Wnt signaling: **The Naked truth?**

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Frizzled receptors can activate two alternative signal transduction pathways: the canonical Wnt pathway or the planar cell polarity pathway. Recent studies of the Naked cuticle protein suggest a mechanism for the inactivation of the canonical pathway and concomitant activation of the planar cell polarity pathway.

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Current Biology 2001, 11:R524–R526

0960-9822/01/$ – see front matter

The development of metazoans depends upon a series of molecular cues and coordinated cellular responses just as a theatrical performance depends upon a 'cast of characters' and their protagonistic and antagonistic relationships. Wnt proteins are secreted ligands that pattern insect and vertebrate embryos by directing the differentiation of different cell fates (reviewed in [1]). Just as some characters are involved in independent, yet related story lines, certain Wnt signaling components also regulate the establishment of planar cell polarity (reviewed in [2]). These results illustrate the use of divergent signaling pathways downstream of common ligands; however, the mechanism whereby cells selectively regulate distinct pathways in response to similar stimuli remains unclear. To this end, Rousset *et al.* [3], Yan *et al.* [4], and Wharton *et al.* [5] have begun to characterize the function of Naked Cuticle (Nkd), a protein that antagonizes Wnt signaling while potentially activating the planar cell polarity pathway.

Genetic studies have identified many of the leading cast members required for Wnt-dependent cell fate assignment (reviewed in [1]). Wnt signaling regulates the accumulation of cytoplasmic β-catenin, the vertebrate ortholog of *Drosophila* Armadillo. In the absence of Wnt signal, cytoplasmic β-catenin is rapidly degraded (reviewed in [6]). Cells that receive Wnt signals accumulate β-catenin, a process that depends upon the cell-autonomous activation of Dishevelled (Dsh/Dvl), as well as the subsequent inactivation of the destruction complex, which includes glycogen synthase kinase-3β, APC and axin/conductin (Figure 1), vertebrate orthologs of *Drosophila* Zeste-white3 kinase (Zw*3*), DAPC/APC2, and DAxin, respectively. As β-catenin accumulates, it affects target gene transcription by interacting with LEF1/TCF transcription factors. Because Wnt signaling depends upon the concentration of β-catenin, defects in genes whose activity antagonizes the function of β-catenin will result in constitutive, and potentially oncogenic, activation of the Wnt signaling pathway.

In addition to the canonical cast of characters associated with Wnt signaling, a number of 'supporting cast members', such as Puckered [7] and Dally [8,9], influence the plot by modulating signaling either directly or indirectly. One supporting cast member whose role has recently been featured center stage is *naked cuticle* (*nkd*), a segment polarity gene first described almost 20 years ago [10]. In *nkd* mutant embryos cells fated to secrete hair-like processes known as denticles instead adopt a 'naked' cuticle fate, a phenotype resembling activation of the Wnt cascade. Consistent with Nkd functioning as a negative regulator of Wnt signaling, misexpression of *Drosophila* Nkd phenocopies loss-of-function of the *Drosophila Wnt* homolog *wingless (wg)* [11]. Furthermore, injection of *Drosophila nkd* RNA into *Xenopus* embryos disrupts anterior-posterior axis specification and blocks XWnt8-dependent induction of secondary axes, two events that depend upon the canonical Wnt cascade [11]. As both *Drosophila* and murine *nkd* mRNAs are up-regulated by Wnt signaling [4,11], Nkd is poised to attenuate Wnt signals on cue through negative feedback.

To begin to define the antagonistic role of Nkd, Rousset *et al.* [3] used RNA interference and traditional genetics to refine the epistatic relationships between Nkd and canonical Wnt cast members. They found that Nkd function is cell autonomous and that Nkd overexpression can suppress the effects of Wg or Dsh overexpression; however, Nkd fails to suppress constitutively active Armadillo. In addition, they showed that Nkd antagonizes Wnt signaling upstream of Zw3, while both Dsh and Armadillo are required for *nkd*-associated phenotypes. Epistasis experiments in cultured mammalian cells also placed murine Nkd1 downstream of Wnt1 but upstream of β-catenin [5]. Therefore, Nkd function depends upon Wnt signaling, as previously shown by others [12], and thus Nkd is distinct in action from Zw3 and company, suggesting that Nkd is not an integral part of the destruction complex. Instead, it appears to desensitize cells to Wg by raising the response threshold. These data further suggest that Nkd antagonizes the Wnt pathway at or upstream of Zw3.

In an effort to understand the mechanism by which Nkd antagonizes the canonical Wnt signaling cascade, Rousset *et al.* [3] probed the physical interactions between Nkd and various Wnt cast members. Through yeast two-hybrid and *in vitro* immunoprecipitation experiments, they showed that Nkd shares the stage exclusively with Dsh. Wharton *et al.* [5] extended these findings by identifying two murine

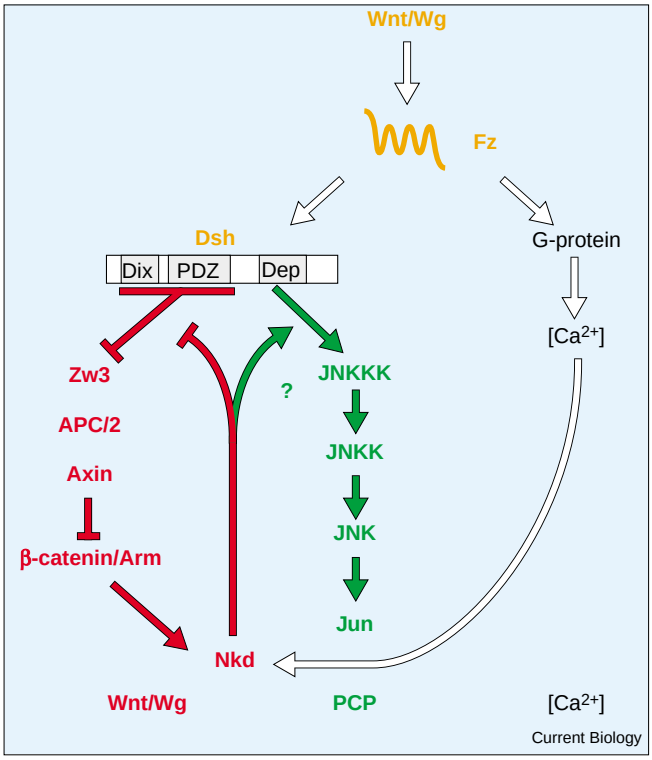
and two human Nkd homologs and showing that murine Nkd1 binds Dsh. In an independent screen for proteins that interact with murine Dvl, Yan *et al.* [4] identified a murine *nkd* homologue. Both groups used a series of fragments of Dsh/Dvl to demonstrate that Nkd specifically binds to the basic-PDZ region of Dsh/Dvl. As this domain is known to bind positive regulators of Wnt signaling such as GBP/Frat [13] and CAM Kinase-1ε [14], these results suggest that Nkd might antagonize Wnt signaling by preventing protagonistic cast members from associating with Dsh/Dvl; however, further experiments will be required to substantiate any potential mechanism.

In addition to the canonical pathway, genetic studies identified an alternative Wnt signaling cascade, the planar cell polarity pathway, that functions both to establish asymmetric cell polarities and to coordinate cell shape changes in response to Wnt signals (Figure 1b; reviewed in [2]). This alternative pathway depends upon the Wnt receptor, Frizzled (Fz), and Dsh/Dvl, but functions independently of β-catenin (Figure 1). Instead, planar cell polarity signaling appears to both affect the organization of the cytoskeleton directly [15] and regulate the activity of the Jun N-terminal kinase (JNK) signaling cascade [16]. As Dsh/Dvl acts in both Wnt and planar cell polarity signaling pathways, and directly interacts with Nkd, Yan *et al.* [4] and Rousset *et al.* [3] both examined the affect of Nkd on planar cell polarity signaling, yet came to different conclusions.

Characterization of the role of Dsh during planar cell polarity signaling in *Drosophila* established that the DEP domain of Dsh is required to both activate the JNK signaling cascade and coordinate planar cell polarity [16,17], while the basic-PDZ motif is dispensable. Nevertheless, Yan *et al.* [4] demonstrated that misexpression of murine Nkd promotes Jun phosphorylation in a dose-dependent manner [4]. Furthermore, they found that overexpression of Nkd in *Xenopus* explants mimics Dsh overexpression as convergent extension movements are arrested independent of β-catenin. Likewise, Rousset *et al.* [3] observed that overexpression of Nkd disrupted planar cell polarity in the *Drosophila* wing in an otherwise wild-type genetic background [3]. Together, these results suggest that Nkd, in addition to antagonizing Wnt signaling, may regulate the planar cell polarity signaling cascade. Yan *et al.* [4] further suggest that Nkd may act as a switch, redirecting signal from the canonical Wnt pathway to the JNK pathway (Figure 1).

Other data, however, are inconsistent with a role for Nkd in directing planar cell polarity. Loss of *nkd* function in the *Drosophila* wing fails to disrupt planar cell polarity, suggesting that Nkd is not essential for this process [11]. The observed effects of Nkd overexpression on planar cell polarity could also be explained if Nkd simply sequestered

Figure 1



Wnt signaling stage. Canonical Wnt signaling 'cast members' shown in red. Planar cell polarity signaling 'cast members' shown in green. Factors common to Wnt and planar cell polarity signaling pathways shown in yellow. Ca^{2+} signaling pathway shown in black.

Dsh in a non-functional complex as suggested by Rousset *et al.* [3] or altered Dsh binding to Axin, another Wnt cast member that activates the JNK signaling cascade [18]. Additional experiments will be required to verify a role for Nkd in regulating planar cell polarity signaling.

There are few clues in the amino acid sequence of Nkd as to how its activity might be regulated during canonical Wnt signaling or during the establishment of planar cell polarity. *nkd* encodes a hydrophilic protein with a single EF hand motif — a calcium-binding domain found in many Ca^{2+}-regulated proteins (reviewed in [19]) — most similar to that found in the Recoverin family of myristoyl switch proteins [11]. The EF-hand and the amino acids immediately surrounding it are well conserved between Nkd and its mammalian homologs [4,5]. Yan *et al.* [4] demonstrated that while the EF-hand of Nkd is not required for Dsh/Dvl binding, putative mutations that disrupt Ca^{2+} binding affect the ability of Nkd to antagonize Wnt signaling. This raises the question of whether Ca^{2+} levels play some role in Wnt signaling. While genetic approaches have not yet identified any obvious Ca^{2+}-regulated Wnt signaling components other than Nkd, it is intriguing that Fz receptors

are seven-pass transmembrane proteins which are structurally analogous to G-protein coupled receptors, many of which induce Ca^{2+} fluxes in response to ligand binding.

Biochemical and ectopic expression studies from Moon and colleagues (reviewed in [20]) have demonstrated that a third, G-protein coupled, pathway may also operate downstream of certain Fz receptors to elevate the intracellular concentration of Ca^{2+} and subsequently activate Protein Kinase C and CAM kinase II. Thus Nkd might serve to limit the duration and level of Wnt signaling by coordinating various story lines downstream of Fz (Figure 1). Further studies will be needed to address both the possible role of Ca^{2+} in regulating Nkd function and the potential cross-talk between the different pathways downstream of Wnt ligands. The expanding cast of characters in Wnt signaling is likely to spur further sequels in this on-going drama.

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