(bkr) and gonzo (gnz) that have forebrain truncations or HPE; gnz mutants may also present with gastrulation defects or eye defects. Both mutations disrupt genes in the glycerophosphatidyl inositol (GPI) biosynthesis pathway: the bkr mutation is a single base substitution within a splice donor of Pgap1 and the gnz mutation in a single base substitution within a splice donor of Pig-N. Interestingly, gnz mutant embryos have alternatively spliced Pig-N mRNAs, and expression of the different splice isoforms correlates with a variable phenotype. GPI anchors are post-translational additions to a diverse group of proteins, and act to localize these proteins to lipid raft domains. We show that gnz mutant embryos and cell lines derived from these embryos display mislocalized GPI-anchored proteins. Genetic analysis of gnz embryos reveals that there is a linkage with the TGFβ co-receptor Cripto and the TGFβ ligand Nodal. Moreover, Cripto activity, as read-out by Smad2 phosphorylation, is reduced in cell lines generated from gnz mutants. Our data suggest that Cripto, a GPI-anchored protein, is the critical target protein that links GPI biosynthesis with TGFβ signaling and forebrain development.

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Program/Abstract # 131
Interaction between Cdx transcription factors and the Retinoic Acid pathway in establishing the hindbrain/spinal cord boundary in the zebrafish
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Although both the hindbrain and spinal cord originate from the posterior neural plate, the hindbrain develops into segmented rhombomeres while the spinal cord remains unsegmented. Cdx transcription factors are ParaHox genes that have been shown to modify hox gene expression patterns. We have previously shown that downregulation of the cdx1a and cdx4 transcription factors in the zebrafish leads to loss of spinal cord identity and the formation of ectopic hindbrain rhombomeres. Conversely, the over-expression of cdx4 under the control of a heat-shock promoter leads to ectopic expression of spinal cord-specific genes within the hindbrain. Recently, it has been shown that a similar phenotype occurs when Retinoic Acid signaling is expanded through chemical inhibition of the Cyp5a which function as Retinoic Acid degradation enzymes. Based upon these findings, we are currently investigating the interaction between cdx transcription factors and the Retinoic Acid pathway in establishing the boundary between hindbrain and spinal cord territories.

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Program/Abstract # 132
Evolutionarily conserved function of Gbx2 expression in cranial nerve V development
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The Gbx class of homeobox genes encodes DNA-binding transcription factors. The amino acid sequence of GBX2 is highly conserved across multiple species. Loss-of-function studies in mice have demonstrated an essential role for Gbx2 in establishing the midbrainhindbrain boundary (MHB) and anterior hindbrain development. However in zebrafish, gbx1, the sole family member of gbx2, has been implicated in establishment of the MHB. The hindbrain is transiently segmented into seven regions known as rhombomeres (r) during development. Hindbrain structures such as, the cerebellum and pons are derived from rhombomeres. Also, motor neuron cell bodies of the trigeminal (nV) and facial (nVII) cranial nerves arise in r2/r3 and r4/r5, respectively. Previous studies in mice have shown that inactivation of Gbx2 results in a complete absence of r1r3. More recent studies in which Gbx2 levels have been reduced to (610) of wild-type levels in mice demonstrate that r2 and its derivatives have a more stringent requirement for Gbx2 expression than r1 or r3. In the present study we focus on the impact of gbx2 expression in anterior hindbrain development of zebrafish. Our results demonstrate that abrogation of gbx2 expression with an antisense morpholino results in an increase in cell death in r2, r3 and r5 but not a failure of development. Furthermore loss of gbx2 function results in abnormal clustering of cranial nerve V cell bodies. Interestingly, the phenotypes are rescued by expression of mouse GBX2 protein. We suggest an evolutionarily conserved role for Gbx2 in cranial nerve V development.

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Program/Abstract # 133
Identification and characterization of a highly conserved Meis-linked gene
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We have identified a previously unstudied zebrafish (Danio rerio) gene, which is linked to the meis2.2 homeobox gene, using a comparative genomics approach. We have temporarily named the gene m2lg (for Meis2 linked gene). We have been able to identify a homologue of this gene in all animals examined and in every vertebrate examined the gene is directly adjacent to Meis2. While zebrafish contain two Meis2 paralogues it appears that they contain only one M2lg as we have been unable to identify one adjacent to meis2.1, or anywhere else in the available zebrafish genome for that matter. We are characterizing the expression of m2lg during zebrafish development using real time PCR and in situ hybridization. Preliminary experiments indicate that m2lg is likely present in the egg as a maternal transcript and is then actively transcribed in specific regions of the developing embryo later in development.

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Program/Abstract # 134
Identification and characterization of T-box transcription factor downstream targets
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The zebrafish no tail (ntl) and spadetail (spt) genes encode members of the T-box family of transcription factors. Both genes, as well as their counterparts in other vertebrates, have been shown to play important roles in the specification and patterning of posterior mesoderm. In the early embryo, ntl is expressed strongly in axial mesoderm (notochord precursors), whereas spt is expressed in paraxial mesoderm (somite precursors). However, ntl and spt are co-expressed at the blastoderm margin, the site of mesodermal cell internalization, and in involuting mesodermal cells of the tail bud. Correspondingly, ntl mutants lack a notochord and tail, spt mutants