner ears of *dreher* mice have poorly developed membranous labyrinths that lack an endolymphatic duct. However, individual sensory patches are present, albeit many are malformed. LIM-only (Lmo) proteins lack the homeodomain, binding only to other proteins to form part of the LIM-hd transcriptional complex. Four Lmo proteins are expressed in the developing mouse inner ear. Notably, *Lmo4* has been shown to be required for vestibular formation. In $Lmo4^{-/-}$ inner ears, the three semicircular canals and their associated sensory structures, the ampullae, are absent. This is consistent with expression of *Lmo4* in the sensory organs during development. *Dreher* mice and $Lmo4^{+/-}$;$Lmx1a^{dr/dr}$ mice display similar phenotypes. In contrast, $Lmo4^{-/-}$;$Lmx1a^{dr/+}$ inner ears exhibit a rescue of the anterior canal and possibly the anterior crista as well. In wildtype mice *Lmx1a* expression is down regulated in the developing sensory cristae, therefore this partial rescue is consistent with the idea that too much Lmx1a negatively affects the sensory organs. Based on expression data and the partial rescue, we posit that Lmo4 normally down regulates Lmx1a in presumptive sensory cristae allowing the development of the vestibular apparatus.

doi:10.1016/j.ydbio.2010.05.173

Program/Abstract # 134
Identification of novel candidate Six1-interacting proteins with potential roles in cranial placode development
Sally A. Moody[a], Pallavi Mhaske[a], Francesca Pignoni[b], Bo Yan[a], Karen M. Neilson[a]
[a]*Dept. Anatomy & Regen. Biology, George Washington Univ, Washington, DC, USA*
[b]*Dept. Ophthalmology, Upstate Medical Center, Syracuse, NY, USA*

Cranial placodes give rise to the sensory organs of the vertebrate head. They arise from an ectodermal zone that borders the anterior neural plate. Two human syndromes, BO/BOR, are characterized by craniofacial defects and hearing loss. About half of these cases carry mutations in the SIX1, SIX5 and EYA1 (a Six1 co-factor) genes; the genetic defects that cause the remaining cases are unknown. Six proteins contain a Six domain (SD) that can bind with co-factors, which increase DNA binding specificity and modulate Six function as either co-activators or co-repressors of transcription. Clustal analysis demonstrates that the SDs of the *Drosophila* Six gene, Sine Oculis, and *Xenopus* Six1 are highly conserved; only 4 aa substitutions are non-

conserved. This suggests that they likely share co-factor binding specificity. In *Drosophila*, 25 proteins that can interact with SO have been identified (Giot et al., 2003; Kenyon et al., 2005). We performed database searches for putative *Xenopus* orthologues of these fly genes and identified between one and four clones with sequence homology to 15 of them. Expression assays show that 20 *Xenopus* orthologues of 10/15 of the fly genes are expressed in the developing placodes as well as other craniofacial tissues. These results suggest that there are novel co-factors that may regulate the ability of Six1 to promote and maintain cranial placodes during development. Functional assays will determine the roles of these proteins in placode development. (Supported by NIH HD055321 and NSF IOS-0817902).

doi:10.1016/j.ydbio.2010.05.174

Program/Abstract # 135
Chicken Scratch2 is expressed during early embryonic neurogenesis
Felipe M. Vieceli, Irene Yan
Department of Cell and Developmental Biology, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil

In invertebrates, the *Scratch* (*Scrt*) genes encode transcription factors that promote neurogenesis during development. The Scrt function in vertebrates is currently unknown, but in mice *Scrt1* and *Scrt2* are specifically expressed in post-mitotic neurons in the embryo and in the adult central nervous system. In this work, we have cloned the coding sequence of chicken *Scrt2 (cScrt2)* and characterized its expression pattern in the embryo with RT-PCR and *in situ* hybridization. The complete coding sequence was cloned and the predicted translation product is a 276-aminoacids protein. This aminoacid sequence shares identities of 70% with rat Scrt2 and 58% with zebrafish Scrt. *cScrt2* transcripts are first detected as a faint signal in the periphery of the neural tube in the hindbrain by HH 15 and in the spinal cord by HH 17. The intensity of the signal increases between HH 19–23, and the expression in the motor domain of the spinal cord is progressively concentrated in the interface between the ventricular and mantle zones. *cScrt2* expression is also observed in the dorsal root ganglia after HH 22–23, particularly in the dorsomedial domain. The expression pattern of *cScrt2* in the neural tube is complementary to that of *Notch1*, which is expressed in neural stem cells, and to that of *SCG10*, a marker for differentiated neurons. Our results suggest that during embryogenesis *cScrt2* is expressed in a population of post-mitotic undifferentiated neurons.

doi:10.1016/j.ydbio.2010.05.175

Program/Abstract # 136
Concentration-dependence of Tcf3's function in Wnt/β-catenin signaling
Chun-I Wu[a], Jackson A. Hoffman[a], Erin M. Ford[b], Laura Periera[a], Elaine Fuchs[c], Hoang Nguyen[b], Bradley J. Merrill[a]
[a]*Dept. of Biochem. and Mol. Genetics, Univ. of Illinois at Chicago, Chicago, IL, USA*
[b]*Dept. of Mol. and Cell. Biol., Baylor Col. of Medicine, Houston, TX, USA*
[c]*Lab. of Mammalian Cell Biol. and Dev., The Rockefeller Univ., New York, NY, USA*

Tcf/Lef proteins, the downstream transcription factors of Wnt/β-catenin signaling, can act as either transcriptional activators or repressors. Loss of function experiments in several organisms were all consistent with the first embryonic function of the Tcf3 protein being independent of β-catenin interaction. In order to identify any embryonic role for Tcf3/β-catenin interaction, Tcf3ΔN knock-in mice were generated. Mice homozygous for theTcf3ΔN knock-in mutation, in which Tcf3 cannot bind to β-catenin, exhibited several morphogenetic defects, including exencephaly, poor vascular integrity, open eyelids at birth and oligodactyly. Interestingly, all these defects were rescued by reducing Tcf3 protein levels in Tcf3-/ΔN mice. Furthermore, Tcf3 overexpressed specifically in the eyelid epithelium of transgenic mice caused eye-open phenotype. These results, combined with BAT-GAL reporter gene activity, indicate that low levels of Tcf3 allowed Wnt/β-catenin signaling while high levels of Tcf3 inhibited Wnt/β-catenin signaling. Reducing nuclear Tcf protein to transduce the Wnt signaling was previously described for the "variant" Wnt pathway in *C. elegans* (Wnt/β-catenin asymmetry pathway). Taken together, these findings suggest this so called variant mechanism of Wnt/β-catenin activation of target genes, by stabilizing β-catenin and decreasing nuclear Tcf protein, may be more broadly conserved than previously appreciated.

doi:10.1016/j.ydbio.2010.05.176

Program/Abstract # 137
Deciphering the mechanism of Engrailed function during mouse cerebellar foliation
Grant D. Orvis, Alexandra L. Joyner
Dept. of Dev. Biol., Sloan-Kettering Institute, New York NY, USA

During mammalian development, the cerebellum (Cb) arises from rhombomere 1 of the developing hindbrain. The mammalian homologs of the fly segmentation gene *engrailed*(*en*), *En1* and *En2*, are required for specification of the cerebellar anlage and have been implicated in late patterning of the developing Cb. However, little is known about the mechanism(s) by which the *Engrailed* genes regulate later cerebellar patterning. Within the developing cerebellar primordium, two main regions of neurogenesis exist, the ventricular zone and rhombic lip, which give rise to Purkinje and granule cells, respectively. During late embryonic and early postnatal development, the Cb undergoes a drastic increase is tissue size accompanied by folding of the Cb along the A–P axis into lobules. The formation of these lobules occurs through the initiation of fissures and the proliferation of granule cells. Between E17–18.5, four primary fissures form and separate the Cb into five lobes. We show that leading up to and during the specification of these fissures, the *Engrailed* genes are expressed dynamically in cells derived from both the ventricular zone and rhombic lip. Using tissue-specific conditional gene inactivation, we show that *En1* and *En2* are required early for specification and/or production of cells derived from the ventricular zone. Furthermore, we show that loss of *En1* and *En2* in ventricular zone derived cells or in granule cells results in severe A–P pattering defects. We propose that the *Engrailed* genes pattern cerebellar foliation along the A–P axis by specifying positional and temporal cues, which result in formation of the four primary fissures.

doi:10.1016/j.ydbio.2010.05.177

Program/Abstract # 138
Characterization of the function of Sox21 during *Xenopus laevis* neural development
Niteace Whittington, Doreen Cunningham, Elena Casey
Dept. of Biol., Georgetown Univ, Washington D.C, USA

Neurogenesis, the progression from progenitor cell to committed neuron, is fundamental for the development of the central nervous system (CNS), yet the mechanisms involved in this process are not well defined. Members of the SoxB family of transcription factors have been shown to play important roles in neurogenesis. SoxB1 proteins are required for induction of the CNS and maintenance of a neural stem cell