member of the Tbx2 sub-family of T-box factors, which in other species includes both transcriptional activators and repressors, and it is required for the development of anterior pharyngeal muscles. We are interested in determining if TBX-2 is a transcriptional activator or repressor, and in identifying TBX-2 targets to characterize its molecular mechanism. To identify targets of TBX-2, we compared mRNA expression levels in wild-type and hypomorphic tbx-2(bx59) mutant embryos using Affymetrix microarrays. Of 19,885 genes examined, we found 980 mRNAs that were significantly up-regulated in tbx-2(bx59) and 175 mRNAs that were significantly down-regulated. We analyzed a subset of genes and found that the gene D2096.6 is directly repressed by TBX-2 at a variant T-box binding site in its promoter. Our previous genetic evidence suggests that TBX-2 function is SUMOylation dependent, as reduction of the SUMO-conjugating enzyme UBC-9 produces pharyngeal phenotypes similar to loss of TBX-2. Consistent with these results we see that ubc-9 (RNAi) causes over expression of D2096.6 similar to what we see in TBX-2 mutants. By comparing mRNA expression levels in wild-type and tbx-2(bx59) mutant embryos we have identified the first direct target of TBX-2 in C. elegans. Our results suggest TBX-2 functions as a transcriptional repressor whose function is dependent on SUMOylation.

Pax6 is a member of the phylogenetically conserved Pax gene family of nuclear transcription factors. Previous transgenic analyses have identified two mouse Pax6 pancreatic enhancers at 1.9 kb and 4.0 kb respectively from the transcriptional start site, but the functional significance of these regulatory regions remain controversial. In this study, we have generated two genomic deletions—one 237 bp deletion eliminating the 1.9 kb enhancer (Pax ΔM/ΔM), another 3.1 kb deletion removing both the 1.9 kb and 4.0 kb enhancers (Pax ΔGM/ΔGM). Immunohistochemistry and quantitative RT-PCR showed that the Pax ΔM/ΔM mice had a significant decrease in Pax6, glucagon, and insulin expression compared to wild type mice, while no further reductions were observed in the Pax ΔGM/ΔGM mice, indicating that only the 237 bp region is required for pancreatic development. In contrast, Pax ΔGM/ΔGM, but not Pax ΔM/ΔM mice, developed ocular defects such as the failure of the lens to detach from the surface ectoderm, lens hypoplasia, and stunted lacrimal gland growth, confirming the existence of a Pax6 lens ectodermal enhancer in the 4.0 kb region. No retinal differentiation defects were observed in both mouse strains, as there was normal development of horizontal, amacrine, and photoreceptor cells. This study thus underscores the importance of the knock out approach in defining enhancer functionality in vivo.

Enhancers are cis-regulatory elements that control the pattern and levels of gene expression. Enhancers can be located upstream, downstream, or even within a gene, often located at substantial genomic distances from the promoters they regulate. Therefore, distal enhancer promoter interactions are a crucial part of transcriptional regulation, but surprisingly little is known about how these interactions are facilitated in the nucleus. In order to better understand the mechanisms by which enhancers activate transcription from a distance, we are performing an in vivo structure-function analysis of the EGF/MAPK- and Notch-regulated D-Pax2 cone cell-specific sparking enhancer (spa). In this study reporter gene expression is driven by the spa enhancer placed either adjacent to, or at a distance from, a heterologous promoter. Using this approach, we have identified a sequence within spa that is required when the enhancer is placed at a distance from the promoter, but is dispensable when the enhancer is proximal to the promoter. To our knowledge, this is the first enhancer sub-element identified that specifically mediates long-range enhancer activity, yet is not required for patterning gene expression, thus we have named it the “Remote Control” Element or RCE. Our current work focuses on determining the functional properties and capabilities of the RCE, as well as the identification and characterization of proteins that interact with the RCE and allow it to perform its essential activities. This study will allow us to better understand the mechanisms by which enhancers engage in long-range transcriptional regulation.

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Program/Abstract # 542
Essential enhancer elements regulate Pax6 in pancreas and eye development
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Program/Abstract # 543
Early and late expression of D-Pax2 during Drosophila external sensory organ development is controlled by separate upstream enhancers
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The development of functional mechanosensory and photosensory organs in Drosophila melanogaster is dependent upon the transcription factor D-Pax2. The role of this protein in external sensory (es) organs and eyes has previously been documented and the regulation of D-Pax2 gene expression in the eye has been meticulously dissected. However, the elements that control the dynamic expression pattern of D-Pax2 in the es organ cells have not been identified. We have examined the region upstream of the D-Pax2 transcription start site using transgenic GFP reporter lines. We demonstrate that early and late expressions of D-Pax2 in the es organ cell lineage are controlled by separate and adjacent enhancer elements of 2 kilobases and 1 kilobase, respectively. The early expression of the gene begins in the sensory organ precursor cell and continues during the divisions and specification of the es organ cells. This early expression is regulated in part by proneural proteins. The late expression is restricted to the differentiating trichogen and thecogen cells of the es organ and involves a positive feedback loop that requires D-Pax2 protein itself.