(oscillator) and xNocturnin (output) expression levels were quantified using real time PCR (qRT-PCR). Stage 45 eyes showed rhythmic expression of xBmal1 and xNocturnin similar to adult eyes. But, rhythmic expression of xBmal1 and xNocturnin were different in stage 38/39 eyes when compared to stage 45. These data suggest that either the central oscillator cannot support a mature circadian rhythm or that controls necessary for proper regulation of output gene expression are not fully in place at stage 38/39. In the future we will analyze the ontogeny of circadian rhythm in the ear, nose, heart, and pronephros to see if the ontogeny of a circadian clock is similar in different tissues.

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Program/Abstract # 382
Downregulation of group B1 Sox genes is essential for patterning of the retinal pigmented epithelium during avian embryo development
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The pigmented epithelium arises from multipotential optic vesicle cells. Although roles of several paracrine factors in this process have been studied extensively, little is known about underlying transcriptional regulations. We show that members of SoxB1 family genes, Sox1, Sox2 and Sox3, are all expressed in throughout the optic vesicle but are downregulated in the presumptive retinal pigmented epithelium. To test the role of SoxB1, downregulation in patterning of the retinal pigmented epithelium, constitutive expression of SoxB1 was introduced in the prospective retinal pigmented epithelium both in vivo and in vitro. The resulting eyes exhibited loss of pigmentation, and concomitantly, induction of elongated cell shape and expression of neural differentiation markers. Further, exogenous expression of a neural retina inducer signal, FGF4, in the prospective retinal pigmented epithelium resulted in continued expression of all SoxB1 family members associated with downregulation of Mitf and Otx2, critical regulators of pigmented epithelial development. These results suggest that the normal patterning of the retinal pigmented epithelium requires SoxB1 downregulation, which depends on the absence of exposure to FGF-like signals.

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Program/Abstract # 383
The roles of fibronectin 1 and integrin alpha 5 during zebrafish lens development
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Lens fiber cells must differentiate, elongate and migrate in order for a fully transparent lens to properly develop. These processes are dependent on adhesion of lens fibers to the anterior epithelium, the posterior lens capsule and the other lens fibers. Key adhesion proteins include fibronectin 1 (fn1), an extracellular matrix protein, and integrin alpha 5 (itga5), a transmembrane protein capable of binding ECM and cytoskeletal proteins. Zebrafish with mutations in fn1 or itga5 show defects in lens development and morphology. Histology and TEM of fn1 and itga5 mutant embryo’s lenses show detachments between lens fibers and the anterior epithelium, the lens capsule, and other lens fibers. Our data also shows disrupted actin organization in the lens of fn1 and itga5 mutants. Normal lens fiber differentiation occurs near the equator of the lens, and is marked by restriction of crystallin A4 expression to this region. In fn1 and itga5 mutants, this spatial restriction is disrupted and mutants express crystallin A4 throughout the anterior epithelium. These results support the model that fn1 and itga5 are important for adhesion within the lens. fn1/ itga5 interactions are known to function in cell signaling pathways and our in situ data support a link between adhesion and preventing premature fiber differentiation within the anterior epithelium. Finally, adhesion is important for elongation and migration of cells processes that require vast cytoskeletal rearrangements. Our data show that in fn1 and itga5 mutants, actin organization is disrupted, which may contribute to defects in fiber elongation and migration, and thus to the overall disorganization of the lens.

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Program/Abstract # 384
FGF20 signaling regulates organ of Corti development
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Congenital hearing loss resulting from inner ear defects is one of the most common hereditary disabilities, affecting more than 1 in 1000 births. Understanding the molecular and cellular mechanisms that regulate inner ear development is key to identifying genetic etiologies for congenital hearing loss and for understanding degenerative and regenerative processes in the inner ear. During organogenesis, fibroblast growth factors (FGFs) regulate cell proliferation, differentiation, and survival. Here we generated null mice for Fgf20 by insertion of a β-galactosidase gene. β-galactosidase activity identified sites of Fgf20 expression in the developing inner ear as early as E10.5 and extending at least through P7. Auditory Brainstem Response tests show that Fgf20 null mice have complete hearing loss. Analysis of inner ear morphology indicates decreased numbers of outer hair cells and increased numbers of inner hair cells in Fgf20 −/− mice. Gaps lacking all hair cells were also present throughout the cochlea that are similar to the morphology of mice conditionally lacking Fgfr1 in the inner ear. These data indicate that Fgf20 is an endogenous functional ligand of Fgfr1 in inner ear sensory epithelial development.

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Program/Abstract # 385
Dorsalventral patterning of the developing inner ear
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The inner ear develops from an ectodermal thickening known as the otic placode. We demonstrated that the ablation of either the anterior (A) or posterior (P) half of the Xenopus placode results in a high rate of mirror image ears, with the remaining half duplicated. In contrast, dorsal (D) or ventral (V) half ablations never result in mirror duplications but cause the loss of corresponding structures, suggesting that there is less regenerative potential along the DV axis. In zebrafish, Hh signaling is involved in AP patterning of the developing ear while in mouse and chick, it is required for ventral patterning and Wnt signaling is important for dorsal patterning. We have shown that blocking Hh signaling results in mirror duplications of anterior
structures, suggesting that Hh signaling is necessary for posterior half identity in Xenopus. Here we explore the role of Wnt in Xenopus ear with gain and loss of function experiments. Conditional blocking of canonical Wnt signaling results in severe reductions in the number of sensory organs and semicircular canals. Of the two most common phenotypes, one resembles ears with mirror posterior duplications, suggesting that Wnt signaling may play a role in anterior half identity. By placing beads containing Wnt3a protein on inner ears after half-ablation, we are testing to see if Wnt signaling is sufficient to rescue the loss of any of these tissues. Our preliminary results suggest that Wnt3a protein alone is sufficient to rescue the severe loss in inner ear structures resulting from dorsal but not ventral half ablations.

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Program/Abstract # 386
Development of the sensory elements of the posterior macula are specifically regulated by Hh signalling in zebrafish inner ear
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The inner ear is responsible for the perception of motion and sound in vertebrates. The functional unit of the inner ear is the sensory patch. Each patch consists of hair cells and supporting cells and is associated with sensory neurons whose cell bodies lie in a ganglion close beneath the ear epithelium (Adam et al. 1998; Fritzsch et al. 2000). In this work we focus on the study of the generation of the key functional units of the inner ear: the sensory patches and analyse the signalling pathway that coordinates the production of the neurosensory elements. Using specific zebrafish transgenic lines to visualize the dynamics of hair cell and neuron production, we show that development of the anterior and posterior maculae is asynchronous and asymmetric, suggesting that they are under the control of different signals. Cell tracing experiments at single-cell resolution using lipophilic dyes demonstrate that otic neurons specifically innervating each macula are spatially segregated within the statoacoustic ganglion (SAG). Finally, we identify the Hh pathway to be crucial in: i) coordinating the production of hair cells, and ii) controlling the specific innervation of the posterior macula.

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Program/Abstract # 387
Wnt4/β-catenin signaling is required for germ cell survival in the fetal ovary
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Female germ cells are essential for ovarian organogenesis since in their absence follicles do not form. When Wnt4 is inactivated in the mouse fetal ovary, most germ cells undergo apoptosis before birth. Moreover, when β-catenin is ablated in somatic cells of the fetal ovary, embryos develop germ cell loss phenotype identical to that in the Wnt4 knockout (KO), suggesting that Wnt4 and β-catenin operate in a common pathway required for female germ cell survival. The goal of this study was to identify the mechanism of female germ cell loss in the absence of Wnt4/β-catenin signaling. In addition to germ cell loss, ectopic androgen production and upregulated inhibin beta b (Inhbb) expression were found in the fetal ovary without Wnt4 or β-catenin. Therefore, we hypothesize that germ cell loss in the Wnt4 and β-catenin KO ovaries could result from ectopic androgen production and/or elevated Inhbb. The persistence of germ cell loss in the β-catenin KO embryos after treatment of antiandrogen flutamide indicated that androgens are not responsible for germ cell loss. We argue that if activin B, the protein product of Inhbb, cause female germ cell death in the absence of Wnt4, removal of Inhbb in the Wnt4 KO background should prevent germ cell loss. Indeed, germ cell numbers were restored in the Wnt4/Inhbb double KO ovary. These results indicate that maintenance of female germ cells is controlled by a pathway operated by somatic cell-derived factors. Wnt4, via the action of β-catenin, antagonizes the production of Inhbb and activin B. Without the Wnt4/β-catenin pathway, activin B causes female germ cell demise and premature ovarian failure.

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Program/Abstract # 388
VEGF mediated endothelial migration is required for testis morphogenesis
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Developing organs must recruit and pattern a vascular supply during morphogenesis. The mammalian gonad is a unique model for organ morphogenesis because it arises as a bipotential primordium and develops into either a testis or an ovary. One of the first morphological events distinguishing the testis from the ovary is divergent vascular development. In the testis, endothelial cells undergo a highly directed migration that avoids presumptive domains of aggregating testis cords. Previous observations indicated that Sertoli cells induce endothelial migration. However, pathways that affect male-specific migration are also critical for multiple other functions, including Sertoli cell specification, making the identification of signals controlling discrete morphogenetic events difficult. Vegf is a potent modulator of endothelial cells and is expressed broadly in the gonad. We blocked VEGF signaling during the bipotential window of gonad development. In treated organ, male-specific vasculature fails to develop and testis cord morphogenesis is blocked. Surprisingly, Vegf is specifically enriched in interstitial cells of the testis, not Sertoli cells. Live imaging after VEGF inhibition revealed defects during interstitial proliferation, although Sertoli cell specification appeared normal. In vivo results show a specific role for the vasculature in supporting the development of a subset of interstitial cells, male specific Leydig cells. Blocking VEGF thus uniquely inhibits male-specific vasculogenesis and interstitial cell proliferation, demonstrating their requirement during testis cord morphogenesis while distinguishing these events from earlier Sertoli cell specification.

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Program/Abstract # 389
Functional and phylogenetic analysis Fgf8 shows that Fgf8 is not involved in external genital development
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In mammalian embryos, male and female external genitalia develop from the genital tubercle. Genital outgrowth is maintained by the urethral epithelium, and it has been reported that Fgf8 mediates this activity in a manner similar to the AER of the limb bud. To test directly whether Fgf8 is required for external genital development, we