inner ear, trigeminal and distal cranial ganglia, as well as in somites and nephrogenous mesenchyme in mice. Cranial sensory organs and ganglia are derived from thickened ectoderm termed cranial placodes, which are derived from pre-placodal region (PPR), a continuous ectodermal region surrounding the neural plate. Six1 is also known as a marker for the PPR and placodes. Analyses of Six1−/− mice revealed the essential roles of Six1 in the development and morphogenesis of the organs where Six1 is expressed. To identify the enhancers responsible for the expression of Six1 during embryogenesis, we compared genome sequences around Six1 loci among vertebrates and found out 16 conserved non-coding sequences (CNSs). The identified CNSs were hooked onto a minimal promoter with EGFP reporter and electroporated into chick embryos to monitor enhancer activities. We identified eight independent enhancers that showed specific expression similar to the endogenous Six1 expression domains. The enhancer activities were confirmed in mice harboring the CNS upstream of minimal promoter with lacZ reporter. Elements for the CNS that showed expression in the PPR were analyzed by mutagenesis, and homodomain protein binding sites in the CNS were identified as essential for the enhancer activity in the PPR. The involvement of Dbx5 and Msx1 was suggested by overexpression and RNAi experiments in chick embryo. The evolution of Six1 enhancers will be also be discussed.

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Program/Abstract # 471
Dual functions of the miR-10 locus miRNAs in refinement of Hox gene expression
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Controlled regulation of gene expression is essential to proper development. This control can be imposed at nearly every step between initiation of transcription and the eventual degradation of a protein. The discovery of miRNAs demonstrated pervasive post-transcriptional regulation by an ever expanding group of small RNAs which can be expressed in temporally and spatially restricted patterns similar to protein coding genes. The mir-10 locus, which resides in between the Hox4 and Hox5 orthologs in most bilaterian animals, encodes two functional miRNAs miR-10 and miR-10′, which have highly conserved complementary sequences in the 3′ UTRs of insect Scrt and Abd-B orthologs respectively. These miRNAs and Hox genes in Drosophila are expressed in highly complementary and largely non-overlapping domains, suggesting that while the mir-10 miRNAs do not contribute to the gross pattern of Hox gene expression, they are responsible for maintaining precise and developmentally robust expression patterns.

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Program/Abstract # 472
Segmental origin and Hox dependence of neural crest-derived otic ganglion
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Classic studies in chick-quail chimeric embryos show that parasympathetic motor ganglia arise from preotic and postotic segments of the developing hindbrain. Although this observation provides a broad view of the segmental origin of parasympathetic ganglia, it suggests that individual ganglion may arise from rhombomere (r)-specific neural crest cells (NCCs). In turn, the NCCs may be controlled by the determinants of rhombomere identity, the Hox genes. To address these issues, we performed genetic fate maps of Hox gene-expressing Cre and ROSA-EYFP lineage reporter mouse lines to label NCCs originating from specific rhombomeres along the rostrocaudal axis. The identification of individual parasympathetic motor ganglion derived from specific Hox lineage reporter lines was subsequently matched with corresponding Hox knockout mice to determine its dependency on Hox gene function. Using a Hoxa3 lineage reporter line, we show that the otic ganglion, whose fate had not been previously mapped, originates from r6. We found that r6 NCC-derived otic ganglion is independent of Hoxa3 and Hoxb3, the Hox3 paralogous (P) genes known to synergize in r6, but instead require the Hox1P genes, Hoxa1 and Hoxb1. In the absence of the Hox1P genes, the otic ganglion is almost eliminated. This defect is associated with increased apoptosis and loss of dorsal rhombomere identity, as indicated by the absence of Kreissler/MafB protein expression, which normally labels r5 and r6. These findings suggest that individual parasympathetic motor ganglion originates exclusively from a single rhombomere and depends on the combined function of Hox paralogous genes.

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Program/Abstract # 474
The role of zebrafish zic genes in neural crest development
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Zic genes encode a conserved family of zinc finger transcription factors. We are focused on zic2a and zic5, which are closely linked and similarly expressed at the neural plate border and throughout the dorsal neural tube during neurula stages. Studies in mouse and Xenopus have identified zic2a and zic5 as important regulators of neural crest (NC) induction and perhaps migration, but have not explored these roles in detail. We have observed a severe reduction in jaw cartilage formation in embryos injected with morpholinos that
knock down Zic2a and Zic5 function, consistent with a requirement for zics during NC formation in zebrafish. Temporal analysis of NC marker expression in zic2a and zic5 morphants has revealed a migratory defect. This migratory phenotype is also present in embryos depleted for another member of the zic family, zic2b. Live imaging studies, currently in progress, will pinpoint the stage of NC development which requires Zics. These studies will define specific roles for zic genes during neural crest induction, maintenance, and/or migration, and will serve as a basis for dissecting the mechanism of zic gene function in the vertebrate neural crest.

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Program/Abstract # 475
Conditional ablation of Dlx3 in cranial neural crest-derived cells results in abnormal development of hair, teeth and craniofacial bone
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During embryogenesis, the homeodomain transcription factor, Dlx3, is involved in the development of structures derived from epithelial–mesenchymal interactions such as hair and teeth, as well as in bone (craniofacial and appendicular). In humans, a frameshift mutation in the coding sequence of DLX3 results in an ectodermal dysplasia known as Tricho-Dento-Osseous (TDO) syndrome. TDO patients have defects in hair, teeth and bone. At E11.5, Dlx3 is expressed in post-migratory cranial neural crest cells (CNC) that are known to contribute to hair, teeth and craniofacial bone formation. In order to assess the role of Dlx3 in CNC, we generated mice lacking Dlx3 in all CNC-derived tissues, using Wnt1-cre and Dlx3-floxed mice. These mice exhibit visible hair defects with a disheveled coat, kinky vibrissae and sparse hair on the head. Analysis of the composition of the coat revealed a change in the proportion and structure of specific hair types. Mutant mice also exhibit major tooth defects: their incisors are small and underdeveloped as compared to their wild-type littermates. Histological analysis of the teeth revealed a dramatic hypoplasia of the dentine that is totally absent on the labial part of the incisors. The structure, size and bone mineral density of the skull are also affected. These data demonstrate that the expression of Dlx3 in CNC-derived cells is essential for normal development of the three structures affected in TDO syndrome.

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Program/Abstract # 476
Role of Dlx3 in hair cycling
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Ectodermal appendages such as hair and tooth are attractive models for understanding the mechanisms underlying epithelial–mesenchymal interactions. Dlx3 belongs to the Distal-less family of homeodomain transcription factors, and an autosomal dominant mutation in DLX3 is responsible for the ectodermal dysplasia termed Tricho-Dento-Osseous syndrome (TDO), characterized by defects in hair, tooth, and bone development. Recently, we assessed the function of Dlx3 as a crucial transcriptional regulator of hair formation and regeneration using a Cre-mediated knockout mouse model. The most striking defect in those mice was complete alopecia due to failure in hair morphogenesis and cycling. However, it is not clear that the failure of hair cycling is due to a direct result from Dlx3 loss or a secondary effect from an incomplete first anagen in the Cre-mediated knockout mouse. To further investigate the specific role of Dlx3 in the hair cycle, we are utilizing a tamoxifen-inducible system by crosses of K14-CreERT2 mice with Dlx3-floxed mice. Knockout of Dlx3 expression is accomplished by the topical application of tamoxifen during the first postnatal catagen, especially to avoid the cumulative effect from an incomplete hair cycle as mentioned above. Our preliminary results establish Dlx3 as an essential regulator of hair cycling, validated by the permanent hair loss in the inducible knockout mice.

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Program/Abstract # 477
Aquaporin-3b and other direct targets of the Zic1 transcription factor
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Zic transcription factors cause multiple birth defects, among them the neural tube defect holoprosencephaly. We conducted a screen for direct targets of the Zic1 transcription factor, which plays multiple roles during early development, for example in patterning the early neural plate, development of the neural crest, and somite development. An aquaporin gene was identified in this screen, which we named aqp-3b due to its high similarity to aqp-3. aqp-3b and aqp-3 are both expressed in adult tissues of Xenopus, but only aqp-3b is expressed during embryonic development. In neurula stages, aqp-3b is expressed very specifically in the anterior neural folds, extending more posteriorly as the neural tube begins to close. Aquaporins are transmembrane proteins that form water channels in the plasma membrane and have been shown to facilitate cell movement and cell shape changes, which are both needed for neural tube closure. Our results suggest that aqp-3b morpholin oligonucleotides disrupt normal neural tube closure in Xenopus embryos, indicating that aqp-3b may be required for proper formation of the neural folds. Very few genes are known to be specifically involved in neural fold formation and we are examining the role of aqp-3b in this process further. Thus, aqp-3b may contribute to the mechanisms by which reduced activity of zic genes cause neural tube defects. Most recently, we identified another gene from our screen, which is also expressed in the neural folds. We are examining this gene further and will present our data.

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Program/Abstract # 478
Identifying the functional domains of FoxD5, a neural fate specifying gene
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FoxD5, an early-expressed neural transcription factor, is required for the appropriate expression of 11 other neural TFs; it up-regulates 3 TFs that promote an immature neural fate (gem, sox11, zic2), expands 2 TFs that maintain proliferative neural progenitors (sox2, sox3), and represses 6 TFs that promote the onset of neural differentiation (zic1, zic3, soxD, Xiro1–3). Using VP16-activating and EnR-repressing foxD5 constructs, we found that it regulates some NFS genes by transcriptional activation (gem, zic2) and others by transcriptional repression (zic1, zic3, Xiro1–3). These experiments, however, could not determine how the sox genes are regulated. Because FoxD5 contains several domains