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

doi:10.1016/j.ydbio.2008.05.509

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

Olivier Duverger [a], Delia Lee [a], Mohammad Q. Hassan [b], Susie X. Chen [a], Frederic Jaisser [c], Jane B. Lian [b], Maria I. Morasso
[a] *Developmental Skin Biology Unit, NIAMS/NIH, Bethesda, MD, USA*
[b] *UMass Medical School, Worcester, MA, USA*
[c] *INSERM U772, College de France, Paris, France*

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.

doi:10.1016/j.ydbio.2008.05.510

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

Amy K. Wehn, Deborah L. Chapman
Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA

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.

doi:10.1016/j.ydbio.2008.05.511

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

L.A. Naiche [a], Ripla Arora [b], Virginia E. Papaioannou [b]
[a] *Cancer and Developmental Biology, National Cancer Institute, Frederick, MD, USA*
[b] *Department of Genetics and Development, Columbia University, New York, NY, USA*

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.

doi:10.1016/j.ydbio.2008.05.512

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

Jason Z. Stoller [a,b], Li Huang [a,b], Jonathan A. Epstein [b]
[a] *Division of Neonatology, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelhia, PA, USA*
[b] *Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, USA*

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