Tbx2 mediates dorsal patterning and germ layer suppression through inhibition of BMP and Activin/Nodal signaling

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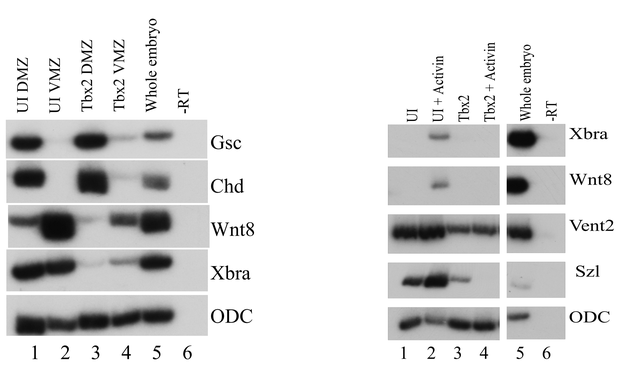
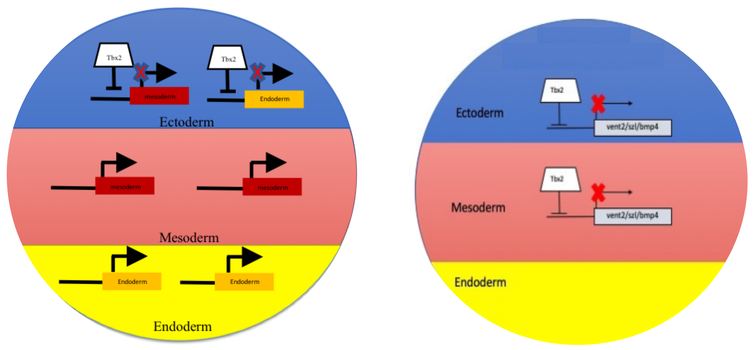
Abstract: During gastrulation, the T-box family of transcription factors regulates the formation and patterning of the three primary germ layers. In embryos of the frog *Xenopus laevis*, the T-box transcriptional activators Brachyury, VegT and Eomesodermin activate expression of germ layer-specific genes in the presumptive mesoderm and endoderm. In the animal pole, the zygotically expressed T-box protein Tbx2 functions as a transcriptional repressor to inhibit signaling through both the Activin/Nodal and Bone Morphogenetic Protein (BMP) branches of the Transforming Growth Factor β (TGFβ) signaling network; overexpression of Tbx2 in the presumptive ectoderm promotes neural fate, and loss of Tbx2 in the animal pole results in ectopic expression of mesodermal and endodermal genes. Here, we report physical association between Tbx2 and intracellular mediators of TGFβ signaling, and present a structure/function analysis to establish the domains of Tbx2 that mediate inhibition of both BMP and Activin/Nodal signaling. This work is supported by PHS Grant R15GM124577 (to DCW), and with funds from the Professional Staff Congress-City University of New York.

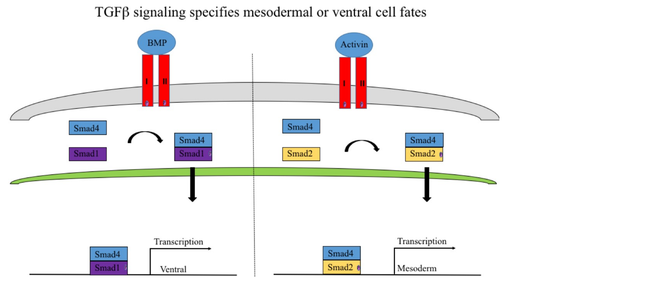
Figure 1. Tbx2 represses mesodermal and ventral markers

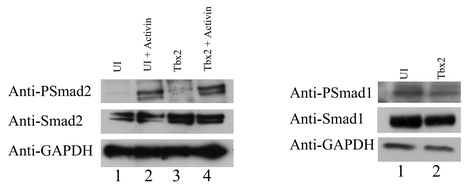


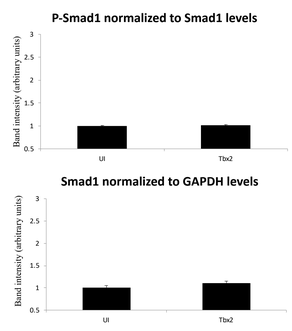


Ectopic expression of *tbx2* dorsalizes the presumptive ectoderm and mesoderm during gastrulation. Ectopic expression of Tbx2 in the marginal zone decreases pan-mesodermal markers and ventral markers. These data suggest that Tbx2 represses the BMP and Activin/Nodal pathways.

Figure 2. Tbx2 does not inhibit C-terminal phosphorylation of Smad1 or Smad2

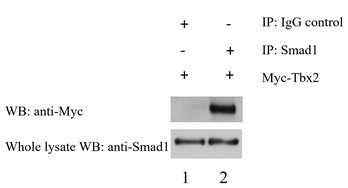


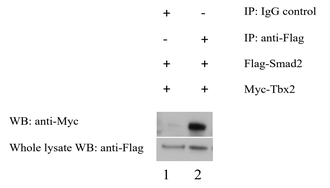




Smad1 and Smad2 C-terminal phosphorylation is required for TGFβ signal transduction. Tbx2 does not inhibit Smad1 or Smad2 C-terminal phosphorylation, indicating that this is not the mechanism through which Tbx2 represses the TGFβ signaling pathway.

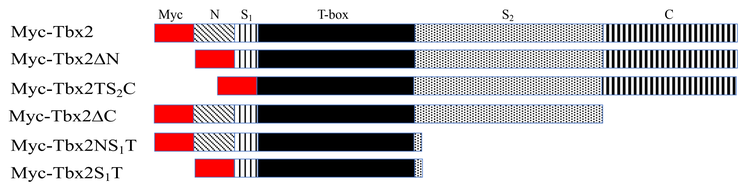
Figure 3. Tbx2 physically associates with Smad1, Smad2, and Smad4





Tbx2 represses mesodermal and ventral markers, suggesting that Tbx2 inhibits both the BMP and Activin/Nodal TGFβ signaling pathways. Tbx2 physically associates with Smad1, Smad2, and Smad4, indicating that Tbx2 does inhibit the association between R-Smads and Smad4.

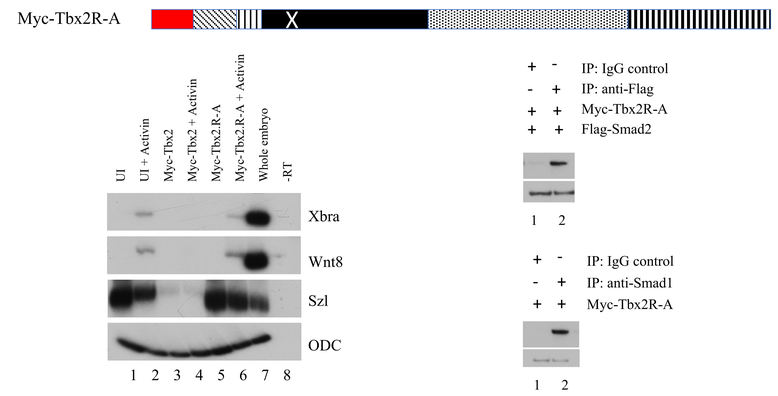
Figure 4. Tbx2 associates with Smad1 and Smad2 via the T-box domain



	Tbx2	Tbx2ΔN	Tbx2TS$_2$C	Tbx2ΔC	Tbx2NS$_1$T	Tbx2S$_1$T
Represses Mesoderm	+	+	+	-	-	+
Represses Ventral	+	+	+	-	-	-
Associates with Smad1	+	+	+	+	+	+
Associates with Smad2	+	+	+	+	+	+

Tbx2 deletion constructs were tested for activity and association with Smad1 and Smad2. The C-terminus is necessary for repression of mesodermal and ventral markers. The data suggest that Tbx2-Smad1/2 interaction is mediated via the T-box

Figure 5. A Tbx2 DNA-binding mutant does not have repressor activity, yet still associates with Smad1 and Smad2



Conclusion: Numerous studies have shown that T-box proteins play important roles in germ layer specification. In the ectoderm Tbx2 represses mesodermal and ventral marker expression. Our experiments show that Tbx2 physically associates with Smads 1, 2, and 4, important intracellular mediators of mesodermal and ventral gene expression. Deletion construct co-immunoprecipitation experiments demonstrate that Tbx2 associates with Smads via the T-box. Future experiments will study the mechanisms through which the Tbx2/Smad complex represses mesodermal and ventral gene expression.