

# When Wnts antagonize Wnts

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**Secreted Wnt ligands appear to activate a variety of signaling pathways. Two papers in this issue now present genetic evidence that “noncanonical” Wnt signaling inhibits the “canonical” Wnt/ $\beta$ -catenin pathway. Westfall et al. (2003a) show that zebrafish embryos lacking maternal *Wnt-5* function are dorsalized due to ectopic activation of  $\beta$ -catenin, whereas Topol et al. (2003) report that chondrogenesis in the distal mouse limb bud depends on inhibition of Wnt/ $\beta$ -catenin signaling by a paralogue of *Wnt-5*. These studies present the first genetic confirmation of the previous hypothesis that vertebrate Wnt signaling pathways can act in an antagonistic manner.**

Wnt signaling regulates differentiation and proliferation of a variety of cell types during animal development and is also implicated in tumor formation. Some Wnts activate a signaling pathway that results in increased levels of  $\beta$ -catenin, which in turn modulates transcription of target genes (Huelsenken and Behrens, 2002). The earliest function of this so-called “canonical” Wnt/ $\beta$ -catenin pathway in vertebrate embryogenesis is to specify dorsal cell fates. Consequently, overexpression of some Wnts (such as Wnt-8) in early zebrafish and *Xenopus* embryos promotes formation of excess dorsal cells at the expense of ventral cell fates. In contrast, overexpression of other Wnts (including Wnt-5 and Wnt-11) interferes with gastrulation movements rather than modulating  $\beta$ -catenin or cell fates (Torres et al., 1996). Genetic evidence for the existence of such “noncanonical” Wnt signaling in vertebrates has been provided by analysis of zebrafish *Wnt-11* mutations, which cause defects in gastrulation movements that cannot be rescued by  $\beta$ -catenin (Heisenberg et al., 2000; Tada et al., 2002).

Intriguingly, for many years there has been evidence that the biological activities of some Wnts might work in an opposing manner. For example, overexpression of a *Wnt-5* paralogue blocks the ability of *Wnt-8* to promote dorsal cell fate in *Xenopus* (Torres et al., 1996), and overexpression of *Wnt-5* can activate the nemo-like kinase, which in *C. elegans* and mammalian cells inhibits Tcf transcription factors to which  $\beta$ -catenin binds to regulate gene expression (Ishitani et al., 2003). However, there has so far been no

genetic evidence supporting the apparent antagonism of some Wnts in vertebrates.

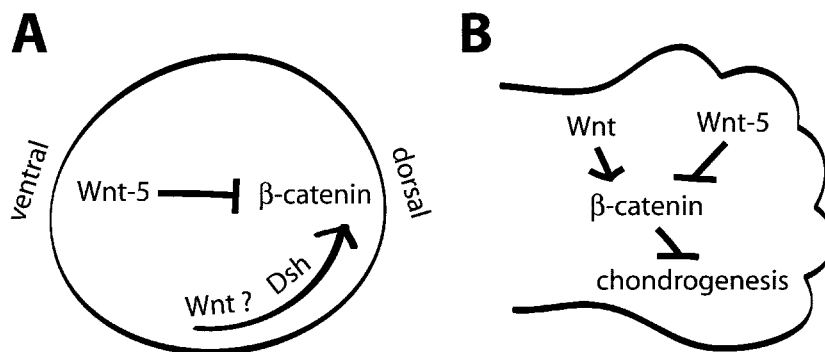
The laboratories of Diane Slusarski and Yingzi Yang now present this genetic evidence (Fig. 1) (Westfall et al., 2003a; Topol et al., 2003). Slusarski’s laboratory shows that removal of both the maternal and zygotic function of zebrafish *Wnt-5* (*MZWnt-5*) not only enhances the morphogenesis defects of zygotic *Wnt-5* mutants, but also results in variable degrees of dorsalization, including formation of a secondary axis (Westfall et al., 2003a). These phenotypes resemble those obtained by overactivation of Wnt/ $\beta$ -catenin signaling, and indeed the authors find ectopic stabilization of  $\beta$ -catenin and ectopic expression of  $\beta$ -catenin target genes in *MZWnt-5* mutant embryos. These findings add strong genetic support to the previously suggested requirement of a Wnt signal for development of ventral cell fates and antagonism of dorsal fates, as has been proposed based on the ability of dominant-negative forms of Wnt-11 and of a frizzled receptor to interfere with ventral cell fates in *Xenopus* (Itoh and Sokol, 1999; Kühl et al., 2000a). Similarly, Yang’s laboratory shows that in the distal tip of mouse limb buds *Wnt-5a* antagonizes Wnt/ $\beta$ -catenin signaling, since in *Wnt-5a*<sup>-/-</sup> limbs higher levels of  $\beta$ -catenin can be detected in the distal tip where a  $\beta$ -catenin-responsive reporter is ectopically expressed (Topol et al., 2003). Chondrocyte differentiation, which is defective in the *Wnt-5a*<sup>-/-</sup> limbs, can be partially restored by transplantation of cells expressing a secreted Wnt inhibitor, which presumably interferes with canonical Wnts only. Thus, in the mouse limb bud, *Wnt-5a* signaling appears to promote chondrocyte differentiation by antagonizing the Wnt/ $\beta$ -catenin pathway.

Which signaling pathway(s) do *Wnt-5* paralogues activate to inhibit Wnt/ $\beta$ -catenin signaling? Noncanonical Wnts have been reported to be able to activate a wide variety of cellular responses upon overexpression in vertebrate embryos or cultured mammalian cells. In some cellular contexts, *Wnt-5* paralogues activate the Wnt/ $\text{Ca}^{2+}$  pathway, which involves  $\text{Ca}^{2+}$  release from intracellular stores, stimulation of protein kinase C (PKC), the  $\text{Ca}^{2+}$ /calmodulin-dependent kinase CamKII, and the  $\text{Ca}^{2+}$ -dependent transcription factor NFAT (Kühl et al., 2000b; Saneyoshi et al., 2002). *Wnt-5* paralogues might also modulate a vertebrate counterpart to

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Abbreviations used in this paper: JNK, c-jun NH<sub>2</sub>-terminal kinase; PCP, planar cell polarity; PI, phosphatidylinositol; PKC, protein kinase C.

**Figure 1. Vertebrate Wnt-5 paralogues antagonize Wnt/ $\beta$ -catenin signaling.** (A) A highly schematized model of antagonistic interactions of maternal Wnt signaling pathways in early *Xenopus* or zebrafish embryos. In response to Dishevelled (Dsh) and perhaps a Wnt signal, high  $\beta$ -catenin levels specify the dorsal side. Maternal Wnt-5 is required for development of ventral cell fates by antagonizing  $\beta$ -catenin signaling, although where Wnt-5 is active remains unclear. (B) A simplistic model of Wnt antagonism in the mouse limb bud. The limb ectoderm and apical ectodermal ridge (AER) meet at the distal limb bud. As several Wnts (e.g., Wnt7a or Wnt3) that are able to activate canonical Wnt/ $\beta$ -catenin signaling are expressed in the limb ectoderm and apical ectodermal ridge, the distal limb bud has a higher level of canonical Wnt/ $\beta$ -catenin signaling, which suppresses chondrogenesis. In the distal limb bud, Wnt-5a signaling decreases  $\beta$ -catenin levels, which allows chondrogenesis to occur.



the *Drosophila* planar cell polarity (PCP) pathway (Tada et al., 2002; Mlodzik, 2002).

Wnt/ $\text{Ca}^{2+}$  signaling has previously been implicated in specification of ventral cell fates in *Xenopus*, since both a dominant-negative Wnt-11 (thought to interfere with non-canonical Wnts) and a dominant-negative CamKII promote dorsal cell fates (Kühl et al., 2000a). In addition, interference with the phosphatidylinositol (PI) cycle—an important signaling pathway that leads to intracellular  $\text{Ca}^{2+}$  release—likewise dorsalizes *Xenopus* and zebrafish embryos (Kume et al., 1997; Westfall et al., 2003b). However, there has been no genetic evidence supporting these observations. Westfall et al. (2003a) now show that zygotic zebrafish *Wnt-5* mutant embryos have slightly reduced  $\text{Ca}^{2+}$  fluxes at early stages and that a constitutively active CamKII can partially rescue the morphogenesis defects of such mutants. In addition, lower doses of PI cycle inhibitors can phenocopy the morphogenesis defects of zygotic *Wnt-5* mutants. These results indicate that Wnt-5 may indeed be required for calcium signaling and might regulate gastrulation movements via the Wnt/ $\text{Ca}^{2+}$  pathway. However, a growing body of evidence also suggests that PCP signaling is required for gastrulation movements (Tada et al., 2002), and at present it is unclear whether these pathways act in parallel or overlap.

The papers by Westfall et al. (2003a) and Topol et al. (2003) also raise a number of questions. Although both groups show that Wnt-5 paralogues are required as repressors of  $\beta$ -catenin signaling, it is not clear if this is through  $\text{Ca}^{2+}$  signaling or by something else activated by Wnt-5. Thus, further research will be needed to elucidate the signaling pathway used by Wnt-5 to antagonize Wnt/ $\beta$ -catenin by rescue and genetic interaction experiments in *MZWnt-5* zebrafish embryos, as well as determining  $\text{Ca}^{2+}$  flux in these embryos. Moreover, Topol et al. (2003) provide evidence that the inhibition of  $\beta$ -catenin they see is not mediated by activation of CamKII, PKC, NFAT, or c-jun  $\text{NH}_2$ -terminal kinase (JNK). Therefore, currently described effectors of Wnt/ $\text{Ca}^{2+}$  signaling do not appear to adequately explain the inhibitory effect of Wnt-5 on  $\beta$ -catenin signaling in cultured mammalian cells. Topol et al. (2003) go on to show that *Siah2*, a component of a known  $\beta$ -catenin destruction complex, is transcriptionally activated by Wnt-5a, concomitant with observed decreases in  $\beta$ -catenin levels. Given the

paucity of information on how Wnt signaling might regulate gene expression in a  $\beta$ -catenin-independent manner, it will be interesting to study how *Siah2* is regulated.

Another question regards the relevance of these findings to cancer biology, given that activation of  $\beta$ -catenin function is observed in many cancers. The finding of Topol et al. (2003) that Wnt-5a inhibits  $\beta$ -catenin levels and transcriptional activity in a colon cancer cell line adds to the sporadic literature suggesting that this Wnt might be involved in some cancers.

Although our understanding of noncanonical Wnt signaling lags far behind our knowledge of the Wnt/ $\beta$ -catenin pathway, the papers of Westfall et al. (2003a) and Topol et al. (2003) make valuable contributions to understanding the involvement of noncanonical Wnt signaling in promoting ventral cell fates in embryos, and chondrogenesis, respectively. Both papers also provide the first genetic hint that distinct vertebrate Wnt signaling pathways may indeed be capable of cross-talk, leading to functional antagonism.

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