but has not been explored during secondary neurulation, typical of lower vertebrates. Intriguingly, mouse zic2 was recently shown to be involved in HP formation (Ybot-Gonzalez et al., 2007). To understand this conserved aspect of zic function, we are conducting experiments, including microarray hybridizations, aimed at identifying transcriptional targets of zic2a. Correct expression of zic genes is essential for their function, yet the mechanism of its regulation is not understood. We are using bioinformatics, site-directed mutagenesis and in vivo transgenic reporter assays to identify transcriptional regulators of zics. This approach has identified CDP/cux/cutl, conserved homeobox TFs, as essential novel controls of zic transcription. CDP/cux proteins regulate neural cell cycle progression in mammals, but have not been implicated in neurulation. We have previously shown that zic2 and zic5 mediate the mitogenic function of canonical Wnt signaling in the brain primordium. Collectively, our data suggest that zic genes are an important conserved node in the genetic regulatory network that coordinates cell cycle progression (growth vs differentiation) with morphogenesis in the developing neural tube.

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Program/Abstract # 161
Requirements for ovo orthologues in zebrafish neural tube and neural crest development
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The neural crest (NC) develops at the border between the non-neural and neural ectoderm of vertebrate embryos, and gives rise to a variety of different cell types such as cartilage and bone of the skull and pigment cells of the skin. In order to arrive at their final locations, NC cells delaminate from the ectoderm and migrate extensively throughout the body. Lineage tracing studies have shown that NC cell fates are specified prior to overt migration in zebrafish, but genetic evidence to support this idea has been limited. Wnt signaling promotes pigment cell fates at the expense of other NC-derivatives. Ovo transcription factors are known to respond to Wnt signaling to regulate several aspects of mammalian development. We have identified two zebrafish ovo orthologues, ovo1 and ovo2, expressed in NC cells and in the neural tube. Injection of morpholino oligonucleotides targeting either Ovo causes defects in migration of pigment precursors but not other NC-lineages. Inhibiting Wnt signaling reduces expression of ovo1, suggesting that ovo genes mediate Wnt functions in pigment specification as well as their migration. Ovo may regulate the progressive emergence of distinct subpopulations of NC cells from the dorsal neural tube. Consistent with this hypothesis, Ovo morphants have compromised neuro-epithelial integrity and open neural tubes. Rhodamine-labeled wild type cells transplanted into the neural tube of Ovo morphants failed to cross the midline, suggesting that Ovo acts non-cell autonomously. One explanation for this comes from our finding that Ovo genetically interacts with N-cadherin, suggesting that it regulates neural tube and NC morphogenesis via modulation of cell adhesion.

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Program/Abstract # 162
The zebrafish dob/fgf20a mutant models human craniosynostotic syndromes with midfacial hypoplasia
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Craniosynostosis is the premature fusion of skull sutures, and in most of the more common craniosynostotic syndromes this condition is coupled with midfacial hypoplasia. Affected individuals may present restricted brain growth, cognitive dysfunction, and problems with hearing, speaking, eating, and breathing. While recent progress has been made in identifying the genetic basis of craniosynostosis and its associated syndromes, the diagnosis and treatment of these disorders is still complicated by their broad etiologies and variable expressivities. Developmental variation is therefore a considerable obstacle in craniosynostotic research. Using morphometric analyses, we were able to quantify the cranial shapes of dob/fgf20a mutant zebrafish and their wild-type siblings, remove the effects of developmental noise, and identify statistically significant mutational effects with a high degree of precision. We coupled this approach with whole-mount in situ hybridization studies of fgf20a expression in wild-type fish in order to compare the regions of anatomical distortion in mutants with regions of fgf20a expression in normal fish. Mutant fish exhibit aberrant suturing, distorted skull vault development, and midfacial hypoplasia that are strongly consistent with Apert’s, Crouzon’s, Pfeiffer’s, and Saethre-Chotzen syndromes. The areas of skull deformation coincide with regions in which fgf20a is expressed in wild-type specimens. Our results offer the zebrafish dob/fgf20a mutant as a new experimentally tractable model with which to examine the developmental pathology of these diseases.

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Program/Abstract # 163
Craniofacial phenotypes of the knypek (glypican4) mutant zebrafish
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The Wnt/PCP pathway controls cell behavior by regulating cell polarity and the cytoskeleton. Glypicans, a class of membrane-linked heparan sulfate proteoglycans, interact with secreted ligands, including Wnts, to modulate their signaling potential. The zebrafish head skeleton is ideally suited for analysis of chondrocyte behavior and skull morphogenesis downstream of this pathway. Here, we describe the craniofacial development of a zebrafish knypek mutant, which is disrupted in the gene coding for glypican4. kny/gpc4 homoyzogotes develop dramatically misshapen head cartilages, similar to malformations observed in the Wnt5 mutant pipe tail. In the early larvae we observed a loss of chondrocyte polarity, failure of cell–cell intercalation, and consequent defects in the elongation of larval cartilage elements. Adult kny/gpc4 mutant fish (>1 y) have persistently abnormal chondrocyte organization and significant malformations of mature cranial bones. Morphogenetic defects include a shortening of the rostral-most skull and frequent loss of the symplectic, an endochondral element in the zebrafish jaw suspension. Finally, we report the unexpected result that all knypek mutant adults lack barbels, paired dermal appendages normally found in zebrafish and many other cyprinid species. To our knowledge, gpc-4 is the first gene to be linked to barbel outgrowth, which does not occur until the juvenile stage. This novel phenotype suggests that late-acting glypicans may be critical in the morphogenesis and repeated evolution of these fish sensory structures.

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