Dandy Walker Malformation, the most common human cerebellar birth defect, is caused by heterozygous deletion of *Zic1* and *Zic4*. In *Zic1/4* single and double null mice, cerebellar phenotypes range from mildly reduced size with normal folial patterning, to severely small and mispatterned cerebella. The developmental basis of these phenotypes remains unknown and little is understood regarding the molecular pathways regulated by the *Zic* genes. We observe reduced granule cell (GC) proliferation in *Zic1−/−;Zic4−/−* mice at post-natal day one. Shh drives GC proliferation during early neonatal development, suggesting the hypothesis that *Zic1* and *Zic4* may act in the Shh pathway. At embryonic day 17.5, *Zic1−/−;Zic4−/−* cerebella demonstrate 2–4 fold reduction in expression of several Shh target genes, including *Gli1* and *Ptch1*, supporting a role for *Zic1* and *Zic4* in the Shh pathway. Several members of the Eph/Ephrin family also have reduced expression, although these genes have not been implicated in Shh dependent proliferation. This may indicate that adult cerebellar foliation defects have a Shh independent component. In situ hybridization at e17.5 shows that, in *Zic* mutants, EphA4 and EphA7 expression patterns are altered in the anterior medial domain of the developing cerebellum. This altered expression presages abnormal positioning of anterior folia, suggesting that *Zic* function is critical for determining the placement of the initial cardinal folia of the anterior cerebellum. Further experiments are underway to determine if size and foliation defects are related.

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Program/Abstract # 430
Genetic and functional interaction between transcription factors MEF2C and Dlx5/6 is required for craniofacial development
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Congenital craniofacial anomalies are among the most common cause of birth defects in infants, signifying the importance of understanding the molecular basis of craniofacial development. Our study focuses on the genetic and functional interaction between the MADS domain transcription factor MEF2C and two Dlx homeodomain transcription factors, Dlx5 and Dlx6. Neural crest-specific inactivation of Mef2c causes craniofacial lethality in mice. MEF2C is required for *Dlx5* and *Dlx6* expression in the craniofacial mesenchyme during development, and can transcriptionally synergize with Dlx5. We hypothesize that MEF2C and Dlx5 form a transcriptional complex essential for craniofacial development. Our current studies focus on elucidating the biochemical basis of the Dlx5–MEF2C interaction and identifying genes that are exquisitely sensitive to the dosage of these two factors. We show a genetic interaction between the *Mef2c* and *Dlx5/6* loci where heterozygosity at either locus ($Dlx5/6^{+/-}$ or $Mef2c^{+/-}$) results in viable mice with no obvious phenotype but heterozygosity at both loci ($Dlx5/6^{+/-};Mef2c^{+/-}$) results in perinatal lethality. $Dlx5/6^{+/-};Mef2c^{+/-}$ mice have defective mandibular outgrowth resulting in micrognathia and a posterior cleft of the palate. This mandibular defect is highly reminiscent of the Pierre Robin sequence (PRS) in humans and $Dlx5/6^{+/-};Mef2c^{+/-}$ mice could serve as a mouse model for PRS. Understanding the interaction between Dlx5/6 and MEF2C will contribute to our understanding of craniofacial development and how interactions between MADS box and homeodomain transcription factors regulate organogenesis.

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Program/Abstract # 431
Functional equivalence between Osr1 and Osr2 in mouse development
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The Odd-skipped gene was originally identified in *Drosophila* as an essential regulator of embryonic development and patterning. Two mouse homologs, odd-skipped related 1 (Osr1) and odd-skipped related 2 (Osr2) have been identified. Interestingly, whereas the *Drosophila* odd-skipped gene encodes a protein with four C2H2-type zinc finger motifs, mouse Osr1 encodes a protein with three zinc fingers and Osr2 encodes both a three-finger and a five-finger protein due to alternative splicing of the pre-mRNA. Targeted disruption of either gene caused distinct developmental defects that largely correlate with their distinct expression patterns. To investigate whether the two genes have evolved distinct functions at the molecular level and whether the two Osr2 isoforms function differently during mouse development, we replaced the endogenous Osr2 coding region with either the Osr1 cDNA encoding for the three-finger Osr1 protein or the Osr2-5F cDNA encoding the five-finger Osr2 protein isoform. Expression of either Osr1 or Osr2-5F from the Osr2 locus similarly rescued cleft palate phenotype of the Osr2 null mutants. These data indicate that the distinct functions of Osr1 and Osr2 during mouse embryonic development result from differential expression rather than distinct molecular function and that the two isoforms of Osr2 protein likely function redundantly during palate development.

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Program/Abstract # 432
The role of Dlx3 in hair development
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The Dlx3 homeodomain transcription factor is crucial for developmental processes including tissue differentiation and organ formation. Tricho-Dento-Osseous Syndrome (TDO) is an ectodermal dysplasia linked to a 4 bp deletion immediately downstream of the DNA-binding homeodomain in the Dlx3 gene and that is characterized by abnormalities in hair, teeth, and intramembranous and ectochondral bones. To examine Dlx3 gene expression throughout development, we generated a knockin mouse line carrying the reporter gene beta-galactosidase (lacZ) under the control of the endogenous Dlx3 promoter. Dlx3 expression is detected in tissues and organs derived from epithelial-mesenchymal interactions such as hair follicles, teeth and limbs, and in craniofacial bones and interfollicular epidermis. We have characterized Dlx3 expression through hair differentiation and growth cycle. Dlx3 expression was initially detected in the matrix of hair follicles directly adjacent to dermal papilla, and then gradually extended to inner root sheath (IRS), cortex, medulla, and cuticle of the differentiating hair. The role of Dlx3 in hair development is being investigated intensively using a Cre-mediated knockout mouse model. The most striking defect in those mice was alopecia due to a failure in hair follicle differentiation. Taken together with pathological

conditions of TDO patients, our results support the hypothesis that Dlx3 is an essential regulator for development of hair follicle.

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Program/Abstract # 433
Molecular consequences of a frameshifted Dlx3 mutant leading to Tricho-Dento-Osseous syndrome

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The homeodomain protein Distal-less-3 (Dlx3) plays a crucial role during embryonic development. In humans, a frameshift mutation in the coding sequence of the DLX3 gene results in an ectodermal dysplasia called Tricho-Dento-Osseous syndrome (TDO). The main features of this autosomal dominant disorder are defects in hair, teeth and bone. To investigate the functional alterations caused by the mutated $Dlx3^{TDO}$ isoform ex vivo, we used tetracycline-inducible cell lines in which the expression of $Dlx3^{WT}$ and/or $Dlx3^{TDO}$ could be regulated. Immunocytochemical analysis revealed that both $Dlx3^{WT}$ and $Dlx3^{TDO}$ recombinant proteins are targeted to the nucleus. However, as demonstrated by Electrophoresis Mobility Shift Assay, $Dlx3^{TDO}$ is not able to bind to the canonical Dlx3 binding site. Furthermore, we demonstrate that the frameshifted C-terminal domain in $Dlx3^{TDO}$ is responsible for the loss of DNA binding activity since the C-terminal domain in $Dlx3^{WT}$ is not required for DNA binding activity. Although $Dlx3^{TDO}$ cannot bind to Dlx3 responsive element it can interact with $Dlx3^{WT}$. Reporter assays showed that $Dlx3^{TDO}$ has a defective transcriptional activity. Moreover, the transcriptional activity of $Dlx3^{WT}$ is significantly reduced in the presence of the mutated isoform. Taken together, these data demonstrate that many of the developmental defects associated with TDO are potentially a consequence of the dominant negative effect of the $Dlx3^{TDO}$ protein on its wild type counterpart.

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Program/Abstract # 434
Role of T and Tbx6 in mesodermal patterning

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Tbx6 and Brachyury (T), two T-box transcription factors, are co-expressed in the primitive streak of the developing mouse embryo and are essential for mesodermal patterning. Tbx6 has an additional expression domain in the presomitic mesoderm independent of T, and T is expressed in the node and notochord independent of Tbx6. The T-box proteins are related through a conserved T-box DNA binding domain, and accordingly, Tbx6 can bind T's consensus binding sequence in vitro. We are further investigating how T and Tbx6 work together and independent of each other to activate common and/or different downstream targets and specify different cellular and morphological properties. Results from these studies will give further insight into how T and Tbx6 function in primitive streak and paraxial mesoderm formation.

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Program/Abstract # 435
The identity and fate of Tbx4-expressing cells reveal previously unknown developmental decisions in the allantois, limb, and proctodeum

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The T-box gene *Tbx4* is critical for the formation of the umbilical vessels as well as for the initiation and proper morphogenesis of the hindlimb. Previous work has shown that it is expressed in broad domains throughout the allantois and the hindlimb, as well as in the lung and proctodeum. We have examined the expression of *Tbx4* in greater detail and used a cre-mediated lineage reporter to examine the eventual fates of cells that express *Tbx4*. Despite the observation that loss of *Tbx4* produces profound defects in the developing allantois vasculature, the presumptive endothelial cells of the allantois do not appear to express *Tbx4*, and lineage trace analysis reveals that much of the umbilical endothelium has never expressed *Tbx4*. These results imply that endothelial and non-endothelial lineages are segregated well before the onset of vasculogenic genes such as *Flk-1*, and also demonstrate a novel role for the peri-vascular tissue in the development of continuous vascular structures. Likewise, examination of the relationship between the expression of *Tbx4* in the posterior mesenchyme and the eventual fate of *Tbx4*-expressing cells suggests that various distinct appendages such as the allantois, hindlimb, and external genital all arise from a single contiguous domain. In addition, although *Tbx4* is normally associated with the hindlimb, we have found and characterized two domains of expression in the forelimb which produce cells that segregate to specific regions of the forelimb.

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Program/Abstract # 436
Ash2l: A Novel interacting cofactor of DiGeorge syndrome transcription factor Tbx1

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DiGeorge syndrome (DGS) is a common syndrome associated with 22q11 deletions. Most patients with DGS are born with severe heart defects. Congenital heart disease is the most commonly occurring birth defect and relatively little is known about the molecular basis of these defects. Mouse models have implicated Tbx1 as a critical gene within the commonly deleted region. Tbx1 encodes a nuclear transcription factor that binds DNA and regulates downstream genes. Tbx1 direct targets and its transcriptional complex are largely unknown. We have identified a potential transcriptional cofactor, Ash2l. Ash2l is known to be part of a histone methyltransferase complex involved in epigenetic transcriptional regulation. Two non-overlapping interacting Ash2l domains were independently found to interact with Tbx1 in our unbiased yeast two-hybrid screen. These interactions were confirmed in mammalian cells. Ash2l mRNA and protein is widely expressed in the mid-gestation mouse embryo, including in Tbx1 expression domains. While Ash2l+/− mice are normal, complete loss of Ash2l is lethal early in embryogenesis.Ash2l physically interacts with Tbx1. Very early embryonic lethality of Ash2l null mice suggests this protein is critically