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Wnt/β-catenin/Tcf Signaling Induces the Transcription of a Tumor Suppressor Axin2, a Negative Regulator of the Signaling Pathway

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Axin2/Conductin/Axil and its ortholog *Axin* are negative regulators of the Wnt signaling pathway, which promote the phosphorylation and degradation of β-catenin. While *Axin* is expressed ubiquitously, *Axin2* mRNA was seen in a restricted pattern during mouse embryogenesis and organogenesis. Because many sites of *Axin2* expression overlapped with those of several *Wnt* genes, we tested whether *Axin2* was induced by Wnt signaling. Endogenous *Axin2* mRNA and protein expression could be rapidly induced by activation of the Wnt pathway, and *Axin2* reporter constructs, containing a 5.6 kb DNA fragment including the promoter and first intron, were also induced. This genomic region contains eight Tcf/LEF consensus binding sites, five of which are located within longer, highly conserved non-coding sequences. The mutation or deletion of these Tcf/LEF sites greatly diminished induction by β-catenin, and mutation of the Tcf/LEF site T2 abolished protein binding in an electrophoretic mobility-shift assay. These results strongly suggest that *Axin2* is a direct target of the Wnt pathway, mediated through Tcf/LEF factors. The 5.6 kb genomic sequence was sufficient to direct the tissue specific expression of d2EGFP in transgenic embryos, consistent with a role for the Tcf/LEF sites and surrounding conserved sequences in the in vivo expression pattern of *Axin2*. Our results suggest that *Axin2* participates in a negative feedback loop, which could serve to limit the duration or intensity of a Wnt-initiated signal.