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Wnt Pathway Activation: New Relations and Locations

Minireview

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Recent advances in the Wnt signaling field reveal new components, such as a G protein and an atypical receptor tyrosine kinase, and novel connections between known components. In addition, different subcellular localization of receptors may help to explain distinctions between canonical and noncanonical Wnt pathway activity.

The Wingless(Wg)/Wnt family of secreted signaling molecules and the downstream components of Wnt signal transduction are highly conserved among animal species. Activation of the Wnt pathway controls a wide variety of processes in embryonic development and adult homeostasis (reviewed in Logan and Nusse, 2004). Wnts bind to cell surface receptors encoded by the Frizzled (Fz) gene family. Receptor activation in turn somehow activates Dishevelled (Dsh), the most proximal cytosolic component known. The precise role of Dsh in pathway activation is still not clear, but it may be intricate because the pathway branches downstream of Dsh. In canonical Wnt signaling, Dsh is involved in turning off a protein complex dedicated to the degradation of β-catenin (β-cat, called Armadillo in *Drosophila*) (reviewed in Jones and Bejsovec, 2003). Inactivation of the destruction complex allows β-cat to accumulate within responding cells and to enter the nucleus where it binds to Tcf, the transcriptional effector of the Wnt pathway. This complex recruits other proteins to drive transcriptional activation of Wnt target genes. In both vertebrates and invertebrates, conventional Wnt signal transduction results in tissue-specific cell fate decisions during embryogenesis and regulates cell proliferation in adult tissues.

Noncanonical Wnt signaling does not involve β-cat/Tcf-mediated transcription (reviewed in McEwen and Peifer, 2000). The best characterized noncanonical pathway activity generates planar cell polarity (PCP). This process was first defined in *Drosophila*, where the misorientation of adult hairs and bristles in mutants gave rise to the *frizzled* and *dishevelled* gene names. These components of the Wnt pathway are required for proper alignment and bundling of actin filaments to produce the normal orientation of epidermal elaborations such as wing hairs or thoracic bristles (reviewed in Adler, 2002). Although no Wnt molecule has yet been connected with the PCP pathway in flies, Wnt-5 and Wnt-11 in zebrafish are associated with the related process of tissue polarization during vertebrate gastrulation. Again, Dsh's role in converting Fz cell surface events into the proper placement of actin bundles during polarization is not clear. With so many pieces still missing from the puzzle of Wnt signal transduction, any new connections and components are of great value. Several recent papers reveal new insight into Wnt pathway mechanics.

In this issue of *Cell*, Katanaev et al. (2005) demonstrate that a *Drosophila* Gαo subunit plays a role in both canonical Wg signal transduction and planar cell polarity. Fz family members are serpentine receptors with seven transmembrane-spanning domains, closely related to G protein-coupled receptors. Previous studies with a chimeric rat Fz protein expressed in cultured cells indicated that heterotrimeric G proteins may play a role in Fz-stimulated Tcf transcriptional response (Liu et al., 2001). Because of the possibility that Fz receptors might act through a G protein, Katanaev et al. examined the six Gα genes annotated in the fly genome to assess their potential involvement in Wg signaling. They found that mutations in *G-oα47A*, also known as *brokenheart*, mimic the effects of mild loss of Wg signal transduction in imaginal disc clones and also generate PCP phenotypes in the mature wing. Overexpression of Gαo mimics the effects of Wg gain of function in embryonic patterning and also generates PCP phenotypes similar to Fz gain-of-function conditions. These phenotypes are also observed with a mutant Gαo that cannot hydrolyze GTP; they are not seen with a mutant form that fails to exchange GDP for GTP. Epistasis experiments with these overexpression constructs suggest that the Gαo acts upstream of *dsh* and that the wild-type, but not the GTP bound mutant form, of Gαo requires *fz* and *fz2* activity. Thus, the data are consistent with Fz acting as a guanine nucleotide exchange factor to activate Gαo and promote downstream events. However, no direct contact between Fz and Gαo was reported in this paper, and the subcellular distributions of the molecules suggest that they may not directly interact. In the wild-type pupal wing epithelium, Fz and Gαo become asymmetrically localized within the cortical region but at opposite sides of each cell. Gαo accumulates at the proximal edge, whereas Fz becomes enriched at the distal edge (Figure 1). Intriguingly, each is mislocalized in clones mutant for the other gene product, indicating that their subcellular localizations are interdependent.

Localization of the Fz receptors may be crucial for the differential deployment of Dsh in PCP versus canonical Wnt signaling (Figure 1). In *Drosophila*, Fz (which I will refer to as Fz1 for clarity) and Fz2 have redundant roles in canonical Wg signaling (Bhanot et al., 1999), although Fz2 has a ten-fold higher affinity for the ligand (Rulifson et al., 2000) and appears to be the primary receptor for Wg in embryonic signaling (Moline et al., 2000). Fz1 is exclusively required for PCP signaling in adult tissues; Fz2 has no role in PCP and no ability to compensate for loss of Fz1 activity in this process. Wu et al. (2004) have shown that Fz1 and Fz2 differ in their subcellular localization. Fz1 is enriched in the apical domain of the plasma membrane, where it colocalizes with components of the adherens junctions. Fz2 is more broadly distributed along the apical-basal axis of the cell. This distibution is controlled by sequences in the C-terminal,

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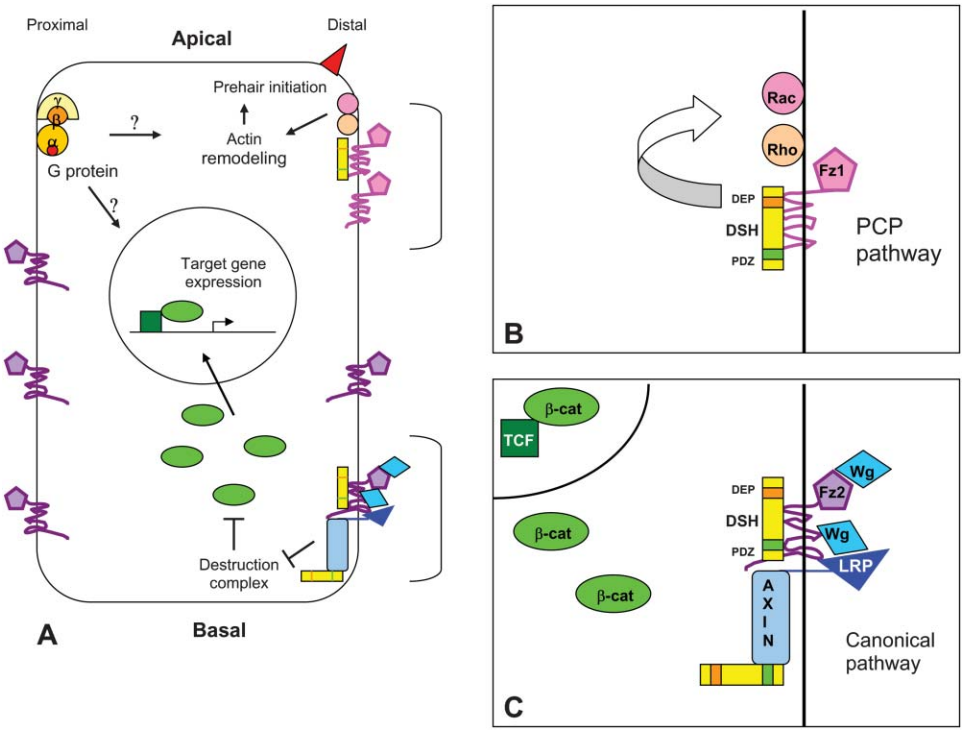

Figure 1. Schematic Diagram of Subcellular Asymmetries in a *Drosophila* Pupal Wing Cell

(A) Fz1 (pink) colocalizes with adherens junctions components in the cell cortex and becomes enriched at the distal edge of each cell. Fz2 (purple) is more evenly distributed along the apical-basal cell axis. The amino-terminal CRD of both Fz molecules is depicted by a pentagon. The Gαo protein localizes to the proximal edge of cells, presumably in connection with β and γ subunits to form a heterotrimeric G protein complex that potentially overlaps with the Fz2 distribution. The Gαo subunit binds GTP, depicted as a red circle.

(B) Apically localized Fz1 triggers the planar cell polarity (PCP) pathway. This involves recruiting Dsh, and some aspect of Dsh's DEP domain is required for stimulating the JNK signaling pathway and for activating Rac and Rho to reorganize the actin cytoskeleton into polarized structures.

(C) The canonical Wg pathway is activated by binding of Wg/Wnt to Fz2 and Arrow(Arr)/LRP, causing these cell surface receptors to cluster. This inactivates the Armadillo/β-catenin destruction complex, allowing Arm/β-cat to accumulate and form a transcriptional activation complex with Tcf. Dsh is absolutely required for this process, but it is unclear how it contributes to destruction complex inactivation. It is known to interact via its PDZ domain with Axin, a component of the destruction complex that can bind to the cytosolic tail of Arr/LRP. The DEP domain of Dsh is not required for canonical signal transduction, but other portions of the molecule, including the PDZ domain, are essential.

cytosolic portion of the molecule. The Fz2 C terminus is 61 amino acids longer than that of Fz1. Simply truncating the Fz2 C terminus at the point where the Fz1 C terminus ends is sufficient to redirect the molecule apically. Transferring the C-terminal 61 amino acids from Fz2 onto the end of the full-length Fz1 molecule prevents apical localization, and the chimeric protein assumes a broad apical-basal distribution. Curiously, this redistributed Fz1 protein still has some PCP activity, perhaps indicating that sufficient Fz1 is present in the apical domain to provide some tissue polarization function. The converse situation, however, has profound consequences for canonical Wg signaling. Overexpression of Fz1, or an apically directed chimeric Fz2, causes disruption of Wg-dependent wing margin patterning. This effect depends on Dsh dosage: it is enhanced in *dsh* heterozygous flies and reversed by extra copies of *dsh*. Wu et al. show that this effect results from changes in Dsh localization when Fz1 is ectopically expressed. Normally, Dsh is evenly distributed within epithelial cells, but overexpression of an apically targeted Fz1 molecule causes it to be recruited apically. Depletion of Dsh from the basolateral portion of the cell correlates with reduced canonical Wg signal transduction.

Previous studies had shown that Fz signaling correlates with membrane recruitment of Dsh (see, for example, Axelrod, 2001). This recruitment requires the DEP domain of Dsh, a portion of the molecule known to be required for PCP but not for canonical Wg signaling (reviewed in McEwen and Peifer, 2000). However, recent work using NMR spectroscopy shows that the PDZ domain of Dsh, which is known to be required for both canonical and noncanonical signaling, also interacts with a membrane-proximal region in the C terminus that is conserved between Fz1 and Fz2 (Wong et al., 2003). Moreover, the Varmus laboratory has identified three conserved residues in cytosolic regions of human Fz5 that, when mutated, abolish canonical Wnt signaling and also disrupt membrane recruitment of Dsh in transfected cells (Cong et al., 2004). Thus, a physical association between Fz and Dsh may be relevant for the canonical pathway as well. On the other hand, recruitment of Dsh to the membrane could be indirect, through other components of the receptor complex. Fz interacts with LDL-

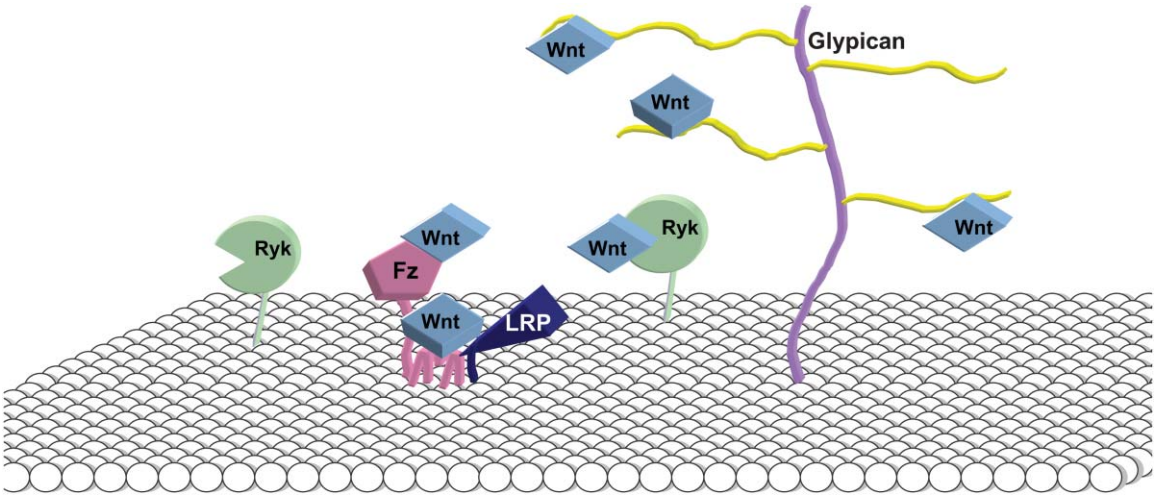

Figure 2. Schematic Diagram of the Extracellular Environment of a Cell Responding to Wnt Signal

The CRD domain that comprises the extracellular amino-terminus of Fz is known to bind Wnt, but may not be required for transducing the signal. This implies that the true ligand binding pocket may be formed by the extracellular loops of the transmembrane-spanning region. The extracellular WIF domain of the Ryk receptor also binds Wnt and this may either transduce signal directly through some novel pathway or may potentiate signaling through the Fz receptor. Sulfated glycosaminoglycans, such as those attached to the glypican proteoglycan core protein, are also known to bind Wnt but are not essential for signaling when ligand is abundant. Thus, they act to concentrate Wnt at the cell surface, perhaps enhancing its interaction with the Fz-LRP receptor complex.

receptor related protein (LRP, called Arrow in *Drosophila*), a single transmembrane-spanning protein that is essential for canonical pathway activation. Previously, the only direct connection between receptor complex and canonical pathway had been through the cytosolic domain of Arrow/LRP, which recruits Axin, a scaffolding molecule that binds Dsh and comprises part of the destruction complex (reviewed in Jones and Bejsovec, 2003). Thus, it will be of interest to test for apical-basal subcellular distribution of Arrow and Axin under normal conditions and during Fz1 overexpression to determine whether Arrow and Axin might also be involved in the apparent association between Fz and Dsh in vivo.

A basolateral focus for canonical signaling superficially seems to conflict with previous data indicating that *wg* mRNA is apically targeted in polarized tissue (Simmonds et al., 2001). This transcript targeting is necessary for Wg protein product to be fully functional in canonical signaling. However, we do not yet understand why this apical localization of message and product in *wg*-expressing cells is important and what effect this has on Wg response in neighboring cells. It is possible that the glycosylases and acyltransferases that modify Wg are enriched in apical domains of the endoplasmic reticulum and Golgi, or that the mature ligand is sorted in this domain into trafficking vesicles that target it for delivery to the basolateral portion of the membrane. Indeed, earlier reports indicate that Wg protein can be detected preferentially in basolateral regions of cells adjacent to the *wg*-expressing cells (Gonzalez et al., 1991). A basolateral focus for canonical Wg signaling also complicates the explanation of Fz1's ability to compensate for loss of Fz2 in embryonic Wg signaling. However, Wu et al. did not examine embryonic stages, and so Fz1 may not be targeted apically in the embryonic epidermal epithelium. Alternatively, embryonic loss of Fz2 activity may alter Fz1 trafficking. *fz2* mutant embryos appear to accumulate higher levels of Wg protein extracellularly, indicating that loss of the high-affinity receptor artificially alters the cellular environment (Moline et al., 2000). The higher concentration of Wg ligand alone, or in combination with an altered subcellular localization of Fz1, may enable Fz1 to participate in canonical Wg signal transduction and rescue the *fz2* mutant effects.

Chimeric constructs have also revealed that the transmembrane-spanning and extracellular regions of Fz direct its participation in the PCP pathway (Wu et al., 2004). Replacement of these portions with the corresponding Fz2 sequences eliminates its gain-of-function PCP activity even though the molecule is apically targeted. The majority of this effect is due to replacement of the transmembrane region alone, suggesting that much of the PCP signal transduction capacity of Fz1 resides in the three cytosolic loops between transmembrane-spanning segments. The effect of chimeras on canonical Wg signaling is complicated by the apical targeting provided by the Fz1 C-terminal tail. However, it is clear that chimeras with the Fz2 extracellular region, when overexpressed, have a stronger dominant-negative effect on canonical signaling than the full-length Fz1 transgene does. This is thought to be due to high-affinity Wg binding mediated by the cysteine-rich domain (CRD) in the Fz2 extracellular region.

The role of the CRD in Wg signaling has recently been called into question. Fz transgenes with this extracellular domain deleted are able to rescue canonical loss-of-function phenotypes observed in *fz1 fz2* doubly mutant embryos or doubly mutant clones in wing imaginal discs. The CRD-deleted form of either Fz1 or Fz2 is able to rescue equally well under the constitutive low-level expression conditions used in this study (Chen et al., 2004). These data suggest that the ligand binding pocket rele-

vant to Wg signal transduction is formed from the extracellular loops of the transmembrane region, whereas the CRD simply stabilizes extracellular Wg and/or increases its local concentration near the signaling pocket (Figure 2). This function would be analogous to the way in which proteoglycans are thought to contribute to Wnt signal transduction (reviewed in Bejsovec, 2000). Mutations that disrupt proteoglycan biosynthesis in *Drosophila* produce embryonic loss-of-function *wg* phenotypes, but these defects can be rescued by ectopically expressing *wg*. Because raising the concentration of ligand bypasses the proteoglycan requirement, proteoglycans are thought to have an accessory rather than essential function in the core receptor complex (Figure 2).

A similar auxiliary role may be played by the Ryk atypical receptor tyrosine kinase, a newly identified receptor for Wnt. A single Ryk gene in mammals and in the nematode *C. elegans* and three Ryk genes in *Drosophila* define this family (Yoshikawa et al., 2003; Inoue et al., 2004; Lu et al., 2004). They are characterized by an extracellular domain with homology to Wnt inhibitory factor (WIF), a single transmembrane-spanning sequence, and an intracellular kinase homology domain that does not appear to possess kinase activity. One of the fly gene products, Derailed, and the mammalian Ryk are associated with axonal guidance in response to Wnt-mediated cues. The mammalian Ryk has been shown to bind through its WIF domain to Wnt-1, Wnt-3a, and the Fz CRD and to interact with Dsh via a cytosolic PDZ domain (Lu et al., 2004). However, in *C. elegans*, intracellular portions of Ryk appear to be dispensable in Wnt signaling (Inoue et al., 2004). The worm Fz, encoded by *lin-17*, and Ryk, encoded by *lin-18*, are both required for proper specification of vulval cell fates. They play nonredundant roles in this Wnt-mediated process and *lin-17*;*lin-18* doubly mutant animals have a more fully penetrant vulval defect. Remarkably, a *lin-18* construct lacking the entire cytosolic domain is able to rescue the *lin-18* mutant phenotype, even when the transmembrane domain is replaced with a heterologous transmembrane domain. Thus, only the extracellular portion of the molecule is relevant to its involvement in Wnt signaling. This is consistent with the idea that the WIF domain interacting with Wnt provides a ligand recruitment function that enhances activation of the core receptor complex. While Ryk/Lin-18 appears to have function independent of Fz/Lin-17, there are other Fz family members in *C. elegans* with which Ryk/Lin-18 might interact during vulval cell specification. It is not clear whether this finding in *C. elegans* will apply generally to canonical Wnt signaling in other systems, however, because none of the downstream canonical Wnt components have yet been connected with vulval cell fate specification. Indeed, the axonal guidance phenotype of *derailed* in *Drosophila* also may not involve canonical Wnt signaling, because Dsh does not appear to participate in this process (Yoshikawa et al., 2003). Thus, the Ryk family may control a signal transduction cascade that is entirely different from known Wnt pathways, in addition to or instead of augmenting the known canonical pathway.

In summary, more pieces of the Wnt puzzle have been discovered, but it is still not clear how they all fit together to promote different cellular responses. The observation that Wnt pathway components partition into distinct subcellular domains is a tantalizing clue, and with the rapid evolution of imaging technologies, the puzzle pieces may soon fall into place.

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