

Identifying a Role for Tbx2 in Heart Development

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Summary and Implications

Functional domains were identified within the Tbx2 protein. We have identified a couple of proteins that interact with Tbx2. Knowledge of how Tbx2 interacts with other proteins is essential in understanding how it inhibits gene expression during heart development.

Introduction

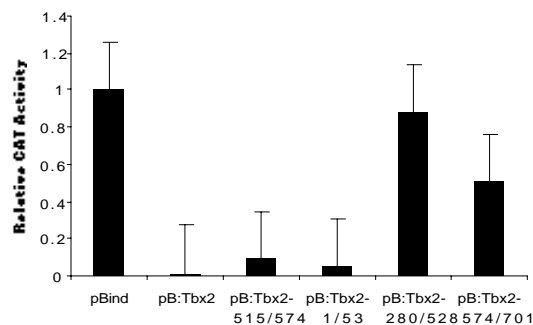
Tbx2 is a strong repressor of gene activity, and can inhibit expression both *in vitro* and *in vivo*. Recent studies have shown that it is capable of inhibiting heart specific genes during development of the heart. In addition, Tbx2 has a very defined expression pattern during heart development. However, we do not know how it represses gene activity. To better understand the mechanism of how Tbx2 works we have identified 1) functional domains within the protein, and 2) proteins that Tbx2 interacts with.

Materials and Methods

Gene Expression Studies

Tbx2 was broken up into specific regions and fused to the GAL4 DNA binding domain. The fusion proteins were introduced into a fibroblastic cell line along with a reporter gene, Chloramphenicol Acetyl Transferase (CAT), and assayed for functional activity of the reporter gene after forty-eight hours. The GAL4 DNA binding domain without a Tbx2 fusion was used as a negative control, and relative CAT activity was determined based on the control values.

Figure 1. Repression of CAT activity by functional domains of Tbx2.



Identification of Protein Interactions

The functional domains of Tbx2 were fused to the human SOS protein. A temperature sensitive yeast cell line was co-transfected with the SOS:Tbx2 expressing vector and a mouse cDNA expression library, and incubated at room temperature. After two days, the yeast plates were replicated, and the replicate plates were incubated at an elevated temperature. The SOS protein complements the temperature sensitive mutation at higher temperature when there is an interaction between the Tbx2 fragment and the library expressed protein. Colonies that grew at the higher temperature were selected and the plasmid DNA was isolated and sequenced. Sequences were screened against the human and mouse genome databases to identify the genes coding for the proteins that interacted with Tbx2.

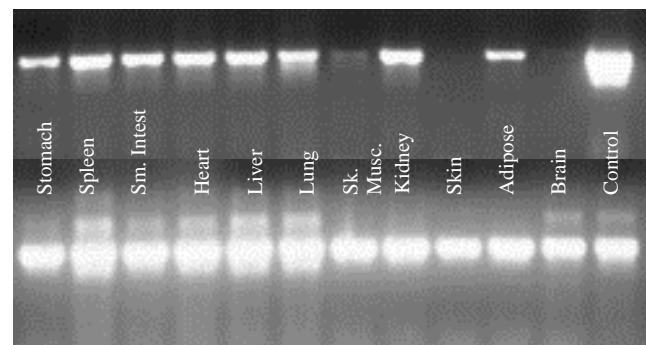
Deletion Mapping

Regions of the target genes were systematically removed to identify the region of target protein that interacts with Tbx2. These interaction studies were also performed in the yeast line.

USP-1 Expression Pattern

RNA was isolated from adult female mice. We made cDNA copies of the mRNA collected from various tissue samples. Using USP-1 gene specific primers, we did a Polymerase Chain Reaction to visualize the expression of USP-1, and identify which tissues it was expressed in.

Figure 2. Expression of USP-1 in adult mouse tissues



Results and Discussion

We have shown that Tbx2 is capable of repressing gene expression in cell culture. Through our fusion protein studies we have identified two unique domains within the Tbx2 protein that are capable of inhibiting expression. One found at the amino terminus and the second in the carboxy-terminal region of the protein. Even though we can show repression, we do not know *how* it represses gene activity.

Figure 3. Functional domains of the Tbx2 protein.

Protein interactions with Tbx2 were identified using a yeast-2-hybrid system. We have shown that the amino terminal repression domain of Tbx2 can interact with Ubiquitin Specific Processing Protease-1 (USP-1) and Coactivator of Activating Protein (AP) 1 and Estrogen Receptors (CAPER). The identified regions of these proteins that interact with Tbx2 were further defined by deletion mapping. In addition, we identified the expression of USP-1 in a number of adult mouse tissues, showing that its expression is fairly ubiquitous.

Ubiquitination of proteins has recently come to the forefront as a mechanism of regulating gene expression. Our findings that Tbx2 interacts with USP-1, which is capable of removing ubiquitin from other proteins, are significant because they suggest that deubiquitination may play a role in Tbx2-mediated repression. Specifically, they pose the question, is deubiquitination by USP-1 responsible for the gene repression seen in our Tbx2 transfection studies? That is the question we are working to answer.

Acknowledgements

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